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Cross-Amplified Polymorphic Microsatellites for Campbell's Monkey

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Abstract
Population genetic analyses are of considerable importance for conservation strategies to protect endangered primates. We tested microsatellites of human origin with an aim to understand the genetic diversity of a West African forest guenon, Campbell's monkey, *Cercopithecus campbelli*. Twelve markers amplified successfully, were polymorphic and were inherited in a Mendelian fashion in a group of 4 individuals kept in captivity. These 12 markers were further amplified from 35 faecal samples collected in Taï National Park. These samples proved to originate from 18 free-ranging monkeys and showed that the 12 markers we developed for this species are polymorphic and suitable for future population genetic and parentage analyses.

When developing hypervariable microsatellite markers in a new non-model species, one has to choose between de novo library construction and cross-amplification of markers from related species. Microsatellite libraries are still quite expensive to develop [Zane et al., 2002], and cross-amplification is not devoid of problems

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Table 1. Human microsatellites identified as polymorphic in Campbell's monkey

Locus	Primer sequence (from 5′ to 3′)	Final [c] μM	Reference	Size range bp	Alleles n	Expected heterozygosity
D1S207*	6FAM-CACTTCTCCTTGAATCGCTT GCAAGTCCTGTTCCAAGTCT	0.2	a	121–141	8	0.84
D3S1766*	HEX-ACCACATGAGCCAATTCTGT ACCCAATTATGGTGTTGTTACC	0.15	b	206–218	4	0.73
D3S1768*	NED-GGTTGCTGCCAAAGATTAGA CACTGTGATTTGCTGTTGGA	0.3	c	187–219	7	0.77
D5S117$	6FAM-TGTCTCCTGCTGAGAATAG TAATATCCAAACCACAAAGGT	0.15	b	133–149	7	0.70
D5S820$	NED-ATTGCATGGCAACTCTTCTC GTTCTTCAGGGAAACAGAACC	0.25	c	170–195	7	0.79
D5S1457$	6FAM-TAGGTTCTGGGCATGTCTGT TGCTTGGCACACTTCAGG	0.2	c	115–127	4	0.76
D6S265$	HEX-ACGTTCGTACCCATTAACCT ATCGAGGTAAACAGCAGAAA	0.1	a	128–134	3	0.62
D6S311$	HEX-ATGTCCTCATTGGTGTTGTG GATTCAGAGCCCAGGAAGAT	0.2	c	186–232	10	0.89
D7S503*	NED-ACTTGGAGTAATGGGAGCAG GTCCCTGAAAACCTTTAATCAG	0.2	a	125–131	4	0.62
D8S166*	HEX-GATTGTGTCATTGCACTCCA ACAAGGAAGTTCCTTTTTGG	0.2	b	116–122	4	0.70
D14S306$	6FAM-AAAGCTACATCCAAATTAGGTAGG TGACAAAGAAACTAAAATGTCCC	0.2	c	157–205	9	0.87
D21S1440*	HEX-GAGTTTGAAAATAAAGTGTTCTGC CCCCACCCCTTTTAGTTTTA	0.1	b	125–141	5	0.61

* and $ designate the two multiplexes for which we give final primer concentrations in the PCR mix. a = Clisson et al., 2000; b = Hatcher, 2007; c = Charpentier et al., 2005.

either. In particular, microsatellites that are polymorphic in one species may be monomorphic, or not be present at all, in a related species. The probability of finding polymorphic microsatellites in cross-amplification is increased for markers that have been tested and proved polymorphic in at least 2 other closely related species due to phylogenetic reasons. This is an advantage when working with apes and monkeys because (1) a huge number of markers derived from the human genome are available [Subramanian et al., 2003] and (2) many of them have already been tested in different non-human primate species [Erler et al., 2004; Roeder et al., 2009].

To develop a set of polymorphic markers useful for population genetic analyses in a species for which no information is yet available [Roeder et al., 2009], Campbell's

monkey *(Cercopithecus campbelli)*, we tested microsatellites of human origin that have already been tested in other *Cercopithecus* species [Clisson et al., 2000; Charpentier et al., 2005; Hatcher, 2007]. We obtained samples from two sources. Four individuals born and kept in captivity at the Paimpont Biological Station (University Rennes-1 and CNRS, Brittany, France) were investigated with 18 markers to test for amplification success, polymorphism and Mendelian inheritance. The 4 individuals were an adult male and female with their son and an unrelated adult female. The markers that proved polymorphic and which exhibited Mendelian inheritance were used to genotype 35 faecal samples collected from a free-ranging population of Campbell's monkeys habituated to human observers [Ouattara et al., 2009] in the Taï National Park (Ivory Coast). The samples were kept dried in silica gel beads after collection and until DNA extraction. DNA was extracted following the procedure described in Vallet et al. [2008]. The microsatellites were amplified in 10-μl multiplex reactions containing 2 μl of DNA extract, 1 × Multiplex PCR Master Mix (Qiagen) and primer concentrations as reported in table 1. Amplification conditions were as follows: 15 min at 95°C; 35 cycles of 94°C for 30 s, 57°C for 90 s, 72°C for 90 s; 30 min at 72°C. PCR products were run on an ABI Prism 3130 XL Genetic Analyser 16-capillary system (Applied Biosystems) and sized with an internal lane standard (ROX500; Applied Biosystems) using the program Genemapper version 4.0 (Applied Biosystems). Because we worked with noninvasive genetic samples, typing was repeated at least twice for each genotype before mutlilocus genotypes were compared to assign them to individuals, as different faecal samples may come from the same individual. These replicates were obtained from the same DNA extract. Gene diversity and the coefficient of inbreeding were computed with FSTAT 2.9.3 [Goudet, 1995], with which we also tested for linkage and Hardy-Weinberg equilibrium for each locus.

Among the 18 markers we tested (listed in table 1 plus MIB, D5S1470, D6S265, D13S318, D13S765, D17S1290), 12 were polymorphic, amplified reliably and were inherited in a mendelian fashion. They were amplified from a set of 35 samples that proved to originate from 18 individuals. Each genotype resulted from the consensus reading of, on average, 3.24 PCR amplifications. We did not observe any genotyping error, either allelic dropout or false allele [Broquet and Petit, 2004]. However, a significant and negative correlation between the coefficient of inbreeding and the number of PCR replicates across loci (Kendall's rank correlation test, $z = -1.9933$, $p = 0.046$) suggested that allelic dropout occurred in this limited data set. Amplifying the same allele from a heterozygous locus in different PCR replicates (as a result of dropout or null alleles) may have led us wrongly to assign a homozygous genotype in such cases. In fact, while heterozygous genotypes were read on average 3.3 times, homozygous genotypes were replicated only 3 times, which might not be sufficient to confirm homozygotes [Taberlet et al., 1996]. A limited number of genotyping errors has however little influence on the evaluation of polymorphism of microsatellites, which was the goal of this study.

In fact, the 12 loci were highly polymorphic in the population of the Taï National Park. These loci had 6 alleles on average (range: 3–10) and an expected heterozygosity of 0.74 (range: 0.61–0.89). These figures indicate that the species ranks among those exhibiting high genetic variability [DeWoody and Avise, 2000], thus providing researchers with relevant tools for population genetic and parentage analyses in this species.

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