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Identification and characterization of microsatellite markers via cross-species amplification from Francois' langur (*Trachypithecus francoisi*)

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Abstract: Analysis of the population genetic structure and reproductive strategies of various primate species has been facilitated by cross-species amplification. We screened 138 human-derived markers to assess their utility in Francois' langur (*Trachypithecus francoisi*). Of the 138 loci, twenty-three produced reliable results and exhibited moderate levels of polymorphism. The number of alleles per locus ranged from three to nine among 28 individuals, and average observed and expected heterozygosities were 0.50 and 0.62, respectively. Seven loci showed significant deviation from Hardy-Weinberg equilibrium. There were null alleles at nine loci, but no linkage disequilibrium between loci was detected. These loci could be useful in the population genetic study of this species.

Key words: Cross-species amplification; Francois' langur (*Trachypithecus francoisi*); Microsatellite primers

近缘种扩增法对黑叶猴微卫星位点的筛选及特征分析

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摘要：微卫星位点近缘种筛选法使得在探讨各种灵长类种群遗传结构和生殖策略上更加便捷。我们利用 138 条人类微卫星引物在黑叶猴中进行筛选，得到了 23 个具有多态性位点。在 28 个检测个体中，每个位点的等位基因数为 3 到 9 个，期望杂合度为 0.62，观测杂合度为 0.50，其中有 7 个位点偏离 Hardy-Weinberg 平衡，9 个位点存在无效等位基因现象。但是各位点之间均未检测到连锁不平衡现象。这些位点将在黑叶猴种群遗传结构的研究中发挥重要作用。

关键词：近缘种扩增；微卫星引物；黑叶猴

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1 Introduction

The Francois' langur (*Trachypithecus francoisi*) is a primate species living in a habitat characterized by karst topography, and is categorized as endangered on the IUCN Red List (IUCN 2009). Due to human activities, their habitats have been severly destroyed, causing large population declines (Li *et al.*, 2007). Proper knowledge of the population and conservation genetics of *Trachypithecus francoisi* will contribute to more appropriate conservation management. However, research on genetic variation in *Trachypithecus francoisi* has been very limited, leaving little relevant information for this species. Microsatellite loci are relatively common in vertebrate genomes, and are highly polymorphic and easy to score, which make them powerful tools for population genetic analysis (Zane *et al.*, 2002). Here, we report a suite of 23 polymorphic microsatellite loci for the Francois' langur.

2 Animals and methods

A total of 28 samples were collected, including 9 blood from Beijing Zoo, 3 muscle from Nanning Zoo in

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Guanxi Province, 5 hair and 11 fecal samples from the wild population in Mayanghe Nature Reserve in Guizhou Province, China. We extracted total DNA from blood and muscle samples via a standard phenol-chloroform method (Sambrook *et al.* 1989). DNA from hair samples was extracted following a modified version of Allen *et al.* (1998) protocol, and the fecal DNA was obtained via the QIAGEN DNA Stool Mini Kit (QIAGEN). We tested 138 human microsatellite loci to select reliable polymorphic molecular markers for this species, which have proved to be useful in other primate species. We first amplified these loci from DNA from the muscle samples. The PCR amplifications were performed in a 20μL reaction volume comprising of 50 ng of template DNA, 50 mM KCl, 20 mM Tris-HCl (pH 8.0), 2.0 mM $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each dNTP, and 0.5U of Hotstart Taq DNA polymerase (QIAGEN). The PCR conditions were as follows: 95℃ for 5 min, followed by 40 cycles of 30 s at 95℃, 30 s at 50-54℃ (depending on the primer set), and 30 s at 72℃, with a final step of 30 min at 72℃. 45 microsatellite loci were amplified successfully and were screened for variation in all 28 samples. The forward primers used in the PCR were fluorescently labeled (FAM, HEX, or TAMRA). To further analyze all successful reactions, we determined allele sizes by genotyping the alleles on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) and comparing the results with GeneScan ROX 350 internal size standard. We assigned allele sizes against the internal size standard and genotyped individuals via GeneScan version 2.0 (Applied Biosystems). We repeated amplification 2-3 times for these samples. Specifically, both alleles of a heterozygote were typed twice before they were accepted, and individuals were only scored as homozygous if all the replicates showed identical homozygous profiles.

3 Results

Of the 45 successfully amplified loci, only 23 were polymorphic. Population genetic parameters, deviations from Hardy-Weinberg equilibrium (HWE) and a linkage disequilibrium (LD) test were estimated with GENEPOP version 4.0 (Raymond and Rousset, 1995). The characteristics of the 23 microsatellite loci are listed in Table 1. All of the 23 loci were highly polymorphic with an average of 4.91 alleles per locus (range from three to nine), expected heterozygosity (H_E) ranged from 0.138 to 0.840, whereas observed heterozygosity (H_O) ranged from 0.143 to 0.929. LD was not detected between these loci. Seven microsatellite loci were found to deviate from Hardy-Weinberg expectations ($P < 0.05$) after Bonferroni correction (Narum, 2006), which exhibited heterozygote deficit. MICRO-CHECKER (van Oosterhout *et al.*, 2004) analysis indicated there were null alleles at nine loci (Table 1). Therefore, the occurrence of null alleles could be the most likely explanation for the heterozygote deficiencies observed in the data set.

Table 1 Characterization of 23 microsatellite loci in *Trachypithecus francoisi*

Locus and GenBank Accession no.	Primer sequences (5'-3')	T_a (℃)	Size range (bp)	N_a	H_O	H_E	P_{HW}	F_n
D2S1326	F: AGACAGTCAAGAATAACTGCCC R: CTGTGGCTCAAAAGCTGAAT	50	201-217	5	0.500	0.756	0.0002*	0.1391
D3S1768	F: GGTTGCTGCCAAAGATTAGA R: CACTGTGATTTGCTGTTGGA	50	158-186	4	0.179	0.263	0.1234	–
D3S2459	F: CTGGTTTGGGTCTGTTATGG R: AGGGACTTAGAAAGATAGCAGG	52	166-186	3	0.143	0.138	1.0000	–
D5S1457	F: TAGGTTCTGGGCATGTCTGT R: TGCTTGGCACACTTCAGG	50	93-129	9	0.893	0.803	0.9058	–
D6S264	F: AGCTGACTTTATGCTGTTCCT R: TTTTCCATGCCCTTCTATCA	50	147-163	6	0.429	0.761	0.0000*	0.1825
D6S287	F: ATATTAGTGCCTTATGCTTCTG R: AAATTGGATATTCATGCTTG	52	129-133	3	0.571	0.579	0.3793	–
D6S311	F: TCATTGGTGTTGTGCATTAA R: TTGGAAGGATGAGAATTAAGG	52	220-232	6	0.464	0.727	0.0149*	0.1455
D6S434	F: TTCAACACACAGGTAGTCCC R: CAGGGCTTATGCCAGTATTA	50	173-193	9	0.571	0.840	0.0123*	0.1391
D6S493	F: ATCCCAACTCTTAAATGGGC R: TTCCATGGCAGAAATTGTTT	50	244-252	3	0.500	0.663	0.1214	–

Continued from table 1

Locus and GenBank Accession no.	Primer sequences (5' -3')	T_a (℃)	Size range (bp)	N_a	H_O	H_E	P_{HW}	F_n
D7S1826	F: TATTTCTCCTGTCATCTC R: TTCCCAAAGTCTGCAATC	51	148 – 168	6	0. 750	0. 784	0. 5493	–
D8S272	F: GAGAACTAATCCCTTCTGGC R: AGCTTCATAAAGAGTCTGGAAAAT	53	221 – 231	5	0. 571	0. 758	0. 1057	0. 0991
D8S505	F: CAAAAGTGAACCCAAACCTA R: AGTGCTAAGTCCCAGACCAA	53	127 – 141	6	0. 643	0. 778	0. 0594	–
D10S1686	F: CTCTTCAGTTCCAACCACAC R: ATAACACAGGGCCATTTAAG	51	185 – 193	4	0. 429	0. 527	0. 2348	–
D10S676	F: GAGAACAGACCCCCAAATCT R: ATTTCAGTTTTACTATGTGCATGC	54	155 – 165	5	0. 643	0. 687	0. 1011	–
D11S2002	F: CATGGCCCTTCTTTTCATAG R: AATGAGGTCTTACTTTGTTGCC	53	251 – 259	3	0. 464	0. 668	0. 0180*	0. 1156
D12S364	F: CAGACCTATGACTCAAAATCCTTG R: TGTGTACACATAAGACGCATATAGG	54	116 – 130	6	0. 536	0. 721	0. 1359	0. 1008
D15S644	F: CCTTCATTGGCAGACTCACT R: GCAGACACCAAGATGATAACG	52	175 – 181	4	0. 214	0. 564	0. 0258*	0. 2187
D17S921	F: GCAACATATTACATGGGGTG R: CTTTATGGCCACCATAATCA	51	160 – 166	4	0. 179	0. 171	1. 0000	–
D20S206	F: TCCATTATTCCCCTCAAACA R: GGTTTGCCATTCAGTTGAGA	50	105 – 113	3	0. 536	0. 629	0. 2895	–
DQCAR	F: GAAACATATATTAACAGAGACAGACAAA R: CATTTCTCTTCCTTATCACTTCAT	51	99 – 111	6	0. 929	0. 770	0. 9626	–
DQ911153	F: CAGCGTAAGCCAGTTGCC R: GGAAAAGTCTGAAACCCACGA	53	113 – 127	5	0. 464	0. 538	0. 2988	–
DQ911152	F: GTGTATTGTGGGGCTATC R: GTGGGCTCTGACCTAGGAATC	51	182 – 196	5	0. 250	0. 486	0. 0409*	0. 1541
MOGc	F: GAAATGTGAGAATAAAGGAGA R: GATAAAGGGGAACTACTACA	51	131 – 135	3	0. 643	0. 649	0. 4820	–

Reported are: locus name and GenBank Accession no; sequences for forward (F) and reverse (R) primers; optimal annealing temperature (T_a); allele size range (bp); number of alleles (Na); observed heterozygosity (H_O); expected heterozygosity (H_E); probability of Hardy Weinberg equilibrium (P_{HW}) and their significance after table – wide Bonferroni correction (* $P < 0.05$); frequency of null alleles (Fn).

4 Discussion

We report here the first set of microsatellites for *Trachypithecus francoisi* that will likely be useful to investigate the population structure in this species, including estimation of intrapopulation genetic structure and interpopulation genetic relationships. In addition, adequate levels of variation at the 23 loci will allow researchers to assess paternity and other important behavioral components such as reproductive strategies, which will establish a sound base of conservation genetics for a proper management strategy for the Francois' langur.

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