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TECHNICAL NOTE

Isolation and characterization of nine microsatellite loci for the takin (*Budorcas taxicolor*)

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Abstract We report on the isolation and characterization of nine microsatellite markers in the takin (*Budorcas taxicolor*) from genomic DNA-enriched libraries. Twenty-eight microsatellites were screened from the libraries, and nine of the screened microsatellites were polymorphic. The number of observed alleles for each locus in 28 individuals ranged from two to seven, and the expected and observed heterozygosity was 0.105–0.758 and 0.071–0.821, respectively. Four loci (TK01, TK02, TK04 and TK08) of nine deviated from Hardy-Weinberg expectation and no significant linkage association was found among all these loci. These microsatellite markers provide useful tool for population genetic studies of the takin.

Keywords Bovidae · *Budorcas taxicolor* · Conservation · Microsatellite DNA · Polymorphic · Takin

Takin (*Budorcas taxicolor*), a large ungulate belonging to Bovidae, is found in China, Myanmar (Burma), India, and Bhutan. Most of its present distribution is in the provinces of Shaanxi, Sichuan, Gansu, Yunnan and Tibet within China. Because takins live in mountainous terrain, their range within these provinces is largely limited to the Qinling, Minshan, Qionglai, Liangshan, Gaoligong, and Himalaya Mountains (Wu et al. 1990). The takin populations have declined drastically within past 30 years due to habitat loss and fragmentation, over-hunting and other human-caused disturbances. The overall population of takin was estimated to be about 20,000 in 2003 (Zeng et al. 2003). At present, the takin is listed as the first-class key species with state-protection in China, as an endangered species in China Species Red List (Wang and Xie 2004), and as a vulnerable species in the 2007 IUCN List category (http://www.redlist.org/). In order to develop adequate conservation and management strategies for this species, it is important to have a reliable understanding of its population structure and evolutionary history, genetic diversity and kinships. This requires a set of microsatellite markers as a powerful tool (Zhang and Hewitt 2003). Here, we report the isolation and characterization of nine novel microsatellite loci in *B. taxicolor*.

A genomic library was produced according to an enrichment method similar to that described by Hammond et al. (1998). The muscle samples of *B. taxicolor* were collected and stored in ethanol and preserved at −20°C. Total DNA was extracted from a muscle sample using a standard phenol–chloroform method (Sambrook et al. 1989) and digested with *Mbo*I restriction enzyme (Promega). Then a 300–1,000 bp DNA fragments was isolated from total genomic DNA and ligated to *Mbo*I linkers made by annealing equimolar amounts of *Mbo*I1 (5′-GATCGCAGAATTCGCACGAGTACTAC-3′) and *Mbo*I2 (5′-CGTCTTAAGCGTGCTCATGATGC-3′). The ligated fragments were enriched and made double-stranded by polymerase chain reaction (PCR) (5 min 72°C, then 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 67°C and 2 min extension at 72°C) using *Mbo*I1 as the PCR primer. The whole PCR products were hybridizated to biotin-labelled probe [biotin-ATAGAATAT $(CA)_{20}$] (Kandpal et al. 1994) and subsequently isolated with the streptavidin-coated magnetic beads (Promega). The enriched microsatellite fragments were ligated into pMD18-T vector

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Table 1 Characteristics of nine polymorphic microsatellite loci in takin (*Budorcas taxicolor*)

Locus	Primer sequence (5′-3′) (F, forward; R, reverse)	Repeat motif	Labelling dye	T_a (°C)	A	Size range (bp)	H_O	H_E	GenBank Accession no.
TK01	F: CGATCTGCTTTCAAACTAGGA[a]	$(GT)_{22}$	FAM	54	2	160–168	0.107	0.512	EU315216
	R: GGAAATGGCAACCCAC								
TK02	F: GAGGAGCCTGGTGGGTTATG[a]	$(CA)_{13}$	HEX	53	5	129–137	0.107	0.408	EU315217
	R: AGAATTCGCACGAGTACTACG								
TK03	F: GGCTCTCCTTCAGTAATCTCA[a]	$(TG)_{17}$	HEX	53	2	151–161	0.071	0.105	EU315218
	R: CGGAGGAAGAGCAGTATTG								
TK04	F: GCACAGTGATGTCTATGGGTT[a]	$(TG)_9TA(TG)_3$	HEX	53	7	154–168	0.321	0.758	EU315219
	R: AATTCGCACGAGTACTACG								
TK05	F: CCACCTGTACCACTGTACACA[a]	$(AC)_{11}$	FAM	53	7	112–132	0.321	0.353	EU315220
	R: CCCAAGTCTGGATACGAC								
TK06	F: CGGAGGAAGAGCAGTATTG[a]	$(TC)_{11}TG(TC)_4$	HEX	53	2	148–160	0.107	0.137	EU315221
	R: GGCTCTCCTTCAGTAATCTCA								
TK07	F: ACGATCACCTTGTGACAATCA[a]	$(TG)_{13}$	FAM	55	2	148–156	0.071	0.105	EU315222
	R: GCAACCCACTCCAGTATTCT								
TK08	F: CCCTGGAGGAGGAAATAGCA[a]	$(AC)_3AT(AC)_8$	FAM	55	2	160–164	0.821	0.493	EU315223
	R: CGCACGAGTACTACGATCACC								
TK09	F:CCCTCACCACTCCACAGTCC[a]	$(AC)_7$	FAM	55	2	156–162	0.071	0.105	EU315224
	R:AATGCAATCAATGGGTAGCAG								

[a] Labelled primer

T_a, annealing temperature; A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity

(TaKaRa) and transformed into competent Top10 cells, then plated up on Luria Bertani (LB) agar containing ampicillin, isopropyl-β-D-thiogalactopyranoside (IPTG) and X-gal. Colonies were screened following the polymerase chain reaction based screening method of Lunt et al. (1999). Of the 288 colonies screened, 71 were positive and were sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI 377 automated DNA sequencer. Sixty-five colonies had repeat sequences. Twenty-eight primer pairs were designed using the software program oligo^{TM} 6.0 (Molecular Biology Insights).

PCR amplifications were performed in 10 µl reaction mixture, consisting of approximately 30 ng of template DNA, 0.2 mM of each dNTP, 1 × PCR buffer (TaKaRa Biomedical), 1.5 mM Mg^{2+}, 0.15 U of *Taq* DNA polymerase (TaKaRa Biomedical) and 0.15 µM of each primer. The reaction mixture was amplified using an MBS Satellite thermal cycler (Thermo Electron Corp., USA), using an initial denaturing step of 5 min at 94°C and 35 cycles of 94°C for 30 s, 50–58°C for 30 s, and 72°C for 30 s, followed by 7 min at 72°C. For genotyping, fluorescently labelled PCR products (Table 1) were electrophoresed along with GeneScan ROX 400 internal size standard on an ABI PRISM 3700 Genetic Analyser (Applied Biosystems). Allele sizes were assigned against the internal size standard and individuals were genotyped using Genescan version 2.0 (Applied Biosystems).

We observed that nine microsatellite loci showed polymorphic in 28 individuals. Population genetic parameters were estimated with Genepop version 3.4 (Raymond and Rousset 1995; available: http://wbiomed.cutin.edu.au/genepop/), all the effects of multiple tests were adjusted by Bonferroni method (Rice 1989). The number of observed alleles per locus ranged from two to seven. Observed heterozygosity (H_O) ranged from 0.071 to 0.821 and expected heterozygosity (H_E) ranged from 0.105 to 0.758. After sequential Bonferroni corrections, four loci (TK01, TK02, TK04 and TK08) of nine showed deviation from Hardy-Weinberg predictions (exact probability test). The departure from HWE is probably caused by random sampling from different regions and we have no information about the relationship among individuals. No significant linkage association was found among all these loci. The result showed that these nine polymorphic microsatellite loci will provide a powerful tool for the population genetic studies and conservation efforts of takin.

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