Conservation Genet Resour (2014) 6:1–3
DOI 10.1007/s12686-013-0005-7

TECHNICAL NOTE

CATS derived SNPs discovery in the golden snub-nosed monkey (*Rhinopithecus roxellanae*)

Chen Cheng · Shuzhen Li · Boshi Wang · Maofang Luo · Yunxia Yang · Guang Yang · Ming Li

Received: 28 June 2013 / Accepted: 19 July 2013 / Published online: 9 August 2013

Abstract In this study, we used a targeted approach to discover single nucleotide polymorphism (SNP) markers in the genome of an endangered primate, *Rhinopithecus roxellanae*. We first performed polymerase chain reactions using reported comparative anchor tagged sequences (CATS) primers, as well as some of the primers modified according to the *Macaca mulatta* orthologs. 118 CATS sequences were attainable. Then a denaturing high performance liquid chromatography and sequencing combining method was applied to check single nucleotide polymorphisms within the sequences. A total of 56 original SNPs were successfully developed, which were further proved to be detectable and polymorphism in more individual samples. These SNP markers are expected to be used in genetic researches, such as population structure, demography and local adaptation of this precious species.

Keywords Single nucleotide polymorphisms (SNPs) · *Rhinopithecus roxellanae* · CATS · DHPLC

C. Cheng · B. Wang · M. Luo · M. Li (✉)
Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, 1-5 Beixhenxi Road, Chaoyang, Beijing 100101, China
e-mail: lim@ioz.ac.cn

C. Cheng · B. Wang · M. Luo
University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China

S. Li · Y. Yang · G. Yang (✉)
Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210097, China
e-mail: gyang@njnu.edu.cn

The Sichuan snub-nosed monkey (*Rhinopithecus roxellanae*) is an Old World monkey in the subfamily Colobinae. It is endemic to three isolated temperate mountainous districts in west-central China: Sichuan and Gansu (SG), Qinling (QL) of the Shaanxi province and Shennongjia forest (SNJ) of the Hubei province. Mainly due to habitat loss and hunting, it has been through a decline of over 50 % in the last few decades (IUCN 2012). Therefore the species is recognized as Endangered status by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, Version 2012.2, and is listed on Appendix I of the Convention on International Trade in Endangered Species (CITES). It is also in the category I of the Chinese Wildlife Protection Act, 1989. Genetic information of the species is being collected by conservation biologists to discover its population history and evolution, so that the relevant conservation management can be directed (Chang et al. 2011, 2012; Li et al. 2007; Luo et al. 2011, 2012). Single nucleotide polymorphisms (SNPs) are new generation genetic markers frequently used nowadays. SNPs variants are abundant and widespread throughout the eukaryotic genomes (Cho et al. 1999), in both defined genic regions and anonymous regions. Their evolution manners can be well described by simple mutation models (Morin et al. 2004). However, SNPs are not commonly used in non-model species those lack accurate genome information, owing to the unavailability of genomic sequences. Yan et al. (2011) had found 23 SNPs by restriction enzyme digesting library and pyrosequencing, though, such SNPs were developed from only 4 individuals, thus were not informative enough in population genetic analysis. Aitken et al. (2004) developed an approach to discover SNPs based on CATS (Comparative anchor tagged sequences) (Lyons et al. 1997). These loci were designed on evolutionarily conserved genic exonic regions,

to span less conserved introns, according to alignments of several mammalian genomic segments. Thus the orthologs can be obtained in many mammalian species (Lyons et al. 1997). In this study, we try to screen SNPs from CATS sequences in *R. roxellanae*. We expect the markers to reveal more about the population structure, genetic dynamics and functional adaptation of this threatened species.

A total of 35 tissue samples (muscles, skins, bloods included) respectively from the three populations (SG, QL and SNJ) were used in the SNPs screening. Genomic DNA was isolated following the phenol/chloroform approach (Sambrook et al. 2001). We amplified 202 CATS loci using primers listed by Aitken et al. (2004). The PCR mixtures contained 100 pmol Tris (pH 8.3), 500 pmol KCl, about 100 ng template DNA, 0.5–2.0 pmol $MgCl_2$, 10 pmol primers, 2 U ExTaq DNA polymerase (TaKaRa) in 30 μL volumes, with some of the components adjusted accordingly. Touchdown PCR programs as described by Aitken et al. (2004) were performed. 88 of the loci yielded unique and clear products. The rest 114 pairs of primers were aligned to the orthologous genic sequences of *Macaca mulatta* genome and modified to accordant with the macaque orthologs, then amplified again. 30 more CATS loci were obtained.

The aforementioned 118 loci were amplified in 24 individuals covering almost all sample locations. Products of each locus were analyzed by DHPLC (Denaturing High Performance Liquid Chromatography) on a WAVE System 4500 (Transgenomic, Inc.) which reveals single nucleotide mutations by varying times needed for the stands to elute from separating column. Thus the 24 individuals were classified into diverse patterns according to the eluting peaks. One or two individuals of each pattern were re-amplified and sequenced on ABI-PRISM3730 DNA sequencer (ABI, Carlsbad, USA) at Sangon Biotech Co. Ltd. Then we used SeqMan (DNASTAR Inc., Madison, Wis.) to align the sequences and checked single nucleotide mutations by eye. The sites that showed obvious variant alleles among individuals were recognized as definite SNPs.

We discovered aggregately 56 SNPs in 40 CATS sequences (Table 1), representing a density of 1SNP/1,098.30 bp in *R. roxellana* genome. All the SNPs were bi-allele polymorphisms. Three types of nucleotide substitutions were detected, with the transition/transversion ratio of 3.19. 50 of the SNPs were afterwards genotyped among 85 *R. roxellanae* DNA samples. An average genotyping success rate of 96.59 % was reached, demonstrating the availability of the originally developed SNP loci.

In conclusion, the CATS deriving approach can be an effective way to develop new SNPs in *R. roxellana* genome. These genic intron located SNP markers are willing to help us reveal the structure and history of the golden

Table 1 List of SNPs developed using CATS approach

Loci ID	GenBank accession no.	Fragment size (bp)	No. of SNPs	SNP types
ACTC	KG699581	963	1	A/G
ADRBK1	KG699582	640	1	A/G
ALDH2	KG699583–KG699588	2,241	2	C/T; C/T
C5	KG699589	549	1	C/T
CHRNA1	KG699590	406	1	A/G
CLU	KG699591	803	3	C/T; G/T; C/T
COL10A1	KG699631	367	1	A/G
CSF2	KG699592	830	1	C/T
CYP2D@	KG699593	913	1	C/T
DRD2	KG699594–KG699595	1,160	2	C/T; A/G
EDN1	KG699596	225	2	C/T; C/T
ETS	KG699597–KG699598	1,040	1	A/G
F10	KG699599	360	1	A/C
FGFR4	KG699600	380	2	A/G; C/T
FGG	KG699601	923	1	C/T
FN1	KG699602	784	4	A/G; C/T; A/G; C/T
G6PD	KG699603	851	1	C/T
GBA	KG699604	349	1	C/T
GH1	KG699605	646	1	C/G
GSN	KG699606	190	1	C/T
IFNB1	KG699607	464	1	C/T
IL6	KG699609	804	1	C/T
KIT	KG699610	694	1	A/G
LAMC1	KG699611–KG699614	2,112	1	A/G
MYH6	KG699616	651	1	C/T
ODC1	KG699617	441	1	G/T
P4HB	KG699618	557	1	C/T
PLG	KG699619	824	2	C/G; A/C
RB1	KG699620	666	1	A/G
REN	KG699621	783	1	A/G
SCN4A	KG699622	981	1	A/G
SFTP2	KG699623	753	3	A/G; A/C; C/T
SPTBN1	KG699624–KG699625	836	1	A/G
SST	KG699626	845	1	C/T
TF	KG699627	837	1	A/G
TNFA	KG699628	423	3	A/G; A/G; A/G
TTR	KG699629	705	1	A/G
VWF	KG699630	634	3	A/C; A/G; A/G
IL1A	KG699608	940	1	A/G
MPO	KG699615	803	1	C/T

snub-nosed monkey populations. Additionally, some of the loci may be function associated, which may reflect the local adaptations of the monkey. Thus, the CATS derived SNPs we have developed are potential to make a contribution to our genetic knowledge about this endangered species, which will guide the strategy of conservation management.

Acknowledgments Our sincere thanks to S. Xu, W. Xie, T. Shen and X. Zhou for technical advice and lab assistance. This project was supported by the Project of Public Benefit (No. 201104073), National Key Technology R&D Program of China (2013BAD03B02), and the Natural Science Foundation of China (No. 31270420).

References

Aitken N, Smith S, Schwarz C, Morin PA (2004) Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach. Mol Ecol 13:1423–1431

Chang Z, Liu Z, Yang J et al (2011) Noninvasive genetic assessment of the population trend and sex ratio of the Shennongjia population of Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*). Chin Sci Bull 57:1135–1141. doi:10.1007/s11434-011-4872-6

Chang ZF, Luo MF, Liu ZJ et al (2012) Human influence on the population decline and loss of genetic diversity in a small and isolated population of Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*). Genetica 140:105–114. doi:10.1007/s10709-012-9662-9

Cho RJ, Mindrinos M, Richards DR et al (1999) Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. Nat Genet 23:203–207. doi:10.1038/13833

IUCN (2012) IUCN red List of threatened species. Version 2012.2. Retrieved from: www.iucnredlist.org. Accessed 24 Nov 2012

Li M, Liu Z, Gou J et al (2007) Phylogeography and population structure of the golden monkeys (*Rhinopithecus roxellana*): inferred from mitochondrial DNA sequences. Am J Primatol 69:1195–1209

Luo M, Liu Z, Pan H et al (2011) Historical geographic dispersal of the golden snub-nosed monkey (*Rhinopithecus roxellana*) and the influence of climatic oscillations. Am J Primatol 101:91–101. doi:10.1002/ajp.21006

Luo M-F, Pan H-J, Liu Z-J, Li M (2012) Balancing selection and genetic drift at major histocompatibility complex class II genes in isolated populations of golden snub-nosed monkey (*Rhinopithecus roxellana*). BMC Evol Biol 12:207. doi:10.1186/1471-2148-12-207

Lyons LA, Laughlin TF, Copeland NG et al (1997) Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nat Genet 15:47–56. doi:10.1038/ng0197-47

Morin PA, Luikart G, Wayne RK (2004) SNPs in ecology, evolution and conservation. Trends Ecol Evol 19:208–216. doi:10.1016/j.tree.2004.01.009

Sambrook J, Fritsch EF, Maniatis T (2001) Molecular cloning. J Biol Chem 2:13757–13765. doi:10.1074/jbc.270.23.13757

Yan C, Shattuck MR, Thimmapuram J et al (2011) Developing SNPs and STR DNA markers for snub-nosed monkeys (*Rhinopithecus roxellana*) using next-generation sequencing technology. Conserv Genet Resour 4:451–453. doi:10.1007/s12686-011-9572-7