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Case report

Establishing the identity of the massacred tigress in a case of wildlife crime

Sandeep Kumar Gupta *, Jyotsna Bhagavatula, Kumarasamy Thangaraj, Lalji Singh **

Centre for Cellular and Molecular Biology, Hyderabad, India

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ABSTRACT

We report a case study, where we have established the identity from a challenging biological sample of a deceased tigress by parentage analysis. A wildlife crime was committed in one of the zoological parks in India in the year 2000, where one young tigress was killed for its claws. This was of media interest for several days and remained an unsolved case for four years. A framed claw and decomposed tiger hide were seized from the accused in 2005. Biological samples of the victim tigress was not available for further forensics examination, therefore; DNA samples of the biological parents and a male sibling were used to establish the identity of the claw using STRs and mitochondrial DNA markers. Our analysis indicates that the seized claw belongs to the victim tigress.

1. Introduction

Despite a very strict wildlife protection act, the poaching rate of tigers (*Panthera tigris*) and leopards (*Panthera pardus*) is still increasing in several countries for body parts for Chinese medicinal products and ornaments. Developments in molecular biology have strengthened wildlife forensic science by virtue of the availability of molecular markers such as mitochondrial DNA (mtDNA) [1–3] and microsatellites [4]. The application of mitochondrial cytochrome-b gene-based species identification has helped tremendously in identification of forensic samples in wildlife offences [5]. Microsatellites are the best available marker for parentage testing and linkage analysis [6]. Control region sequences of mtDNA provide improved phylogenetic resolution grouping in the big cats i.e. *Panthera* spp. [7]. There are 10 different haplotypes of tigers in India based on control region and coding regions of mtDNA, which can be used to find maternal lineage [8]. In the present study, we were successful in providing evidence that the source of the claw is of the missing progeny of the same tiger family of the zoological park.

2. Case report

2.1. Case history

In 2000, a carcass of a female tiger was found outside of its cage in the "Tiger Safari" of a Zoo Park in India. The death of the tigress was a highlight in media for several days and a wildlife crime was reported, which was pending for the last four years.

Unfortunately, no biological sample was made available for us from the deceased tigress for the DNA profiling. After four years, a team of Police Officials of the same city had seized a claw, encased in silver frame, and pieces of decomposed hide from a local person. These samples were sent to our laboratory to establish whether they belong to the illegally taken tigress.

2.2. DNA analysis to establish the identity

2.2.1. DNA isolation from claw and skin

Samples taken from: a thin stratum of the ventral portion of the claw and pieces of the decomposed skin were separately washed twice with normal saline. The washed material was subjected to DNA extraction [5]. The DNA extracted from the putrefied hide was degraded, hence not found suitable for genotyping. The DNA extracted from the claw was used for genotyping and further analysis. The DNA sample of the biological parents and a male sibling of the victim tigress were obtained from our DNA bank.

* Corresponding author. Present address: Wildlife Institute of India, Dehra Dun 248 001, India. Tel.: +91 135 2640111/15x353; fax: +91 135 2640117.
** Corresponding author. Tel.: +91 40 27192634; fax: +91 40 27160252/27160591.
E-mail addresses: skg@wii.gov.in (S.K. Gupta), lalji@ccmb.res.in (L. Singh).

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Table 1
Allelic detail of the all microsatellite locus used in this study. The three digit number given in the table are the size of allele in base pair.

Marker	Claw	Biological mother	Biological father	Sibling brother	Allelic frequency
C34	188	188	188	188	0.13
C6	146		146	146	0.12
	152	152		152	0.12
D10	134	134			0.11
	140		140	140	0.22
		146		146	0.12
D15	120	120	120		0.30
			128	128	0.04
		138		138	0.13
E21	156		156	156	0.05
	158	158	158	158	0.31
		160			0.21
E7	132	132	132	132	0.02
		144			0.38
	146		146	146	0.27
E22	169	169	169	169	0.42

2.2.2. DNA typing and Sequencing

A total of seven microsatellite loci, along with hypervariable region of mtDNA [7], were used in this study for establishing the identity of the claw. All the seven loci (E7, C34, D10, E21, E22, C6 and D15) used in this study are tiger-specific and four (E7, C34, D10 and E21) out of seven loci are already discussed in our proceeding article [4]. The forward primers of all the above markers were labeled with fluorescent dye to detect the amplicons in the automated DNA Analyzer (ABI 3730). The amplification using each primer pair was carried out in 20 μl reaction volume containing 1 μl (~5 to 10 ng) of the DNA, 100 μM each of dNTPs, 4 pmol of each primer, 1.5 mM $MgCl_2$, 0.5 unit of AmpliTaq Gold (Perkin-Elmer-Cetus, USA) and 1× PCR buffer (10 mM Tris–HCl, pH 8.3, and 50 mM KCl). The PCR conditions used were as follows: initial denaturation at 95 °C for 10 min, followed by 35 cycles each of denaturation at 95 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. The final extension was at 72 °C for 10 min. After successful amplification, PCR products were analysed using automated DNA Analyzer (ABI 3730, Applied Biosystems, USA). The raw data were analysed using GeneMapper (Applied Biosystems, USA) software for obtaining the alleles of each locus. The hypervariable region of mtDNA was amplified and sequenced using the primer described by Jae-Heup et al. [7] by automated DNA Analyzer (ABI 3730, Applied Biosystems, USA).

2.2.3. Statistical analysis

The effect of family relatedness on match probability provides a reliable method that makes full use of available genetic data [9]. Genotype data obtained from claw, alleged parents and sibling was use to calculate the relationship by using SoftGenetics GeneMarker 1.9. The allele frequency of 34 unrelated individual including 21 captive and 13 wild tigers (Table 1) was used to calculate the family relatedness.

2.2.4. Implication of our report

According to the Wildlife Protection Act 1972, Government of India, the tiger is a Schedule-I animal. On the basis of our report the forwarding authority has filed a case in court of law against the person involved, from whom the claw and skin were seized.

3. Results and conclusion

In the present study, seven tiger-specific microsatellites were used to identify whether the seized claw is that of the tigress killed at the same zoological park. Interestingly, the alleles of the loci C6 (146 and 152) are specific to this family and not observed in unrelated tigers from different zoological parks in India, therefore; the minimum allele frequency is calculated by the formula 5/2N [10]. The sharing allele that is characteristic of a patent/offspring relationship was observed between the suspected mother, father and the tested claw (Table 1). Likelihood ratio (LR) score for the relationship of claw with alleged family members i.e. mother, father and sibling were 4.42E + 02, 5.15E + 03 and 1.07E + 06, respectively. These LR ratios are high and signifying the precision of the alleged relationship, therefore; the claw belongs to an individual with the direct relation to this alleged family. Furthermore, we have confirmed the maternal origin of the claw by the mtDNA control region sequence comparison of claw and alleged mother. This observation also strongly suggests that the claw belongs to a tiger with a shared maternal relative. Since no other individual of this alleged family is missing, we strongly believe that the claw is of the victim tigress.

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