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Curtailing Human-Leopard Conflict Using Wildlife Forensics: A Case Study from Himachal Pradesh, India

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Abstract

Recent changes in the land use pattern have severely impacted wildlife, specifically large carnivores like leopards, by reducing natural habitat and prey base. Being highly adaptable, with a distribution more outside than inside the protected areas, leopard very often attacks human and livestock. In human-leopard conflicts, once an animal is declared as man-eater, it is either translocated or killed by officials as per Wildlife (Protection) Act 1972 of India. Identification of conflicting leopard is very difficult and sometimes lead to the castigation of innocent animal. Here, we describe the individualization of a leopard from one such human-leopard conflict in Bilaspur district of Himachal Pradesh, India using modern molecular genetic techniques. The methodology suggested in this study would be of great importance in correct identification of conflicting animals.

Keywords: Leopard; Conflict; Individual identification; Microsatellites; Wildlife forensics

Introduction

A plethora of developmental activities (anthropogenic pressure) in and around forested areas are imposing serious threats to the survival of wild animals, specifically the big cats [1]. The natural habitats of big cats are becoming fragmented, and their natural preys are being out-competed by the livestock [2]. Consequently, big cats start predating on livestock near human settlements [3] and sometimes on humans [4] and thus the situation of human-carnivore conflicts arises. The existence of human settlements in the proximity of wildlife habitat makes human-carnivore conflict incidents more frequent in India [5].

Leopard (*Panthera pardus*) is one of the widely distributed felid species in India, having more distribution outside than inside the protected areas [6]. Leopard often live on forest fringes as well as marginal habitats and account for the major proportion of human casualties incurred due to the big cats conflict in India [7,8]. The human casualties resulted due to leopard attack are high in the states of Uttarakhand, Maharashtra, Himachal Pradesh, Gujarat and Madhya Pradesh [9]. According to Joshi and Aggarwal [10], 180 people were killed, and 343 were injured by leopards in Uttarakhand from 2000 to 2008. Similarly, 186 leopard attacks were reported in Himachal Pradesh in 2008-2009 [11].

Among the mitigatory approaches used to minimize human-leopard conflicts, translocation, and elimination (by killing, once declared as man-eater as per Wildlife Protection Act 1972 of India) are most common in practice by authorities in India [4,6]. The elusive and nocturnal behavior of leopard produces a significant challenge in establishing the identity of conflicting individual reliably. In the absence of established protocol to delineate conflicting individual from non-conflicting, sometimes innocent individuals were penalized. The recent advancements in the field of wildlife forensics permitted species, sex and individual identification from a variety of biological samples [4,12,13]. In the present study, we demonstrate to establish genetic ID of the conflicting individual (from scats collected) and compared with the hunted leopard using tools of wildlife forensic genetics.

Materials and Method

Case history

On January 02, 2012 leopard attacked and killed a child in Bharari range of Bilaspur Forest Division, Himachal Pradesh (Figure 1). The partially consumed (face and neck eaten) victim's body was recovered on January 09, 2012 from the small grotto in a ravine. The authorities also collected two leopard scats at a distance of about 400 meters from the victim's body. In a bid to avoid such incidents, a search operation was conducted, and alleged man-eater leopard was shot dead by the authorities on January 15, 2012 at a distance of about 4 KM to 5 KM away from the incident site. The authorities sent scats (H1, H2) along with tissue sample collected from the killed leopard (H3) to the Wildlife Institute of India, Dehradun to confirm the individual identity of all the samples (i.e., whether they belong to the same individual or not).

Isolation of genomic DNA, identification of species and sex

The total genomic DNA was extracted from the scat samples using QIAamp DNA Stool Mini Kit (Qiagen, Germany) and from the tissue sample using DNeasy Blood and Tissue kit (Qiagen, Germany) as per manufacturer's protocol. During DNA extraction, necessary precautions were taken to avoid contamination as recommended while working with non-invasive samples. The species-level identity and molecular sexing of each of the analyzed sample was confirmed using PCR-based assay suggested by Sugimoto et al. [14]. Species and gender determination PCR assay were performed in a total reaction volume of 10 µl following Sugimoto et al. [14]. The duplicate (for species

identification) and triplicate (molecular sexing) independent PCR attempts were undertaken for data accuracy and precision.

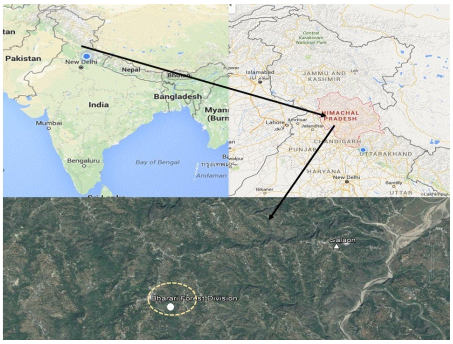

Figure 1: Map of Bharari forest division showing location from where child body (white dot) was recovered and place where leopard was hunted (white triangle). Circle with dotted lines shows probable location scat collection site.

Microsatellite genotyping and individual identification

Microsatellite based individual identity was established for all three samples using twelve polymorphic microsatellite loci (PttA2, PttA4, PttC6, PttD5, PttE5, PttF4, Pun82, Pun100, Pun327, Pun225, Pun124 and F41) [15,16]. The selection criterion for microsatellite markers was polymorphism and amplification success rate with scats. The PCR amplifications (in triplicate) were carried following Mishra et al. [15,16]. The amplified microsatellite PCR products were subjected to fragment analysis on an ABI 3130 Genetic Analyzer (Applied Bio systems, USA) using POP7 polymer. Since allelic ladders were not available to fill the guideline of International Society for Forensic Genetics, we followed the approach as published by Ogden [17]. We used standard PCR product to detect a likely shift in allele size. Alleles were manually scored using Gene Mapper software version 3.7 (Applied Bio systems, USA). A consensus genotype was created from these repeat results, and all allele sizes were rounded to odd or even number for each locus which were <0.5 bp differences.

Results and Discussion

All three samples (H1, H2 and H3) tested with the species identification multiplex PCR [14] showed the presence of leopard specific bands of 156 base pairs (Figure 2). Similarly molecular sexing also suggest male origin (two bands of 205 base pairs and 156 base pairs corresponding to Zfx and DBY genes) of tested samples (Figure 3). Consensus genotypes were obtained for all the three samples using multiple tube approach (Table 1). The twenty different alleles were detected with twelve highly polymorphic microsatellite loci. The genetic ID of all three samples was found identical. Based on the above findings, we conclude that the tested samples (both scats and tissues) belong to same male leopard.

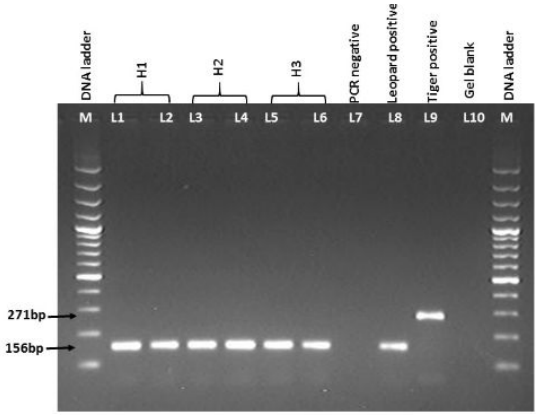

Figure 2: Multiplex PCR assay based species identification.

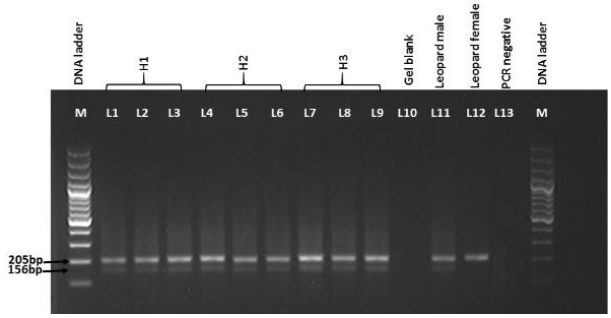

Figure 3: Gender identification. 156 base pair (bp) band corresponds to male specific DBY7 fragment and 205 bp band corresponds to female specific Zfx fragment.

Sample ID	Microsatellite loci												
	Type	Ptt.A2*	Ptt.A4*	Ptt.C6*	Ptt.D5*	Ptt.E5*	Ptt.F4*	Pun82#	Pun100#	Pun327#	Pun225#	Pun124#	F41!
H 1-3	Scat	182/182	161/161	176/182	236/248	–	182/184	117/119	96/96	88/88	189/191	100/104	178/186
H 2-3	Scat	182/182	161/161	176/182	236/248	196/198	182/184	117/119	96/96	88/88	189/191	–	178/186
H 3-3	Tissue	182/182	161/161	176/182	236/248	196/198	182/184	117/119	96/96	88/88	189/191	100/104	178/186
"-" indicates loci not amplified, *Sharma et al. [20], #Janecka et al. [21], !Menotti-Raymond et al. [22]													

Table 1: Consensus genotyping table of the microsatellite loci amplified in the analysed samples (scats and tissue) sent by authorities of Himachal Pradesh, India.

The human-leopard conflict has become a severe problem in the districts of Bilaspur, Hamirpur, Mandi and Kangra and parts of Kullu, Shimla, Sirmaur and Solan [18]. The human-carnivore conflict and subsequent casualties (both humans and livestock) results in fear and negative perception of local communities. The Ministry of Environment, Forest and Climate Change guideline for human leopard conflict management [19] emphasize on establishing correct identity of conflicting (man-eater) individual before making any mitigation strategy (trapping or in adverse case shooting the conflicting leopard as per Wildlife Protection Act 1972 of India). The present study is a pioneer attempt to establish a complete protocol for the genetic identification of leopard in the conflict-prone state of Himachal Pradesh. In the present study, we describe the use of wildlife forensic genetics in establishing species, sex, and individual identity of a leopard which may be involved in the human-leopard conflict in the Himachal Pradesh and more specifically in the areas of Bilaspur and Mandi. Hence, this study may provide significant information for the management of human-leopard conflicts in India. The correct identification of conflicting individual not only reduces the fear of local people but also provide documented proof to management authorities for taking the right decision.

Our results reveal that the three samples were belonging to one individual leopard. However, inquest to establish (with greater accuracy) the killed leopard was man-eater, extensive and intensive collection of non-invasive samples is needed which has so far not in practice in India while examination of such sites by the officials. Because of this, no such samples from victim body were collected. Therefore, we emphasize on the collection of saliva from the places where animal might have eaten victim body or other samples such as urine and fallen hairs to establish the link the actual animal involved in the attack on human with the killed one. We also suggest the use of wildlife forensic genetics in such cases of carnivore attacks across India for correct identification of conflicting individual by matching non-invasive samples collected in and around conflict area with the biological samples of captured or eliminated individual. We also suggest a need for establishing genetic IDs of leopards in Himachal Pradesh. Such comprehensive data would be of great utility for authorities in formulating better management strategies to deal with human-wildlife conflicts.

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