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ORIGINAL ARTICLE

Understanding Human–Tiger Conflict around Corbett Tiger Reserve India: A Case Study Using Forensic Genetics

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Abstract

Human–tiger conflicts have been a major issue in the Terai-Arc Landscape (TAL), in northern India, ever since human settled in the region. For managing such issues, authorities are compelled to eliminate/relocate tigers whenever there is recurrent killing of human beings. But identifying the individual tiger/tigress, involved in conflicts has remained a challenge for wildlife manager as misidentification may lead to the elimination of the wrong individual that do not mitigate the conflict at all. In the present study, we demonstrate the utility of molecular tools by using whatever pre-operational evidences could be collected from a tiger that was eventually killed as a man-eater through a case study from the TAL. In this instance, a tiger attacked and killed four human in different incidents over a period of 3 months (November–December 2010 and January 2011) in and around Corbett Tiger Reserve (CTR), India. The tiger was finally eliminated because it was declared as man eater. Intensive collection of biological samples during conflict period was not done, hence, only a few samples were collected. These were collected from attack sites where the tiger was wounded (n=2) and the site where it was killed (n=2). For each of the sample, we could determine the species, sex and individual identity of the animal involved using molecular markers, i.e. mitochondrial, sex and microsatellite. We found that all the samples came from a male tiger and that it was the same individual that had been involved in the attacks, been wounded and shot dead. We suggest that biological samples, such as hair that had been shed and scats be collected intensively from resting places and blood stains across conflict zones. We also suggest that the genetic identity of the tigers in Uttarakhand be established and so as this information may be used to establish a link with individuals involved in conflicts so that effective strategies may be developed to manage human–wildlife conflicts in TAL corridors, where such conflicts are relatively more frequent.

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Introduction

Human developmental activities and anthropogenic pressure in and around natural habitats are posing serious threats to the survival of wild animals, particularly the large cats. These habitats are becoming degraded and fragmented, and the natural prey species are being outcompeted by the livestock in these areas (1). As human–carnivore interface broadens, the large cats start predating on livestock near human settlements and occasionally on humans, which results in conflicts. Attacks by tigers on humans are often reported from India, especially the Terai (foothills of Himalaya), West Bengal and central India (2). Many such cases have been reported in the past also, and Jim Corbett describes in his book Maneaters of Kumaon the Champawat tigress, which killed 200 people in Nepal and 236 people in India (3). Tigers killed 57 people on average each year during 1975–1984 in the Sunderbans, in West Bengal and Bangladesh (4, 5), and 88 people between 1979 and 2006 in Chitwan National Park (6). Fatal attacks by other cats, viz. leopards and lions, have also been reported (7, 8). Because of such conflicts and retaliatory killings of animals by humans, the populations of many cat species such as the lion (*Panthera leo persica*), African cheetah (*Acinonyx jubatus*), tiger (*Panthera tigris*) and leopard (*Panthera pardus*), have declined substantially over the years (9).

When dealing with conflicts with wildlife, especially elusive and nocturnal carnivores, it has been a great challenge to ascertain the identity of the individuals involved. There is very little information available in the literature on understanding the causes of these conflicts and linking evidence related to individuals involved in conflicts for developing appropriate management strategies a few detailed studies (10). This is due to the lack of appropriate tools for addressing such issues, and many times this has also resulted in a number of non-conflict individuals being killed. The use of modern techniques such as molecular technology may provide better insights in understanding human–wildlife conflicts through the use of biological samples (11).

The development of wildlife forensic genetic methods in recent years permits species identification from varied biological samples (12, 13), sex identification (14), paternity assessment (15) and establishment of the origin of a particular individual (16) from hair, scats, saliva, bloodstains, urine, etc. The potential use of wildlife forensic genetics in dealing with problems involving particular individuals in conflicts has been illustrated by the establishment of the genetic identity of a serial killing wolf (17), a bear involved in a fatal human–wildlife conflict (18), a Sardinian mouflon (19) and a crop-raiding elephant (20).

In this paper, we document for the first time the feasibility of using forensic genetics in India to establish species, sex and individual identity, tracing and linking evidence related to a tiger that was involved in repeated attacks on and killings of humans around Corbett Tiger Reserve, Uttarakhand, India. We used the limited number of samples collected by the authorities between November 2010 and January 2011. This study also provides an example of the feasibility of using molecular tools to develop effective management strategies when dealing with human–wildlife conflicts in India.

Fig. 1: Locations of tiger attacks (white triangles) on humans in Corbett Tiger Reserve, Uttarakhand, India. The numbers in the paraenthesis indicate that samples collected i.e. 1 (WII ID 3002), 2 (WII ID 3005), 3 (WII ID2992) and 4 (WII ID 183) (Maps modified from http://www.mapsofindia.com and https://earth.google.com).

Case history

A woman was killed and partially eaten by a tiger in compartment 9 of Dhangarhi buffer beat, Eastern Dhulwa, Sarpduli range in Corbett Tiger Reserve (CTR), India when she was returning to her home in Sundarkhal village on 12 November 2010. When the authorities searched the area around the site where her body was discovered, they found pugmarks 400 m away. These measured 11.5 cm × 10.5 cm in size, and the stride was 122 cm. Another woman met the same fate in compartment 12 of Garjiya beat, Eastern Dhulwa on 29 December 2010. When the surrounding area was searched, pugmarks with the same dimensions and stride as those of the previous tiger attack were found, but these were 4.0 km from the site of the incident. The authorities concluded on the basis of the pugmarks and other associated evidence that the two attacks were made by a tiger and that it was probably a female. Another fatal tiger attack, in compartment 13 of Garjiya beat, on a woman was reported on 10 January 2011 (Fig. 1). In this case, the dimensions of the pugmarks were 11.5 cm × 10.5 cm, and the stride was 122 cm. The pugmarks were found 200 m from the site. Thereafter, the authorities declared the tiger as a man-eater and decided to eliminate it. A search operation was conducted. An attempt was made to shoot the tiger on 11 January 2011. It was injured, but unfortunately, it escaped. When forest patches in Garjiya beat were searched and examined thoroughly by the authorities, a blood smear was found by them on a leaf on 17 January 2011. The authorities continued their search operation to locate the injured tiger, and on 19 January 2011 they found another blood smear on a stone, where the tiger might have rested. Both these samples (WII ID 3002 and WII ID 3005) were sent to the Wildlife Institute of India (WII), Dehradun for DNA-based examination. On 26 January 2011, one man was killed in the Kunkhet beat of Kosi range, Ramnagar Forest Division, Uttarakhand (Fig. 1). After this incident the forest

authorities searched this region and on 27 January 2011 eliminated a suspected tiger. This tiger had an old, partially healed wound on its body. The authorities found that they had killed a male tiger, whereas a female had been suspected. Two biological samples, i.e. a tissue sample (WII ID 2992) and blood-soaked cotton (WII ID 183), were also sent to WII to confirm the sex and individual identity of the tiger that had been killed by comparison with the earlier samples (WII ID 3002 and 3005).

Methods

Collection of biological samples

A limited number of biological samples were collected during the search operation conducted for the alleged man-eater. These were a bloodstain on a leaf (WII ID 3002), a bloodstain on a stone (WII ID 3005), a tissue sample (WII ID 2992) and a blood sample (WII ID 183), the last two being from the tiger that was shot.

Species identification

DNA was extracted from the blood (WII ID 183, 3002 and 3005) and tissue (WII ID 2992) samples using a DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. The DNA extracted from these four samples was used to identify the species using a multiplex panel (ND490 + ND509) of tiger specific markers developed by Mukherjee et al (21). We also used other tiger-specific primers developed by Sugimoto et al (22).

Sex identification

The Zfx–Zfy molecular marker (Goyal et al unpublished) was used for sex determination. This marker differentiates male and female tigers on the basis of two bands of DNA of size 197 bp and 132 bp on the X and Y chromosomes, respectively. Two bands of the X and Y chromosomes appear if the tiger is male, whereas one band of 197 bp is present if it is female. The use of this primer was tested for its accuracy using tiger tissue samples of known sex (n=40), and no sex was misidentified. Apart from this, we also used another primer (Zfx and DBY7) suggested by Sugimoto et al (22) to reconfirm the gender of the tiger involved in the conflict. DBY7–Zfx gives two bands of the X and Y chromosomes of size 271 bp and 156 bp, respectively. Two bands of the X and Y chromosomes appear in a male, whereas one band of the X chromosome of size 271 bp is present in a female.

Individual identification

For multi-locus genotyping-based individual identification, we used 11 highly polymorphic microsatellite loci, of which seven (PttA2, PttA4, PttC6, Ptt10H, PttF4, PttE5 and PttD5) were tiger specific (23) and four, which were originally designed for the domestic cat (Fca304, Fca272, F41 and F85) (24). All PCR amplifications were carried out in an ABI 9600 Fast Thermal Cycler (Applied Biosystems, USA). Each PCR reaction volume of 10 µl contained 50 ng of the DNA, 5 µl of the 1× multiplex PCR Master Mix buffer (QIAGEN Multiplex PCR Kit, Germany), 1× BSA and 0.2

μM of each primer. The thermal profile for amplification of the microsatellite loci was initial denaturation at 94°C for 15 minutes, followed by 40 cycles of denaturation at 94°C for 35 seconds, annealing at 53°C for 1 minute and extension at 72°C for 90 seconds, with a final extension for 30 minutes at 72°C. One positive (DNA sample of known tiger) and one blank sample were included in each PCR amplification reaction. The amplified PCR products were subjected to fragment analysis in an ABI 3130 Genetic Analyzer (Applied Biosystems, USA) using POP-7 polymer. The use of allelic ladders is recommended by the International Society for Forensic Genetics. But since these are not available for wildlife species, we followed the guidelines suggested by Ogden (25) and use standard PCR products to detect a likely shift in allele size. A similar approach has been used in a study on the brown bear (26). In order to assess and ensure the quality of genotyping, we (i) included control PCR products while running samples for genotyping to determine any allelic shift due to electrophoresis, (ii) injected each PCR product four times for capillary electrophoresis and (iii) typed all samples three times through independent PCR runs because a multi-tube approach is commonly recommended to minimize errors due to allele dropouts and false alleles (27). Alleles were manually scored using GeneMapper, version 3.7 (Applied Biosystems, USA) for assignment. A consensus genotype was created from these repeat results, and all allele sizes were rounded to an odd or even number (±0.5 bp differences) for each *locus*. The quality of the allele was ascertained on the basis of the peak height and was selected so as to be of RFU height between 200 and 6000 for all the *loci*.

Results and Discussion

All four samples (WII ID nos. 3002, 3005, 2992 and 183) tested with the multiplex primers of Mukherjee et al (21) clearly showed the presence of tiger-specific bands of sizes 225 bp and 164 bp (Fig. 2A). Likewise, the tiger-specific marker of Sugimoto et al (22) also revealed the presence of a tiger-specific band of size 271 bp (Fig. 2B). Therefore, we concluded that all the four samples provided by the authorities and examined by us were from tigers. The presence of two bands of the X and Y chromosomes of size 197 bp and 132 bp, respectively, indicated that all four samples were from male tigers (Fig. 2C). The presence of the two bands of Zfx and the DBY7 chromosome at 205 bp and 156 bp also clearly indicates that all four samples were from male tigers (Fig. 2D). The gender of free ranging tigers in India is commonly identified on the basis of pugmark characteristics. If a pugmark is rectangular in shape, the tiger may be female, and if it is square, it may be of a male (28). Recent sex discrimination studies based on the length and width of pugmarks have clearly revealed that there is a probability of misidentification of sex when these measurements are used (29).This was probably the reason why the sex of the tiger was misidentified by the authorities. The inconsistency observed in identification of sex using pugmarks is due to variations in soil texture and other associated factors. Therefore, it is recommended that molecular techniques be considered for sex identification and samples be collected using invasive and non-invasive techniques for better accuracy (29).

All the four samples were successfully amplified with 11 microsatellite *loci* using a multi-tube approach. The observed profiles of all the *loci* indicate a high-quality

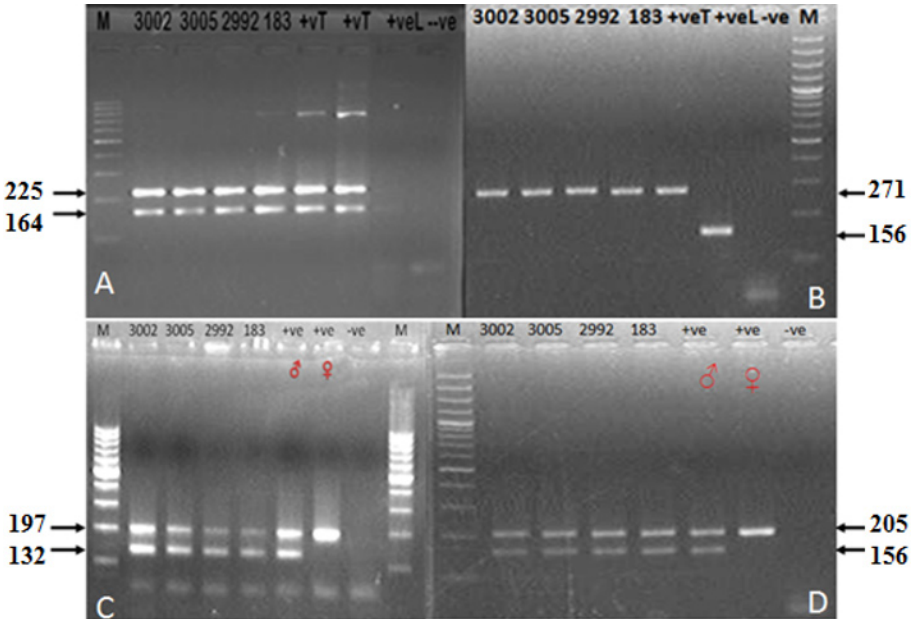

Fig. 2: Identification of species (A and B) and sex (C and D). M = 100 bp ladder, +ve = positive, -ve = negative.

genotype (RFU >200, <6000) (Fig. 3). The genetic identity was determined on the basis of the allele sizes of the 11 highly polymorphic microsatellite *loci* (Table 1). Eighteen different alleles were detected with 11 highly polymorphic microsatellite loci, and all 18 alleles (100%) were found in each sample. The probability of identity (PID), which is the probability of two individuals drawn at random from a population sharing the same genotype, is affected by the population size and the level of heterozygosity. The PID values amongst siblings, exclusively for the tiger population of CTR, was determined using 9 *loci* was calculated as suggested by Waits et al (30). Our analysis of the allele frequencies of the 9 *loci* indicates that seven or eight loci are adequate for individual identification at the PID (sib) (2.64×10-4) level (31). We believe that this level of PID (sib) is acceptable for discriminating tiger individuals within the population of CTR, which consists of 214 (190–239) individuals (32). Other studies on the Bengal tiger of different populations have also indicated that six or seven *loci* are adequate for individual identification at the PID (sib) (5.0×10-4) level (33, 34). Thus in the present study, we considered that the use of 11 highly polymorphic microsatellites was adequate for individual identification even at the PID (sib) level and finding out whether all the four samples were from the same individual. On the basis of these results, it was concluded that all four samples collected from the zone of conflict (wounded tiger and shot tiger) were from the same individual.

Understanding the causes and the consequences of human–wildlife conflicts are of great importance both for the conservation of a species and for maintaining a state of sustainability between humans and wild animals. The place where these series of incidents occurred is in the vicinity of CTR in Uttarakhand, which is amongst the first nine tiger reserves created in India and has an area of ca. 1318 km^2 (32). This

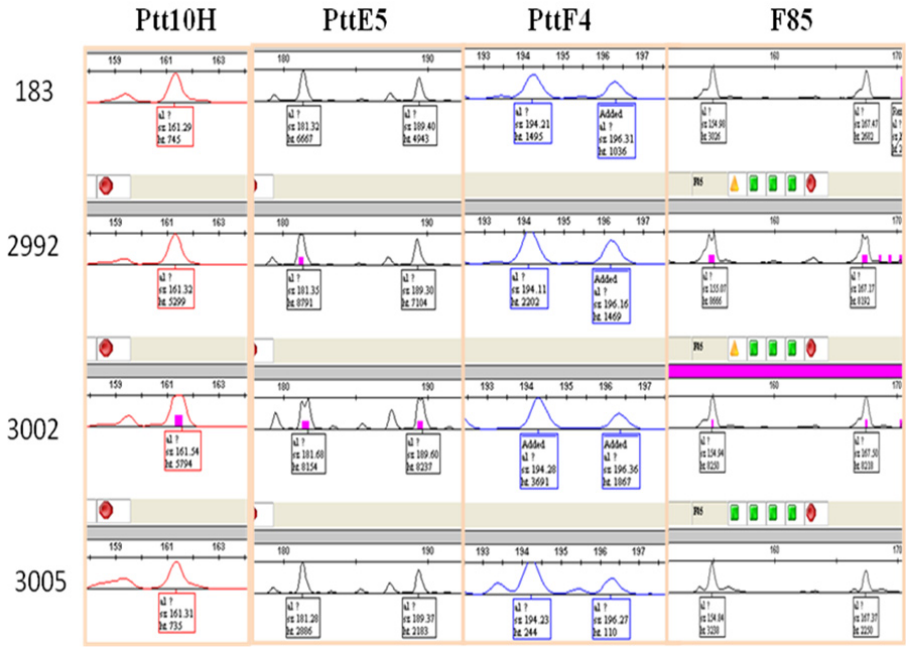

Fig. 3: Electropherogram of the four selected polymorphic microsatellite loci (Ptt10H, PttE5, PttF4 and F85) with three blood samples (183, 3002, and 3005) and a tissue sample (2992).

Table 1: Multi-locus microsatellite genotype of the four samples.

Sl. no.	WII sample ID	Sample type	Loci used										
			PttA2[1]	Ptt10H[1]	PttD5[1]	PttE5[1]	PttF4[1]	PttC6	PttA4	F41[2]	F85[2]	Fca304[2]	Fca272[2]
1	183	Blood in cotton	188/188	162/162	204/208	182/190	194/196	174/176	145/151	170/170	154/168	121/121	120/122
2	2992	Tissue	188/188	162/162	204/208	182/190	194/196	174/176	145/151	170/170	154/168	121/121	120/122
3	3002	Blood on leaf	188/188	162/162	204/208	182/190	194/196	174/176	145/151	170/170	154/168	121/121	120/122
4	3005	Blood on stone	188/188	162/162	204/208	182/190	194/196	174/176	145/151	170/170	154/168	121/121	120/122

[1][21].
[2][22].

tiger reserve is constituted by Corbett National Park, Sonanadi Wildlife Sanctuary, parts of Kalagarh Forest Division and Ramnagar Forest Division. The established tiger population of 214 (190-239) individual of CTR is a source population in the Terai-Arc Landscape (TAL) (32). Corridors play a vital role in wildlife species management, and a number of corridors have been identified around CTR (35). The most important of these are a 20 km belt between CTR and Ramnagar Forest Division. These corridors connect Corbett and the eastern tiger and elephant populations in Uttar Pradesh (Kishanpur and Dudhwa) and Nepal (Suklaphanta and Bardia national parks). There are about 92 villages present within 2–3 km of the reserve (32), and some of the villages are in corridors. The human populations of the villages in the corridors and surrounding areas are dependent on CTR for their fuel, fodder and timber although obtaining these from the forests is illegal. The associated human activities are imposing a tremendous pressure on the tigers, restricting them tightly within the region. Because of these disturbances, the rate of encounters with humans is relatively

higher than other areas.

The connectivity between forest patches is limited due to the presence of villages in the corridors and increased tourism around CTR and along the route from Ramnagar to Almora. As a result, the free movement of tigers between CTR and Ramnagar Forest Division has been restricted. Therefore, it has been suggested that Sunderkhal village, which is located in a crucial corridor in the northern part of CTR (36), be relocated so that conflict is minimized. Such relocations are essential for maintaining the functionality of the corridors for the tiger populations and for ensuring that free movement without any conflict and gene flows across the landscape are possible and for managing the tiger populations as one panmictic population.

Poaching of the natural prey of the tiger (36, 37) has also resulted in increased tiger–livestock conflicts and occasionally attacks on humans. The probability of such conflicts are relatively high when humans venture deep into the forest alone or in small groups for collection of fuel and fodder and for livestock grazing, as happened in this case in CTR. In view of the increased tiger–human conflict around tiger reserves, the National Tiger Conservation Authority (NTCA) has proposed guidelines for understanding such situations and taking appropriate management action.

In the incident in which a suspected man-eating tiger was shot on 27 January 2011, the animal was found at a human kill in CTR. The authorities successfully selected the right male tiger. This was despite their belief, based on pugmarks found at the sites of the initial attacks on the women, that the man-eater was a female. The tiger had an old, incompletely healed wound. In the present case, only these evidences (viz. the finding of the tiger at a human kill and a wound on its body) identified the wounded tiger as the man-eater. Thereafter, no incidences of conflict were reported subsequently.

In the present case of tiger–human conflict, the behaviour and the movement pattern of the wounded tiger could have been understood and explained more effectively if some more biological samples, such as saliva, hair, urine or scats, could have been collected for establishing the identity of the animal involved in the attacks. The minimum territory size of a male tiger is of 33 km^2 in Chitwan National Park, calculated on the basis of the number of resident males (38), and the total area used by the individual involved in the conflict was ca. 17 km^2. Therefore, we suggest that biological samples should be collected intensively from an area of ca. 20–40 km^2 in conflict zones.

The problem of tiger–human conflict is not confined to this region but it is present in other tiger habitats in India as well as in other countries. Thus, we suggest samples such as saliva, hair, urine or scats should be collected extensively from zones of conflict for understanding and tracing individuals involved in conflicts. Because DNA forensics has the potential to provide better understanding and suggests appropriate measures in managing such problems, as it has been used in identifying a tigress (15), a serial-killing wolf (17), a bear (18), a Sardinian mouflon (19) and a crop-raiding elephant (20). We suggest that the genetic identities (Genetic ID) of the tigers present in all tiger habitats in Uttarakhand and other parts of India be established. We believe that knowledge of the exact identities of animals involved in conflicts, established through the use of genetic data, will provide better and appropriate strategies in managing these human–wildlife conflicts and will permit funds to be disseminated correctly under different compensation schemes by the authorities.

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