

Trace DNA from kill sites identifies individual predators[☆]

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ABSTRACT

Predation ecology and evidence-based conflict management strategies require reliable and accurate identification of individual predators. Identifying predators is, however, complex, as they are secretive and individual identification is difficult. Trace DNA that predators leave behind at kill sites might provide an effective strategy to identify them but remains poorly assessed. We use non-invasive genetic samples from kill sites to assess their utility for predator identification. We systematically investigated 198 livestock kills in two of Central India's critical source tiger populations: Kanha and Bandhavgarh Tiger Reserves. We collected 342 saliva, 33 scat and 395 shed hair samples as potential sources of predator DNA. Individual tigers were identified using up to 123 SNP markers. All sample types were highly successful (>95 %) in identifying tigers. Individual tigers were identified at 85 % of all kill sites using at least one sample. Shed hair samples were most effective for individual identification, followed by saliva and scat. Sample type and sampling season were the primary determinants of the number of SNPs typed per sample and individual identification success. Based on the collection site and type of sample in identification, we classified species and individuals into three categories: true predator (high confidence as predator), circumstantial predator (medium confidence) and predator uncertain (low confidence). Individuals were classified as a true predator at 72 sites, circumstantial predator at 34 sites, and predator uncertain at 49 sites. We suggest that when paired with systematic kill site investigation and sample collection, these methods can be effectively used to understand predation ecology better and facilitate evidence-based conflict management.

1. Introduction

Variation in predation behaviour within a population can be significantly influenced by predator specific traits, such as sex, age, morphology/phenotype as well as individual behavioural specialization (Berezowska-Cnota et al., 2023; Bolnick et al., 2003; Dickman and Newsome, 2015; Estes et al., 2003; Scholz et al., 2020; Voigt et al., 2018). Identifying such variation and understanding its causal mechanisms can further our understanding of ecological and evolutionary processes. However, this is challenging because of difficulties in reliably attributing predation events to specific individuals. Large carnivores

often range over vast home ranges, have overlapping territories (both with individuals of the same species as well as other carnivore species), exhibit sociality and engage in scavenging and kleptoparasitism (Balme et al., 2017; Chundawat et al., 2016; Périquet et al., 2015). These traits and their elusive nature and absence of distinct individual markings (in some species) make it difficult to attribute a predation event to a specific individual.

Human carnivore conflicts, such as large carnivores' attacks on livestock and humans, present another case where reliable predator identification is critical (Goodrich, 2010; Swan et al., 2017; Treves and Karanth, 2003). Globally, conflict mitigation involves the removal of

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individuals through translocation or lethal measures (Linnell et al., 1999; Swan et al., 2017). However, a challenge with these approaches is unreliable identification and the consequent removal of non-target individuals (Treves and Karanth, 2003). This can leave conflict unsolved, cause social disruptions in carnivore communities, increase conflict frequency, and invite further public criticism (Athreya et al., 2011; Sinha, 2018; Woodroffe and Frank, 2005). Therefore, for such management interventions to be effective, it is important to make evidence-based decisions starting with reliable individual identification.

Physical evidence at the predation event site can provide vital clues for predator identification. Such evidence can be categorized depending on the stage at which it was deposited. Predation event by large carnivores such as tigers and lions, can be broadly classified into three phases: pre-predation, predation and post-predation. Pre-predation phase involves detecting and stalking of prey, cumulating in an ambush when the prey is within catching distance. When successful, the second phase involves capture and killing of prey during the predation phase. Finally, the post-predation phase involves securing the prey by moving it to a safer location for feeding. While detecting on-field signs and sample collection from pre-predation stage can be difficult, the latter two phases are more amenable to identification and sample collection. Large carnivores such as tigers and lions bring down the prey by usually biting the neck or throat region, resulting in strangulation and/or breaking of the vertebrae. This fatal bite can be distinguished from other bite marks based on the occurrence of haemorrhage. Kill location can be identified based on signs of struggle, pugmarks and hoofmarks, blood splatters, anomalous vegetation, etc. These sites are often valuable sources of shed hair samples, likely deposited on broken vegetation, areas around claw marks, and other nearby surfaces during a predation event. Furthermore, kill locations also serve as a starting point for drag marks as carnivores move their prey to secure areas from other predators or scavengers and to feed. The feeding site can be identified from prey remains, such as bones or meat, with feeding marks like bite marks, lick marks, etc., on the carcass. These sites are often well guarded and can be used as resting sites, resulting in accumulated scat, urine, and shed hair.

Conventionally used methods, such as visual analysis of prey remains and deployment of camera traps at kill sites, can lead to unreliable identification. Field assessments are restricted to identifying species, are highly dependent on the field expertise of the examiner, and limit retrospective use of data (Mumma et al., 2014). They can suffer from inaccuracy when species overlap in killing and feeding characteristics (Verzuh et al., 2018). Camera traps can be highly efficient in identification if deployed at a predation site before a predation event, such as when monitoring bird nests (Steffens et al., 2012); however, their use is limited in post-predation events where they might capture visiting, scavenging species/individuals (Steffens et al., 2012). Further, identifying individual predators using images becomes a challenge when individuals lack unique natural marks for identification.

Trace DNA of the predator left at the kill sites in the form of its saliva, shed hair, urine, scat, etc., can often be the only evidence for conclusive predator identification. Non-invasive genetic sampling, therefore, presents a promising approach to reliable and accurate identification of predators from kill sites (Blejwas et al., 2006; Fotedar et al., 2019; Nichols et al., 2012; Sundqvist et al., 2008). This approach has advantages over conventional methods as it is more sensitive and safer (for researchers and animals), can distinguish individuals of a species even without natural markings, and, most importantly, can distinguish a predator from a scavenger when systematically sampled. While being practical, the success of genetic samples can strongly be influenced by

factors such as abiotic conditions (e.g., temperature, rainfall, light), biotic factors (e.g., microbial activity, prey and predator species, maggot infestation), as well as sample collection procedures and storage methods (Harms et al., 2015; Nakamura et al., 2017; Piaggio et al., 2020; Reddy et al., 2012). If not collected systematically, genetic samples may also lead to incorrect attribution of predation events to a scavenger.

In this study, we attempt to evaluate and use non-invasive genetic samples from kill sites for predator identification. We do this by systematically sampling suspected tiger kill sites at two source populations in the central Indian tiger landscape. By collecting saliva, shed hair and scat samples of potential predators, we identify individual tigers using next-generation sequencing methods. We chose to sample livestock kills in these tiger reserves to evaluate molecular techniques for the following reasons: (i) Livestock kills are frequently reported for financial compensation to the forest department, thereby providing opportunities to collect samples in comparison to wild prey kills, which are challenging to detect, (ii) livestock kills are quickly reported (usually <24–48 h) providing an opportunity to collect fresh samples, (iii) multiple sample sources (saliva, shed hair, scat) can be collected from kill sites for comparison and, (iv) importantly, predator identification from livestock kill sites has management importance.

Specifically, we examined the influence of environmental factors such as season and kill site conditions on the number of SNPs typed and individual identification success. Based on our results, we propose a framework to identify and classify individual predators identified from a kill site and recommend sampling strategies for individual identification. Finally, we discuss the significance of our results, both methodological and conceptual, for conservation and management.

2. Materials and methods

Fieldwork for this study was carried out in two stages. The first stage was conducted in Kanha Tiger Reserve (hereafter, Kanha) from January to October 2017. During this period, we aimed to assess the availability and abundance of genetic samples and evaluate varied approaches to kill site investigation, including sample collection techniques. The second stage involved extensive sampling in Bandhavgarh Tiger Reserve (hereafter, Bandhavgarh) from April 2021 to March 2022. In both reserves, we systematically investigated livestock kills reported to be made by tigers by the livestock owners and forest department staff. We collected saliva, shed hair and scat samples as potential sources of predator DNA.

2.1. Study area

Kanha and Bandhavgarh have been classified as tropical moist deciduous forests with four main habitat types: grasslands, pure sal forest, miscellaneous forest and bamboo mixed forest (Awasthi et al., 2016; Champion and Seth, 1968). Kanha has an area of 2074 km² and is divided into two management units: the inviolate core (940 km²) with minimum human presence and a multiple-use buffer (1134 km²). Bandhavgarh spans 1537 km² with a core of 717 km² and a buffer of 820 km². The reserves harbour over 100 tigers each (Kanha: 129, Bandhavgarh: 165) and are critical source populations for tigers in the central Indian landscape (Qureshi et al., 2023). The local economy is largely dependent on agriculture, livestock rearing and tourism. Kanha reserve has an estimated population of 137,600 humans and 91,900 cattle in the core and buffer area (Negi and Shukla, 2011). Bandhavgarh has at least 130 villages in the park's buffer area with ~110,000 cattle

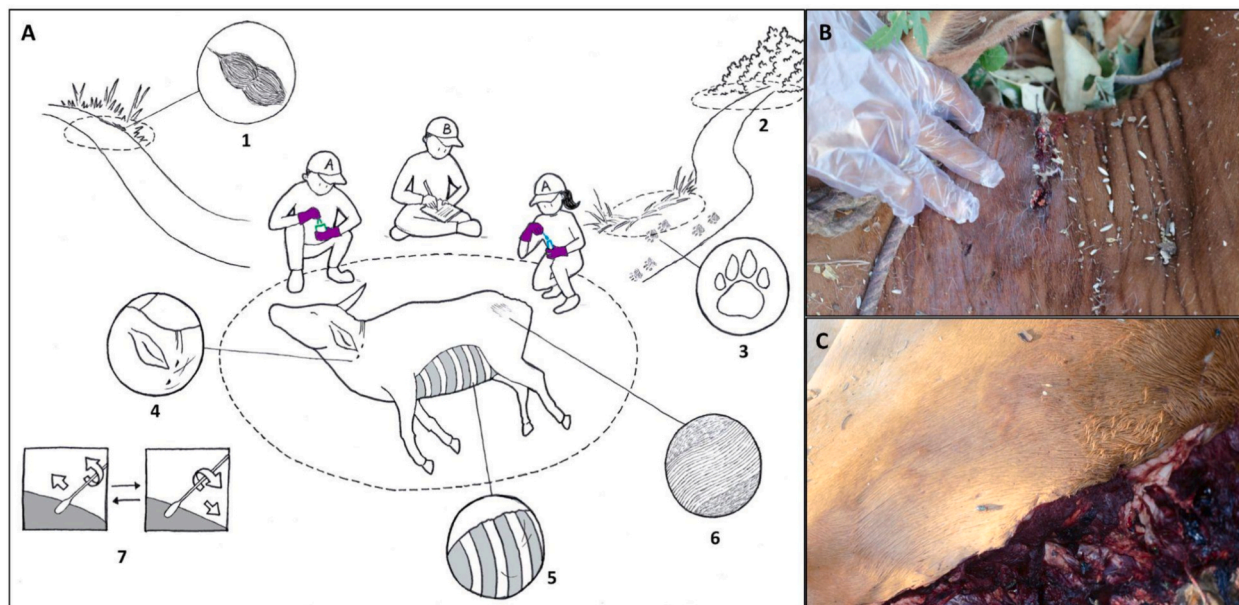


Fig. 1. A) Field sampling scheme illustrating various types of genetic samples of potential predators collected at a kill site: 1) scat sample collected using swab 2) inferred kill location is a source of shed hair sample 3) resting sites are a source of shed hair samples, 4) predation marks in the neck region are a source of saliva samples, 5) feeding area source of saliva and shed hair samples, 6) lick marks characterized by unidirectional bend of hair are source of saliva samples and 7) showing swabbing technique. Samples collected from 2 and 4 were classified as predation samples, whereas the rest were classified as post-predation samples. B) a photograph of a predation wound in the neck region with visible saliva deposit around the puncture region (darker colour), and C) a photograph of carnivore lick areas with saliva deposit.

heads. The resulting overlap of high human use area with that of high tiger density often results in conflicts mainly in the form of livestock depredation (annual livestock kills in Kanha range from 400 to 600 (Negi and Shukla, 2011), while in Bandhavgarh they range from 2000 to 2500 (V. Rahim, personal communication)) and, sometimes, human and tiger mortalities. Addressing conflict, therefore, is a key imperative for reserve management.

2.2. Kill site information

We investigated livestock kill sites reported by the livestock owners to the forest department for financial compensation. Both reserves have a similar, established system of reporting kills. When a livestock carcass is located and reported to the guard of the area by the livestock owner, the guard then verifies the claim by physically visiting the kill site and confirming the involvement of a carnivore in making the kill. Predator identification by the forest department from the kill site is based on field signs such as pugmarks, scrape marks, scat etc. Once verified, the beat guard passes the message to the concerned range office, which then shares the information with the park field director's office to initiate compensatory payment. This process, from initial detection by the livestock owner to final reporting to the field director's office, usually takes 24 to 48 h.

At each kill site, we first noted the GPS location, date, and time of sample collection, and the identity of the investigating team and the sample collector. We collected information on prey species, age, kill date, time, and rainfall from the livestock owner. We recorded the forest department's assessment of the predating species. This was done before our investigation to avoid influencing the department's assessment. Additional observations such as drag distance (in metres, from the inferred kill location to the location of the carcass, wherever visible), percentage consumption (based on visual estimate), feeding pattern (parts consumed, removal of tail and intestines, etc.), maggot infestation (visual estimate, categorized as high/medium/low) and canopy cover over the carcass (visual estimate in %) were noted. Photographs of the carcass were taken at the site.

At each kill site, we began our examination by conducting a quick 'walk through' to gain an overview, identify sites with evidence and infer the kill location. An intensive search for evidence was carried out along the drag marks between the carcass location and the inferred kill location. We scanned animal trails and potential carnivore resting areas for shed hair and faecal (scat) samples. Our search around the kill site and on the trails was limited to a distance of 30 m. This was done to reduce the chances of encountering other carnivore samples since both reserves have high carnivore density areas. Carnivore resting areas were identified based on anomalous vegetation and impressions on soil. Pugmarks, scrape marks and scats detected near the kill sites were assigned to carnivore species based on characteristics like shape, size, etc., following published field manuals (Karanth et al., 2002). Active care was taken not to disturb evidence during this search. However, the forest department/livestock owner had, in some cases, cleared the surrounding vegetation to ease access to the carcass before our arrival. Two people conducted searches independently to maximize sample collections and reduce detection bias. Sites, where carnivore pugmarks of different characteristics (shape, size), multiple resting sites (of varying sizes), irregular lick marks and multiple scat samples were found were noted as kill sites with multiple carnivore presence. Once initial scene documentation was completed, we decided on the collection order and initiated sample collection. We collected saliva samples first (starting from predation wounds) and then scat and shed hair to reduce the chances of sample contamination. We tried to decrease the time spent and disturbance caused at a kill site so as not to disturb the kill site.

2.3. Non-invasive genetic sampling

We collected genetic evidence from salivary marks, shed hair, and scats at each kill site. Sampling kits were not opened until all ancillary information was collected. To minimize the risk of on-field sample contamination, we changed gloves while collecting different samples and stored samples in different zip lock bags. Shed hair samples were collected independently from the various locations using sterile forceps and stored separately in zip lock bags or sterile 2 ml tubes to avoid

sample contamination. Traces of potential carnivore saliva from the carcass and scat samples found near the kill site were collected using sterile polyester swabs. Efforts were made to collect at least three saliva swabs (one each from different parts of the carcass with at least one swab from the predation bite marks whenever available), multiple shed hair samples and all the scat samples detected at the kill site. The number and type of samples collected from each site also varied based on kill site conditions such as the presence of carnivores, scavengers and proximity to villages.

For swab collections, swabs were first briefly soaked in Longmire lysis buffer (Longmire et al., 1997) and then rolled over the target area for 10–12 seconds following Mumma et al., 2014. For scats, swabs were rolled over the outer, shiny areas preferred for host DNA. Samples were categorized as either predation or post-predation samples (Fig. 1). Predation samples were samples that we suspected to be deposited during a predation event. These include saliva samples collected from predation wounds (distinguished from other bite marks based on the occurrence of haemorrhage) and shed hair samples collected from the inferred kill location (where the predation event occurred). Post-predation samples, i.e. samples likely deposited after a predation event had occurred, include saliva samples collected from lick and feeding marks, shed hair samples deposited at suspected resting sites and sites near the carcass, and scat samples. Swab samples were stored in 2 ml tubes with 1.5 ml Longmire lysis buffer.

2.4. DNA extraction and species identification

DNA extraction was done in a dedicated low-concentration DNA extraction facility at the National Centre for Biological Sciences (NCBS), Bangalore. DNA extraction was done using QIAGEN® DNA Tissue and Blood extraction kits, following the protocol suggested by the manufacturer with modifications. Extraction controls (negatives) were set to detect any contamination. DNA extraction of different sample types was done separately to avoid contamination. All samples were screened using tiger-specific primers to identify positives (Bhagavatula and Singh, 2006). PCR controls were set to detect contamination while setting up the PCR reaction.

2.5. Individual identification

Samples genetically confirmed to be tigers were genotyped using a panel of 123 SNPs, as described in Natesh et al. (2019). This mainly involved a three-step process – a multiplex PCR, an indexing PCR, and library preparation and sequencing. Multiplex PCR of Kanha samples was performed using a unified pool of 123 primers. Primers were split into two pools (based on amplicon size) for Bandhavgarh samples. Indexing PCR was performed to add a unique combination of i5 and i7 Illumina indexes to samples. The indexed products were pooled and bead-purified to retain only amplicons of targeted size. The resulting library was then sequenced on an Illumina Miseq platform to obtain 75 × 2 paired-end reads. All the samples were processed in replicates.

Data filtering and subsequent individual identification were done independently for both study sites. Raw reads were filtered using the program TrimGalore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with a quality value of 30 on phred33 scale to remove adapters and short reads. Filtered reads were mapped to the tiger genome assembled for Indian tigers (Shukla et al., 2023) using bwa mem (Li, 2013) with a penalty value of 3. Variant calling was done using bcftools (Li, 2011). Variants were filtered to retain only the targeted loci, following which genotype quality (10 or above) and depth (10 or above) filters using GATK (DePristo et al., 2011). Missing data filters were applied to remove loci missing in >15 % of samples and samples with <40 SNPs. After filtering, we retained 95 and 101 SNPs for Bandhavgarh and Kanha, respectively. Data filtering steps were uniform for both Kanha and Bandhavgarh. Individuals were identified based on pairwise relatedness (PI-HAT) values calculated using Plink (Purcell et al., 2007).

Pairwise relatedness values of known sample replicate were used to determine the minimum relatedness value for recapture identification, following Natesh et al. (2019) and Sagar et al. (2021). After determining the minimum relatedness value for recapture, data of known sample replicates were merged to minimize missing data, resulting in a single data file for each unique sample. The merged files were then re-analyzed to obtain pairwise relatedness between the unique samples.

Samples with pairwise relatedness greater than the recapture cut-off were identified as the same individual. Unique samples that had pairwise relatedness >0.5 and lower than the recapture cut-off with any sample were categorized as uncertain and discarded to avoid ambiguity. All other samples were identified as unique individuals without recaptures. Individual identification was done using Program R v 4.0.2.

2.6. Predicting SNP typing and individual identification success

We conducted standard Generalized Linear Models (GLM) analysis using Program R v 4.0.2 to assess how season and sample type influence the number of SNPs typed (amplified) and individual identification success. We also evaluated the impact of sampling lag (number of days from predation event until sampling) and canopy cover (in percentage), but they were excluded from the final model as there was data limitation. Both rainfall ($X^2 = 31.189$, $df = 2$, $p\text{-value} = 1.688\text{e-}07$) and maggot infestation ($X^2 = 109.88$, $df = 6$, $p\text{-value} < 2.2\text{e-}16$) showed a significant association with season and were therefore excluded from further analysis. We used only Bandhavgarh samples to develop our models since there weren't enough samples from Kanha across the seasons, and the lab protocols for individual identification differed from those of Bandhavgarh. The SNP count used for the analysis was the number of SNPs retained following filtering for genotype quality and depth. Similarly, any sample having >40 SNPs following filtering (depth (10), quality (10) and removing missing SNPs) was classified as successful for individual identification.

2.7. Predator assignment

Trace DNA samples deposited during a predation phase are more likely to be of predator. These include saliva collected from the predation bite marks, usually in the neck region, and shed hair samples deposited during the struggle to subdue the prey at the kill location. Samples deposited after a predation event may originate from either a predator or scavenger. They are usually deposited during feeding, resting near or guarding the kill. These include saliva traces on lick marks, feeding marks and shed hair deposited near the feeding area and on resting spots. Based on the type of sample used for identification (predation or post-predation), we classified tigers into three categories: True predator, circumstantial predator, and predator uncertain. Each of these assignments was at both species and individual levels. True Predators are species, individuals that were solely identified using predation samples, i.e. saliva samples from predation wounds on the carcass and shed hair samples collected from the inferred kill location. Circumstantial predators are species and/or individuals that were likely to be predators but were not identified from the predation samples either because predation samples were not collected or were ineffective in identification. Circumstantial predators were identified only when multiple post-predation samples were collected from a site, and all resulted in the same identification. Any sample where species and/or individuals identified from a kill site that did not fit the above categories were assigned as predator uncertain (schematic flow chart in Supplementary Figure S1). The highest confidence was assigned to true predator, followed by circumstantial predator and the least for predator uncertain, when ranking the likelihood of a species or individual being involved in the predation event (Table 1).

Table 1

Predator classification scheme with conditions for assignment.

Classification	Level	Sample used for identification	Condition	Confidence assignment
True predator (TP)	Species	Predation	1. Species identified using only saliva samples from predation marks or shed hair samples from inferred kill location.	High
	Individual	Predation	1. Individual identified using only saliva samples from predation marks or shed hair samples from inferred kill location.	
Circumstantial predator (CP)	Species	Post-predation	1. No predation mark/inferred kill location sample	Moderate
	Individual	Post-predation	2. All/multiple samples from the kill site should identify only one species from the kill site. 1. No predation mark/ inferred kill location sample for species identification. 2. Individual identified from samples at the kill site, but not predation mark/ inferred kill location. 3. All/multiple samples identify a single individual from the kill site.	
Predator uncertain	Species	Post-predation	1. Criteria for either TP or CP cannot assign species. 2. More than one potential predator species is identified at the kill site using samples other than predation samples. 3. When the species has been identified from just one sample, another than the predation sample.	Low
	Individual	Post-predation	1. Individual cannot be identified using criteria for TP or CP. 2. More than one potential predator individual identified at the kill site using samples other than from predation mark/inferred kill location. 3. Has been identified from just one sample other than the predation mark/ inferred kill location.	

3. Results

3.1. Sample availability and species identification

We sampled 342 saliva swabs, 395 shed hair, and 33 scat samples from 198 kill sites (Bandhavgarh: 142 and Kanha: 56) (Fig. 2, Table 2). Shed hair and saliva samples were the most abundant source of DNA and were collected from 85 % and 72 % of the kill sites sampled, respectively. Shed hair samples were the only source of DNA for 52 kill sites,

while saliva samples were the only source of DNA for 27 sites. Scat samples were found at 10 % of the kill sites. At least one predation sample (saliva from the predation wound or shed hair from the inferred kill location) was collected from 153 (78 %) kill sites. Season influenced the availability of saliva samples from predation wounds, with samples collected from fewer kill sites in monsoon (28 %) compared to winter (60 %) and summer (60 %).

Tigers were detected at all kill sites with species identification success with 98.5 % saliva swabs, 98.2 % shed hair and 96.9 % scat samples.

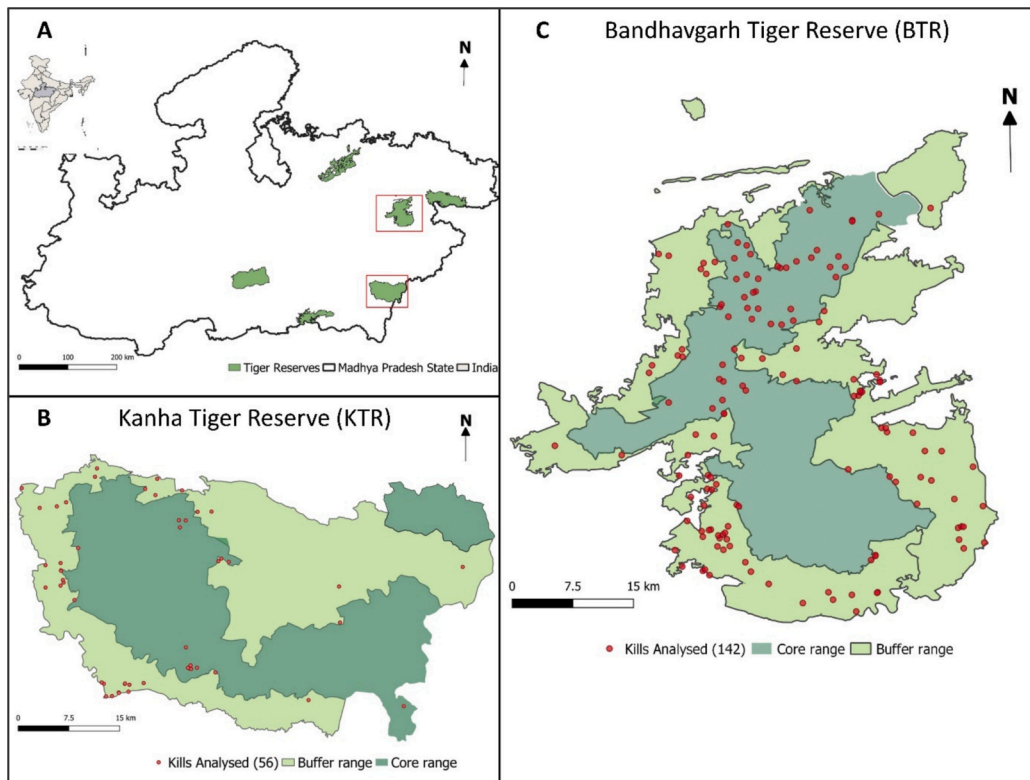


Fig. 2. Study area map with sampling locations. A) Boundary of Madhya Pradesh state in India with study sites highlighted in red box. B) Kills sampled in Kanha Tiger Reserve ($N = 56$) and C) Kills sampled inside Bandhavgarh Tiger Reserve ($N = 142$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Summary of samples collected across kill sites in Kanha (January to October 2017) and Bandhavgarh (April 2021 to March 2022) Tiger Reserves.

Source	Season	BANDHAVGARH TR		KANHA TR	
		Kill sites	Samples	Kill sites	Samples
Saliva	Winter	47	118	7	21
	Summer	29	60	15	40
	Monsoon	17	29	28	74
Shed hair	Winter	53	151	5	7
	Summer	42	96	8	14
	Monsoon	46	112	14	15
Scat	Winter	6	6	1	5
	Summer	3	4	2	5
	Monsoon	4	8	3	5

Using tiger-specific primers limited our detections to tigers; therefore, no amplification doesn't indicate the absence of carnivore DNA. Season did not influence the success of the species identification of any sample types.

3.2. Impact of season and sample type on the number of SNPs typed

Using saliva samples from the winter season as the reference group, our models indicate a 30 % and 53 % decline in SNPs typed in summer and monsoon, respectively (Table 3). For shed hair (again, hair in winter as reference), an 11 % and 37 % decline in SNPs typed was observed in summer and monsoon, respectively. Shed hair samples typed more SNPs in comparison to saliva samples across seasons. In winter, the number of SNPs typed was highest for saliva and shed hair samples. A stronger seasonal impact was observed for saliva samples than shed hair samples.

3.3. Individual identification success

After applying missing data filters, we set 40 SNPs as a cut-off for individual identification. Data filtering and subsequent individual identification were conducted independently for each study site. Of 757 unique tiger-positive samples processed, 449 samples (59 %) had >40 SNPs and were used for individual identification (Table 2). PI-HAT scores of known replicates ranged from 0.64 to 1 for Kanha and from 0.69 to 1 for Bandhavgarh (Supplementary Figure S2). A recapture cut-off of 0.81 was set for Kanha and Bandhavgarh based on the 97.5 % percentile of the relatedness distribution (between replicates). Seventy-

two tigers (Kanha 19 and Bandhavgarh 53) were identified with varying numbers of recaptures (pairwise relatedness of Bandhavgarh individuals in Supplementary Figure S3). Four individuals from Kanha and 11 from Bandhavgarh had no recaptures and were detected only once. The probability of identity (P_{ID}) for two randomly chosen individuals was 1.1×10^{-19} for Kanha and 4.3×10^{-19} for Bandhavgarh. Similarly, P_{ID} -sibs was 7.3×10^{-10} for Kanha and 1.5×10^{-9} for Bandhavgarh. Unique individuals from both populations had genotype mismatches at over 10 loci, indicating high confidence in individual identification. Seventy-one samples were classified as uncertain as they didn't have >0.81 relatedness with any sample and/or had >0.55 relatedness with multiple individuals. These samples were discarded to avoid ambiguity.

At least one tiger was identified at 85 % of all kill sites sampled. Shed hair samples were the most successful source (82 %) for individual identification across seasons, followed by saliva (59 %) and scat (58 %) at kill sites (Table 4). Shed hair samples were the only source of identification at 80 kill sites, while saliva samples were the only source at 29 sites. Predation samples identified individuals at 53 % of the sites where they were collected. At 11 kill sites, saliva samples from predation marks and shed hair from kill locations independently identified individual tigers. Of these, individual identity concurred between the sample types at 10 sites, while there was a mismatch at one site.

Our models indicate a higher probability of individual identification with shed hair samples when compared to saliva samples. Using the winter season as the reference group, the probability of individual identification declined by 10 % and 30 % in summer and monsoon, respectively, for saliva samples (Table 3). Similarly, for shed hair samples, the probability of individual identification decreases by 21 % and 34 % in summer and monsoon compared to winter.

Based on the estimated mean confidence interval, we infer that achieving a 90 % probability of individual identification from a kill site will require 16, six and four samples saliva samples during monsoon, summer, and winter, respectively (Supplementary Table S1). Shed hair are more effective in individual identification, requiring only five, three and two samples in the monsoon, summer and winter, respectively to achieve similar individual identification success.

3.4. Preliminary insights on individual engagement in depredation

While samples from 169 kill sites had >40 SNPs, individual tigers were identified at 155 of the 198 kill sites where tigers were detected. In total, we identified 87 unique individuals from both the study areas.

Table 3

Results of Generalized Linear Models (GLM) examining the influence of sample type (saliva and shed hair) and season (winter, summer and monsoon) on the number of SNPs typed and individual identification success.

Model	Number of SNPs typed			Individual identification success		
	Estimate	Std. error	Pr(> z)	Estimate	Std. error	Pr(> z)
Saliva * Winter (intercept)	3.71379	0.01469	<2e-16***	0.05311	0.18821	0.18821
Saliva * Summer	-0.3505	0.02816	<2e-16***	-0.9004	0.3388	0.00787**
Saliva * Monsoon	-0.7567	0.04481	<2e-16***	-1.6217	0.52639	0.00206**
Shed Hair * Winter	0.42914	0.01788	<2e-16***	1.16223	0.2692	1.58e-05***
Shed Hair * Summer	0.23086	0.23086	<2.52e-12***	0.34647	0.44633	0.4376
Shed Hair * Monsoon	0.2907	0.0485	2.05e-09***	0.46355	0.59352	0.43479

Table 4

Summary of individual identification success for various sample types (saliva, shed hair and scat) collected from Kanha and Bandhavgarh Tiger Reserves across seasons (winter, summer and monsoon).

Source	Season	KILL SITES		SAMPLES	
		Processed	Success (in %)	Processed	Success (in %)
Saliva	Winter	53	64	134	53.7
	Summer	44	56.8	100	47
	Monsoon	45	57.8	103	43.7
Shed hair	Winter	57	79.5	157	78.3
	Summer	50	90	108	70.4
	Monsoon	59	69.5	123	54.5
Scat	Winter	7	57.1	11	72.7
	Summer	5	20	8	37.5
	Monsoon	7	85.7	13	61.5

Multiple individuals were detected at 19 sites. Forty-six per cent of the individuals were detected in only one kill site, while almost a third (29 %) were at two kill sites. In other words, 75 % of all tigers found at kill sites were at only one or two kill sites. Twenty individuals (23 %) were detected at three to five kill sites, and only two individuals (2 %) were detected at seven kills. Our sampling of kill sites was more intensive in Bandhavgarh, and we attempted to investigate spatial distributions of

individual predators here (Fig. 3).

3.5. Predator assignment

We compiled predator assignments for both Bandhavgarh and Kanha. Tigers were detected at all 198 kill sites. By our defined categories, we identified the ‘true predator’ at 151 kills, the ‘circumstantial predator’ and ‘predator uncertain’ at the species level for 34 and 12 sites, respectively. Individual tigers were assigned as ‘true predators’ for 72 kills and as ‘circumstantial predators’ at 34 sites. Individuals were identified using multiple predation samples at 11 sites. All samples identified a unique individual in ten of these 11 sites. Two unique individuals were detected at one site using different predation samples, including saliva and shed hair. One of these individuals was detected at three other sites, while the second wasn’t recaptured at any other site. At 49 kill sites, individuals were identified as predator uncertain. Within these sites, individuals were classified as predator-uncertain when identification was obtained from only one sample (38 kills) or when multiple individuals were detected (11 kills). Moreover, using the predation sample, we successfully differentiated between predator and predator-uncertain individuals at seven sites where multiple individuals were detected.

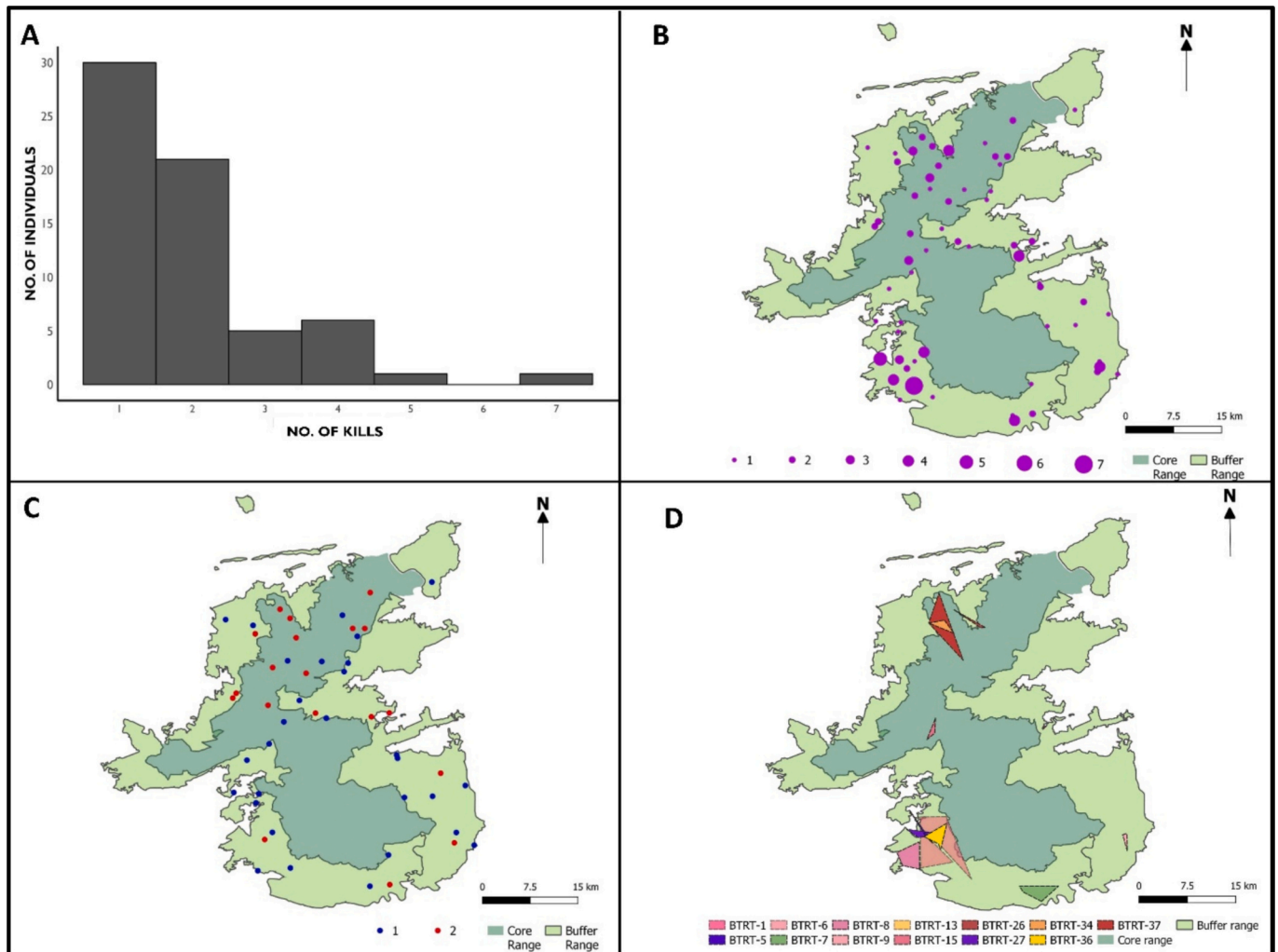


Fig. 3. Summary of individuals identified across kills in Bandhavgarh Tiger Reserve. A) Histogram of individuals found across kill sites indicating majority of individuals were identified at one or two sites, B) frequency of individual occurrence across all kills, C) individuals identified ($N = 51$) at one or two kills, D) occurrence polygon of individuals ($N = 13$) identified at 3 or more kills.

4. Discussion

Individual predators often differ in their hunting strategies, prey selection, and spatial patterns of habitat use. These differences can promote population stability by reducing intraspecific competition, influence prey populations through selection pressures, and ultimately drive ecological diversification within predator-prey systems. An important pre-requisite to understanding these processes is reliable identification of both the species and individual involved in predation. Our study assessed the utility of trace DNA samples in predator identification while examining factors that influence success. We successfully identified tigers at all sampled sites, achieving a 100 % success rate in species identification, and identified individual tigers at 85 % of all kill sites. We find that the sample type and the sampling season are determinants of SNP typing and individual identification success. We develop a framework to assign confidence in predator assignments based on sample type used for individual identification. At 46 % of kills, we identified true predator (high confidence), circumstantial predator (medium confidence) at 22 % of sites, and predator uncertain (low confidence) at 32 % of sites at the individual level. We conclude that trace genetic samples and systematic kill investigation can successfully identify predators.

4.1. Season and sample type determine identification success

Shed hair samples consistently typed more SNPs and had higher individual identification success than saliva and scat samples, regardless of the season. Winter was observed to be the most favourable season for sampling. As predicted, the individual identification success rate per sample was lower during the monsoon season. We could not test the independent impact of rainfall and sampling time lag on success as rainfall was strongly associated with season, and we had a low sample size for delayed sampling events (> three days). While earlier studies in controlled settings have shown the negative impact of rainfall and time lag on DNA quality (Harms et al., 2015; Piaggio et al., 2020; Reddy et al., 2012), testing independent effects in field studies becomes challenging due to deposition of fresh saliva across feeding bouts. Despite declining individual identification success per sample, the success per kill remained consistent across seasons. This implies that collecting multiple samples from various sources at a single kill could help alleviate the seasonal impact. We therefore recommend the collection of more samples during the monsoon season, followed by summer and winter. We endorse previous suggestions to collect samples promptly to prevent degradation and minimize the influence of scavengers (Ganz et al., 2023; Mumma et al., 2014). Additionally, we strongly recommend collecting multiple sources of samples, including predation samples (such as saliva from fatal wounds and shed hair from the point of kill), at all sites, as these samples aid in differentiation between predators and scavengers, ultimately strengthening confidence assignment in identification. We recognize that processing more samples can increase costs and recommend prioritizing sample processing, such as predation samples, over others to reduce costs.

4.2. High proportion of individuals engage in depredation

Human-wildlife conflict poses a significant threat to conservation initiatives, and targeted removal of individuals is widely used as a strategy for conflict management. This approach, however, assumes unequal contributions of specific “problem” individuals in conflict engagement, essentially drawing from intra-species variations (Swan et al., 2017). We identified 87 individual tigers from 155 sites in Kanha and Bandhavgarh that were present at livestock kills. Considering the recent findings from the All India Tiger Estimation (Qureshi et al., 2023), which estimated Bandhavgarh's tiger population at 165 individuals, our study identified ~40 % of these individuals at livestock kills. Seventy-five percent of individuals identified in both Kanha (2017)

and Bandhavgarh (2021) accounted for 58 % of all kills but were involved in less than three kills each. Establishing the presence of problem individuals would require information on intra-population variation in diet and livestock depredation frequencies by individual tigers, resulting in economic losses and the determinants of this variation. We acknowledge the limitations of our study in commenting on the existence of “problem” individuals, and further research is required to validate their presence, especially in systems with large numbers of livestock kills such as ours. Given the high frequency and spatial scale of livestock depredation and the number of individual tigers involved in depredation in our study area, we propose differential assessments of cases involving livestock depredation and human attacks when identifying potential problem individuals. We suggest that removing individual tigers might not effectively reduce depredation risks (Elbroch and Treves, 2023). Instead, management interventions should prioritize alternative approaches such as zoning, limiting livestock numbers, etc. and providing adequate compensation to livestock owners affected by depredation. In contrast, attacks on humans are often localized, and removing specific individuals may be effective if their involvement is conclusively established. Removal of individuals has consequences on population dynamics and raises ethical considerations. Therefore, for such interventions to be effective, it is crucial to establish the existence of problem individuals in the system and accurately identify the predator before resorting to targeted removal. Otherwise, targeted removal will be a tool for balancing conservation and political goals without serving its intended purpose of conflict reduction.

4.3. Conclusive identification of predators will require an adaptive and systematic sampling design

Genetic methods are more effective in predator identification than field-based methods (Ganz et al., 2023; Mumma et al., 2014). Samples can also be collected from human predation sites, where using conventional methods becomes challenging and requires ethical considerations (Pandey and Sharma, 2016). They are distinctly advantageous when trying to identify the “true predator” for a predation event. Trace DNA deposited during a predation event (such as saliva from predation wounds and shed hair at kill locations), essentially serve as sole evidence of a predation event. While camera traps can help in visual identification of individuals such as in case with tigers, they are typically deployed post a predation event. The photographed individual may not necessarily be the predating individual. Both conventional and genetic methods are prone to misidentification, especially when multiple potential predators and scavengers are at the kill site (Ganz et al., 2023; Steffens et al., 2012; Verzuh et al., 2018). Integrating multiple approaches like field investigations, camera trapping, genetic data, and data from collared individuals can not only enhance reliability of identification by reducing errors but also provide comprehensive understanding of the predator itself such as its sex, age, physical condition, relatedness to other depredators in the landscape etc. Once a predation event is confirmed (see Cristescu et al., 2022), the kill site should be systematically explored and documented, followed by meticulous sample collection. To enhance the reliability of assessments, we recommend assigning confidence levels based on the information source and associated errors. We provide a framework for confidence assignment using genetic data by categorizing samples according to their source and collection site. The highest confidence is attributed to samples likely deposited during a predation event, such as saliva samples from fatal wounds (indicated by haemorrhage) and shed hair samples from the inferred kill location. However, in some cases, kill locations might be indistinguishable and can be misidentified; also, the presence of multiple individuals can lead to accidental deposition of hair. We recommend cross-verification of individual identification from both saliva samples from predation wounds and shed hair from the kill location. In our study, among 11 sites where both sample types identified individuals, the identification was concurrent at 10 sites. This framework can be

adapted and modified in future studies to encompass various other sources of information, such as camera trap images from kill sites, prior knowledge of individual territories, and radio telemetry data. We align with Cristescu et al. (2022) in recognizing that reporting such evidence and subsequent assessments will enhance transparency and foster public support in decision-making.

4.4. Integration of molecular approaches into management

The broader acceptance and utilization of genetic tools face challenges due to cold storage, extended processing times, and high sample processing costs (Khan and Tyagi, 2021). However, lysis buffers allow sample storage at room temperature, addressing the cold storage requirement. Advancements in next-generation sequencing methods can further diminish processing time and costs (see Natesh et al., 2019). These methods also enable the real-time generation of high-quality data using non-invasive samples (Urban et al., 2023). We propose establishing a reference genetic database, particularly for individuals involved in conflicts. Furthermore, acknowledging that genetic information does not align with the visual identification typically required for management actions, this database can be linked to the physical identification of individuals. This can be achieved by simultaneously sampling kills for trace DNA and deploying camera traps at kill sites in addition to opportunistic sampling during animal sightings and the capture of individuals. This comprehensive database of individuals will enable a better understanding of predation ecology through robust individual identification and promote evidence-based conflict management.

CRediT authorship contribution statement

Himanshu Chhattani: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Abishek Harihar:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Rounak Dean:** Methodology, Data curation. **Ajay Yadav:** Methodology, Data curation. **Kaushal Patel:** Methodology, Data curation. **Divyashree Rana:** Software, Methodology. **Awadhesh Pandit:** Methodology. **Sanjay Kumar Shukla:** Resources. **Vincent Rahim:** Resources. **Uma Ramakrishnan:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2025.111117>.

Data availability

Data sheets, genotypes of individual tigers and scripts for variant calling and filtering, and predator assignment are available on Github (<https://doi.org/10.5281/zenodo.15178770>).

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