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Case report

Before they are gone – improving gazelle protection using wildlife forensic genetics

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ABSTRACT

Throughout their habitats gazelles (genus *Gazella*) face immediate threats due to anthropogenic effects and natural environmental changes. Excessive poaching plays a major role in their populations decline. Three unique populations of gazelles currently live in Israel: mountain gazelle (*Gazella gazella*), Dorcas gazelle (*Gazella Dorcas*) and acacia gazelle (*Gazella arabica acacia*). Ongoing habitat degradation and constant pressure from illegal hunting has caused a continuous decrease in the last 10 years, stressing the need for drastic measures to prevent species extinction. Wildlife forensic science assists enforcement agencies in the escalating arms race against poachers. Wildlife forensic genetic tests being implemented in our laboratory offer both species and individual identification, which rely on two mitochondrial genes (12S rRNA and 16S rRNA) and nine nuclear Short Tandem Repeats (STR), respectively. The current study, presents a poaching case in which mitochondrial DNA-based species identification revealed the presence of mountain gazelle DNA on the seized items. Subsequently, STR markers linked the suspect to more than one gazelle, increasing the severity of the criminal charges.

1. Introduction

Over the past two decades the intensity and spatial extent of bushmeat and wildlife trade has increased substantially worldwide [1,2] and has led to extensive defaunation, which poses an immediate threat to sustainable conservation of biodiversity [2–6]. Poaching and illegal trade are motivated by various factors including cultural legacy, food necessities and organized crime [2,7,8].

Combating and monitoring poaching and illegal trade in wildlife requires law enforcement agencies and scientists to be able to identify the species in order to assess whether a crime was committed. Species identification is mostly based on DNA identification rather than morphology, especially as traders and consumers try to disguise the origin of the artifact by changing its appearance [9]. Species identification is a vital and primary wildlife forensic genetics test [10]; however in some cases more information is required to fully answer the questions of the investigators. Several such questions deal with individual identification, determining how many specimen were involved or if two crime scenes are connected [11]. Yet, while these tests are often valuable for poaching and trade investigations, they are not available (in forensic standards) for many of the wildlife species in question. Unlike human forensics, which deal with solitary species and therefore the development and screening of genetic tests are performed only once; wildlife forensics requires each of these steps to be performed for every species independently [12]. The limited number of wildlife investigations, compared to human investigations, and the limited resources and genetic information, mean the wildlife forensic science is still in its preliminary steps for many species.

The majority of species targeted by poachers are large animals, which supply more meat, are more popular as trophies and are traditionally believed to have mystical powers (i.e. elephant, rhinos, sharks etc.) [13–15]. In the southern Levant the most commonly hunted mammals, throughout history, are the gazelles, and more specifically the mountain gazelles (*Gazella gazella*) [16–18]. Up until the 20th century these elegant antelopes were abundant in the region, as evident from the reports of pioneer zoologists Tristram and Aharoni [19–21]. However, habitat loss, fragmentation of populations and uncontrolled hunting, especially

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since the introduction of firearms since World War I, has extirpated the mountain gazelles from most of their natural habitat (Lebanon, Syria, Jordan) [22]. The "Wildlife protection law", legislated in Israel in 1955 and its amendments prohibit the hunt, trade, possession or transfer of protected wildlife species and their parts. With the enforcement of the wildlife protection law, the mountain gazelle population in Israel grew from an estimated 500 individuals in the 1960s to ~10,000 in 1985 [21]. Nevertheless, ongoing habitat degradation and constant pressure from illegal hunting caused a continuous decrease in the last 10 years, stressing the need for drastic measures to prevent species extinction [23]. Here we present a case study demonstrating the efficacy of the gazelle STR panel to determine the minimum number of gazelles illegally harvested in a wildlife offence in Israel.

2. Materials and methods

2.1. Specimens

As part of an Israel Nature and Parks Authority (INPA) investigation of illegal hunting, seized evidence from a crime scene and suspected poachers were submitted to Molecular Evolution laboratory, at the Hebrew University of Jerusalem, for wildlife identification using genetic tests. The case consisted of nine exhibits including blood stains on various items, animal hair and tissue (Table 1). Following wildlife forensic guidelines all samples were documented prior to DNA analysis.

2.2. DNA extraction

DNA extraction was carried out using the Guanidine thiocyanate (GuSCN) [24] followed by a silica- based purification [25] methods. The extractions were carried out in a dedicated laboratory for wildlife forensic genetics following international standards and guidelines developed by the Scientific Working group on Wildlife Forensic Science (SWGWILD, accessed from www.wildlifeforensicscience.org/swgwild).

2.3. Species identification

Species identification was based on two mitochondrial gene regions, 12S rRNA (167 bp) and 16s rRNA (153 bp) as we found in our mammalian database these genes are more conserved and show less variation between populations. PCR amplifications were conducted using published primer sets [26] following conditions described in Hadas et al., 2015 with the addition of 0.75ul of fluorescent dye (SYTO9, Invitrogen (50 μM)) to the reaction mix. Real-time PCR and High Resolution Melt (HRM) analysis were performed using QIAGEN Rotor-Gene Q Thermocycler (Qiagen) and Rotor-Gene 6000 software (Corbett Research). After amplification, the samples were heated from 50° C to 99° C with a rise in 1° C/s. Following the melting step a hybridization step in which the samples were cooled back to 50° C with a decrease of 1° C/s was carried out. The HRM analysis was conducted according to the melting step with a gradual rise in temperature of 0.1-0.4° C/s [27].

Table 1
Species identification and STR profiles for the case samples.

Sample ID	Sample Description	12S fragment[a]		16S fragment[a]		Nuclear Microsatellite Markers[b]															
		IWDD	NCBI	IWDD	NCBI	OarFCB193		OarFCB48		ETH10		ILSTS029		BM2113		OarAE54		TGLA122		BM4505		INRA40	
A1	Plastic bottle	Gg	St	Gg	Gt	A	B			E	F			M	O	P	P	T	T	U	V		
		100%	98%	100%	99%																		
A2	Plastic bottle cap	Gg	St	Gg	Gt													T	T	U	V		
		100%	98%	100%	99%																		
A3	Tissue from shoe	Gg	St	Gg	Gt																		
		100%	98%	100%	99%																		
A4	Blood from shoe	Gg	St	Gg	Gt																		
		100%	98%	100%	99%																		
A5	Stain on coverall	Gg	St	Gg	Gt	A	A			E	F							T	T	V	V		
		100%	98%	100%	99%																		
A6	Stain on coverall	Gg	St	Gg	Gt																		
		100%	98%	100%	99%																		
A7	Stain on coverall	Gg	St	Gg	Gt													T	T				
		100%	98%	100%	99%																		
A8	Stain on sack	Gg	St	Gg	Gt																		
		100%	98%	100%	99%																		
A9	Stain on sack	Gg	St	Gg	Gt																		
		100%	98%	100%	99%																		
A10	Blood on paper	Gg	St	Gg	Gt					F	F	H	H	N	N	Q	R	T	T				
		100%	98%	100%	99%																		
A11	Ear tissue	Gg	St	Gg	Gt	A	B			F	F			N	N	Q	R	T	T	U	V	Z	Z
		100%	98%	100%	99%																		
A12	Rifle holster A	Gg	Pp	low quality sequence		A	B			F	F			N	N	Q	R	T	T				
		100%	89%																				
A13	Rifle holster B	Gg	St	Gg	Gt					E	G			L	L	P	Q	T	T				
		100%	98%	100%	99%																		
A14	Rifle holster B	Gg	St	low quality sequences												P	Q	T	T				
		100%	98%																				
A15	Hairs	Gg	Gt	Gg	Gt																		
		100%	98%	100%	99%																		
A16	Hairs	no DNA amplification																					
Positive control	Tissue					A	C	D	D	E	F	i	J	K	L	R	S	T	T	W	X	Y	Z

Gg = *Gazella gazella*, St = *Saiga tatarica*, Pp = *Procapra przewalskii*, Gt = *Gazella thomsonii*.

[a] Percent of identity between case sample sequences and reference sequences deposited in genetic databases: Israel Wildlife DNA Database (IWDD) and National Center for Biotechnology Information (NCBI). Calculations executed by BLAST algorithm.

[b] STR alleles are presented as letters for ease of comparison.