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Review

Forensic DNA analysis for animal protection and biodiversity conservation: A review

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ABSTRACT

The use of DNA analysis in forensic investigations into animal persecution and biodiversity conservation is now commonplace and crimes such as illegal collection/smuggling, poaching, and illegal trade of protected species are increasingly being investigated using DNA based evidence in many countries. Using DNA analysis, it is possible to identify the species and geographical origin (i.e. population) of a forensic sample, and to also individualise the sample with high levels of probability. Despite extensive literature in animal species, there is unfortunately a serious lack of information on plant species, with only a handful of recent studies. In this review, I detail the applications and diverse forensic investigations that have been carried out to date whilst also highlighting recent developmental studies which offer forensic potential for many species in the future.

Introduction

Forensic DNA analysis in investigations of crimes such as animal cruelty and poaching, and illegal collection and trade of flora and fauna has seen rapid growth in recent years mainly due to technological advances made in the field of human forensic genetics which has allowed transfer of methods and applications to various non-human species. However, progress has been more gradual than with human forensic genetics for a number of reasons. Firstly, unlike human forensic genetics, forensic DNA analysis for animal protection and biodiversity conservation has to deal with a plethora of species leading to a lack of optimal genetic markers for many. Secondly, animal/plant/wildlife geneticists have for many years worked in isolation from forensic geneticists, resulting in markers and methodology without the extensive validation procedures required for forensic casework. Thirdly, much of the poaching and illegal collection of natural resources occurs in poor countries where financial resources are limited. And finally, animal/plant/wildlife crime always takes lower priority to crime involving humans. Thus, with the exception of a few domesticated animal and plant species, there has been limited input of resources into other species. More than 20,000 species are currently listed in the IUCN Red List of Threatened Species. The list encompasses diverse species of terrestrial plants such as cycads and cacti, in addition to vertebrates such as fish, amphibians, reptiles, birds, and mammals, and invertebrates such as lobsters, crabs, and corals (IUCN 2013). The application of forensic DNA analysis techniques in these species has obvious potential.

Types of biological evidence

When investigating crimes against animals or protected natural resources, there is an overabundance of the types of samples that may be encountered. From the analysis of more standard samples such as tissue, hair, and feathers, DNA analysis has also been carried out using samples such as tusks, claws, tanned leather, bile crystals, scales, shells, processed animal parts and derivatives within Traditional Chinese Medicines (TCMs), and objects made out of animal parts such as hankos, shawls, idols and handbags. See Table 1 for a full listing of forensic cases to date with information on the DNA marker/s used and the type of investigation carried out. Although several cases involving diverse animal species have been reported to date, there are noticeably no reported forensic cases involving plant species. A large number of developmental studies with good potential for forensic applications have also been reported (see Table 2 for a full listing) once again, incorporating unusual types of biological evidence, and fortunately, a few are on plant species.

Forensic applications

Studies to date have reported diverse types of forensic investigations including poaching, illegal trade, detection of protected species within TCMs, livestock depredation, and illegal smuggling of animals (Table 1). Unlike in human forensic genetics where the predominant objective is the individualisation of a sample (e.g. for establishing the significance of a match obtained between an

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Table 1
Types of forensic investigations carried out to date.

Type of biological evidence	DNA marker	Type of investigation	Species	Reference
Animal				
Meat, skin	Southern blotting and hybridisation	Species identification (poaching)	Chinese water deer	Fang and Wan (2003)
Cooked meat, intestine, dried tissue from chopping board	mtDNA sequencing (cyt *b*, 472 bp)	Species identification (poaching)	Indian peafowl	Gupta et al. (2005)
Skin, blood	mtDNA sequencing (CR, 1079 bp)	Sub-species identification (poaching)	Chinese Sika deer subspecies	Wu et al. (2005)
Blood stains from knife, carcass	STRs	Individual identification, (poaching, animal cruelty)	Wild boar	Lorenzini (2005)
Tissue from dried fins	Nuclear ribosomal ITS2, 560 bp; cyt *b*, 511 bp)	Species identification (illegal trade)	Great white shark	Shivji et al. (2005)
Meat, hair	mtDNA sequencing (cyt *b*, 900 bp)	Species identification (poaching)	Roe deer	An et al. (2007)
Meat	Sexing (CHD-1, 230–280 bp)	Sex identification (poaching)	Pheasant	An et al. (2007)
Tusks	STRs	Determination of geographical origin of seized ivory (illegal trade)	African elephants	Wasser et al. (2007)
Hankos	STRs	Determination of geographical origin of seized hankos (illegal trade)	African elephants	Wasser et al. (2008)
Traditional east Asian medicine	mtDNA species-specific amplification (cyt *b*)	Species identification (identification in traditional East Asian medicine)	Tiger	Linacre and Tobe (2008)
Solid tissue	mtDNA sequencing (CR, 363 bp)	Species identification (contravention of CITES and the US Marine Mammal Protection Act)	Beaked whale (*Mesoplodon ginkgodens*)	Dalebout et al. (2008)
Bile crystals	mtDNA sequencing (cyt *b*, 175 bp)	Species identification (identification of in traditional East Asian medicine)	Asiatic black bear	Peppin et al. (2008)
Solid tissue, swabs, clothing, blood stained carpet	mtDNA sequencing (CR, 503 bp)	Species identification (mixed forensic samples)	Several mammal species	Fumagalli et al. (2009)
Meat	mtDNA sequencing (cyt *b* 774 bp), STRs	Species and individual identification (poaching)	Guanaco	Marín et al. (2009)
Hair, blood stain	STRs	Individual identification (illegal hunting)	Northern European brown bear	Eiken et al. (2009)
Meat (sashimi)	mtDNA sequencing (cyt *b*, 400 bp)	Species identification (illegal trade)	Whale species	Baker et al. (2010)
Muscle	mtDNA sequencing (cyt *b*, 300 bp; COI, 600 bp)	Species identification (food traceability/illegal trade)	Commercial fish products in Italy	Filonzi et al. (2010)
Crocodile skin handbag	mtDNA sequencing (COI, 645 bp)	Species identification	Crocodile	Eaton et al. (2010)
Blood	STRs, population assignment tests	Determination of geographical origin (illegal animal smuggling/hunting)	Chimpanzees	Ghobrial et al. (2010)
Teeth	STRs	Individual identification (illegal killing)	Wolf	Caniglia et al. (2010)
Claw and decomposed skin	mtDNA sequencing (CR), STRs	Individual identification (illegal killing)	Tiger	Gupta, Bhagavatula, et al. (2011)
Ivory idol	mtDNA sequencing (CR, 137 bp)	Species identification (illegal trade)	Asian elephant	Gupta, Thangaraj, et al. (2011)
Blood stains from scene of crime, carcass	STRs	Individual identification (poaching)	Sardinian mouflon	Lorenzini et al. (2011)
Embryonic tissue	mtDNA sequencing (12S, 230 bp; cyt *b*, 500 bp)	Species identification (illegal smuggling)	Parrots and cockatoos	Coghlan et al. (2011)
Meat, carcass	mtDNA sequencing (COI, 650 bp)	Species identification (poaching)	Reedbuck	Dalton and Kotze (2011)
Mites	STRs	Population assignment of *Sarcoptes* mites (illegal trade while infected)	Wildebeest	Alasaad et al. (2011)
Blood	STRs (mtDNA sequencing not useful due to past hybridisation events)	Species identification (illegal smuggling)	South American camelids	Di Rocco et al. (2011)
Solid tissue	STRs	Determination of geographical origin (illegal hunting)	Moose	Ball et al. (2011)
Meat	mtDNA sequencing (cyt *b*, 1070 bp)	Species identification (poaching)	Lowland tapir	Sanches et al. (2011)
Hair, tanned leather	mtDNA sequencing (COI, 708 bp; CR 279–744 bp; cyt *b*, 554 bp)	Species identification (establish wild/captive bear, illegal trade)	Asiatic black bear, suspected felid	Jun et al. (2011)
Blood, solid tissue	STRs	Individual identification (investigate intentional release on nature reserve)	Fox	Wesselink and Kuiper (2011)