DEVELOPMENT OF DNA ANALYSIS FOR FORENSIC ANIMAL INVESTIGATIONS

by

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Development of DNA Analysis for Forensic Animal Investigations

A research project submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

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Bachelor of Science
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Abstract

DEVELOPMENT OF DNA ANALYSIS FOR FORENSIC ANIMAL INVESTIGATIONS

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George Mason University, 2012

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DNA Analysis has been an important tool for forensic investigations, used to scientifically link an individual to a crime, and provide statistical relevance to the possibility that any other person could have contributed that biological evidence. This analysis has predominantly focused on human biological evidence, but animal-sourced evidence can provide equally compelling information to an investigation. Such analysis could be useful in cases that involve trace transfer of animal hairs (such as from a pet of the perpetrator onto a victim), cases that involve an animal directly (such as a animal cruelty cases), or cases that involve wildlife (such as the trade of endangered animal parts). Studies show that ~50% of US households contain a dog or cat, and that wildlife trade is estimated at $20 billion a year. However, animal DNA evidence has been used in only a small number of courtroom cases. This research seeks to outline the potential, limitations, and necessary development in research and technology to apply DNA analysis to animal evidence.

1. Introduction

Deoxyribonucleic acid (DNA) analysis is a specific analytical technique that has gained increasing importance in the field of forensic science since its development in the 1980's. The premise of DNA analysis is to distinguish the variations in genetic code from one individual to another in order to establish identity (or exclusion of an individual from an unknown evidentiary sample) (Butler, 2005). Application of DNA analysis in the field of forensic science began with and continues to focus on human identification, in order to link a suspect to a crime and successfully display clear and convincing evidence of guilt beyond a doubt in the court of law. However, the use of DNA analysis in application to animal based evidence has yet to become a widely used tool to assist forensic investigations (Halverson & Basten, 2005b). Approximately 50% of U.S. households own a dog or cat, and animal hair is a common finding at crime scenes that is often overlooked (Halverson & Basten, 2005a). Additionally, victims and offenders of crimes are not limited to the human arena. Animal cruelty cases and illegal wildlife trade investigations rely on animal identification at the root of proving a conviction (Tobe & Linacre, 2010). DNA evidence has been shown to be essential to human identification, and with continuing development of animal DNA analysis, animal-sourced evidence will provide increasing value for forensic investigations.

DNA analysis of human genetic markers is now commonly accepted as a relevant and reliable scientific method, qualifying to Daubert and Frye criteria in a legal context, but has been expanded and increasingly developed since the initial testing introduced by Alec Jeffries (Butler, 2005). This begs the question, what state of development has animal DNA testing reached for use in forensic science? If this type of testing is lacking in development, what limitations are impeding the advancement of this tool? If DNA testing is not being widely used for animal identification, can it successfully be seen as relevant and reliable methodology when it is applied to a forensic investigation to be used in a legal context? If not, what kind of studies will be needed to reach that goal? If there is sufficient scientific and technological capability to develop animal DNA testing, is it worth the investment by the forensic community to pursue that development? What does such advancement yield for forensic investigations? These questions must be addressed to assess the value of DNA testing of animal derived evidence, lest key information of evidentiary value be lost in negligence. It is the responsibility of the forensic community to explore and exhaust all avenues of investigation, and scientific development is at the forefront of expanding the extent of information that can be extracted from collected physical evidence.

1.1 Goals and Objectives

This research paper aims to underline the importance of DNA analysis in application to forensic investigations that rely on animal sourced physical evidence. In addition, the research goal includes defining limitations in current technology and analytical systems available for DNA experts, as the field of DNA analysis is heavily

oriented around human specific identification. The objective is to evaluate whether further development of animal specific DNA analysis is needed, if such development would provide significant value to the field of forensic science, and if such an approach is a feasible proposal. The objective also includes postulating future research that will be neccessary in order to expand animal DNA analysis, in addition to research required for qualifying this developed technology in a legal context. The importance of this research lies in the untapped potential for using DNA analysis of animal sourced evidence to contribute to forensic investigations.

Thorough assessment of the current status of animal DNA analysis is needed to establish what limitations are interfering with the utility of this type of evidence, and what development is needed to counter those limitations. The field of forensic science requires complete analysis of all physical evidence and information it may contribute to the facts of a case, and the field of DNA analysis has the potential to provide more clues to investigators for crimes involving animals based evidence. The ultimate significance of this research lies in the underlying issue that underdeveloped scientific techniques should not be a barrier to the evidentiary value of available physical evidence. Supposing this research shows that animal based evidence can be successfully utilized for animal identification, it is in the best interest of the forensic community to develop the science to the level that is able to fully appreciate the information that can be provided by such evidence. DNA analysis for human identification was expanded from simple blood typing to STR tandem repeats to obtain the level of information needed by triers of fact. In the same sense, DNA analysis for animal typing should be expanded appropriately to provide

investigators, juries, and judges with the full scope of available information to the fullest extent from the evidence at hand. This information can be applied to several types of cases, which will be considered throughout this research.

1.2 Trace Casework Analysis

In crimes that involve exchange of trace animal evidence, such as pet hairs from an offender to a victim, DNA can further be used as circumstantial evidence to build the case surrounding a crime. Such evidence can provide further information to the investigator and the triers of fact when direct evidence is not readily available.

1.3 Crimes that Involve Animals

In crimes that involve animals, such as animal attacks, DNA is able to provide direct evidence of the presence or exclusion of a specific animal. DNA analysis can also provide information about a perpetrator in animal cruelty cases, or link multiple cases that originate from a multifaceted crime ring (such as dog fighting).

1.3 Wildlife Investigation

In wildlife investigations, such as poaching for bushmeat or animal parts for traditional Chinese medicine, the criminal nature of the act can only be proven by identification of the animal species to indicate its protected status.

2. History of Animal DNA Typing

Animal DNA typing began in the 1970's with agricultural applications to registering cattle and horses. This typing started with blood grouping, due to its simplicity and cost effectiveness. However, blood groupings did not provide enough differentiation for dog breeds, and the Irish Coursing Club became the first canine breed registry to adopt DNA typing using restriction fragment length polymorphism (RFLP) with the probes developed by Alec Jeffreys (Halverson & Basten, 2005a). In the 1980's, human DNA research began to examine tandem repeat sequences in the genetic code for use as unique identifiers in forensic applications. It was noted that all eukaryotic organism demonstrate the same type of repeat regions within their DNA, with similar variation and inheritance patterns as humans (Cassidy & Gonzalez, 2005). This indicated the same potential for forensic identifications by using DNA typing for animals, using the same science that had been invested in human DNA analysis. By the 1990's microsatellite DNA markers were introduced in human genomics for typing, and similar genetic regions were found in cattle, horse, and canine genomes (Halverson & Basten, 2005a). While initial concerns arose that inbreeding of domestic animal species would limit the genetic variability in the microsatellite regions, testing showed this method held higher discrimination power, leaving blood typing behind (Halverson & Basten, 2005a). Advancement of animal typing continued to adopt technologies in human typing

throughout the 1990's, as short tandem repeats (STR) and multiplex kits became the mainstream method of testing.

2.1 Canine and Feline Typing

By 1994, Zoogen Inc. began developing the first canine multiplex for STR tetranucleotide markers, combining 10 loci in one typing kit (see table 1). A similar canine STR kit was developed by Applied Biosystems, and tested extensively from 1998 through 2001 by the American Kennel Club on over 9,500 dogs from 107 breeds (DeNise et al., 2004). Applied Biosystems also developed an STR typing kit for felines in 2002. The MeowPlex multiplex kit seeks to replicate the 13 CODIS loci used to database humans, by selecting key loci that could be uniformly adopted for typing felines, while conveniently using technology already in place at most forensic laboratories (Butler, David, O'Brien, & Menotti-Raymond, 2002).

Mitochondrial DNA testing has been developed for dogs and cats by sequencing the D loop control region; however, this typing is limited to distinguishing the inclusion or exclusion of an animal from a reference sample versus individual identification. This is due to lower haplotype variation (see figure 1 and 2), in which there are less haplotypes and more common frequency of certain types amongst each of these domestic pet species, compared to human typing (Halverson & Basten, 2005a). This information remains useful in compilation with other evidence, when STR analysis is not an option.

Table 1. Loci Included in the Stockmarks Canine Typing Kit. This table details the two multiplex tests used in combination to test 17 loci for canine DNA. Reproduced from Halverson & Basten, 2005b, 4.

Stockmarks	Locus	Forward Primer	Reverse Primer	Repeat Motif	*Size Range (bp)		†Map Location	Reference
Canine I	$CATA_1$	GG CTG TCA CTT TTC CCT TTC	CAC CAC AAT CTC TCT CAT AAA TAC	CATA	95	136	‡ cfa 7	17
Canine I	PEZ03	CA CTT CTC ATA CCC AGA CTC	CAA TAT GTC AAC TAT ACT TC	AAG	95	154	cfa 19	16
Canine I	PEZ05	GC TAT CTT GTT TCC CAC AGC	GTC ACT GTA TAC AAC ATT GTC	AAAG	97	121	cfa 12	16
Canine I	PEZ06	AT GAG CAC TGG GTG TTA TAC	ACA CAA TTG CAT TGT CAA AC	AAAT	166	215	cfa 27	16
Canine I	PEZ08	TA TCG ACT TTA TCA CTG TGG	ATG GAG CCT CAT GTC TCA TC	AAAT	230	260	cfa 17	16
Canine I	PEZ12	GT AGA TTA GAT CTC AGG CAG	GTA GGT CCT GGT AGG GTG TGG	AAAG	250	317	cfa 3	16
Canine I	PEZ20	CC TAA ATT AGA GGT CTA ACC	GTA AGC GGG AAT GTG CTC CTC	AAAT	152	202	§Unmapped	16
Canine I	FHC2010	AA ATG GAA CAG TTG AGC ATG C	CCC CTT ACA GCT TCA TTT TCC	ATGA	220	248	cfa 24	3
Canine I	FHC2054	GC CTT ATT CAT TGC AGT TAG GG	ATG CTG AGT TTT GAA CTT TCC C	GATA	140	184	cfa 12	3
Canine I	FHC2079	CA GCC GAG CAC ATG GTT T	ATT GAT TCT GAT ATG CCC AGC	GGAT	263	299	cfa 24	3
Canine II	PEZ10	CT TCA TTG AAG TAT CTA TCC	CCT GCC TTT GTA AAT GTA AG	AAAG	230	330	cfa 14	16
Canine II	PEZ11	AT TCT CTG CCT CTC CCT TTG	GTG TGG ATA ATC TCT TCT GTC	AAAG	123	175	cfa 8	16
Canine II	PEZ13	AG TCT GGT GAT TTA ATT CGG	GTC TAG TCC CCA GTC TAG TTC ACT GCC C	AAAG	171	322	cfa 4	16
Canine II	PEZ15	CT GGG GCT TAA CTC CAA GTT C	CAG TAC AGA GTC TGC TTA TC	AAAG	193	284	‖Unassigned	16
Canine II	PEZ16	GC TCT TTG TAA AAT GAC CTG	GTG GGA ATC GTC CTA AAA CCC	AAAG	263	334	cfa 27	16
Canine II	PEZ17	CT AAG GGA CTG AAC TTC TCC	GTG GAA CCT GCT TAA GAT TC	AAAG	196	245	cfa 4	16
Canine II	PEZ21	AA CCG GTT GTG ATT TCT GGG	GTC TGT GTC ATT AGT GAC ATC	AAAT	71	109	Unmapped	16

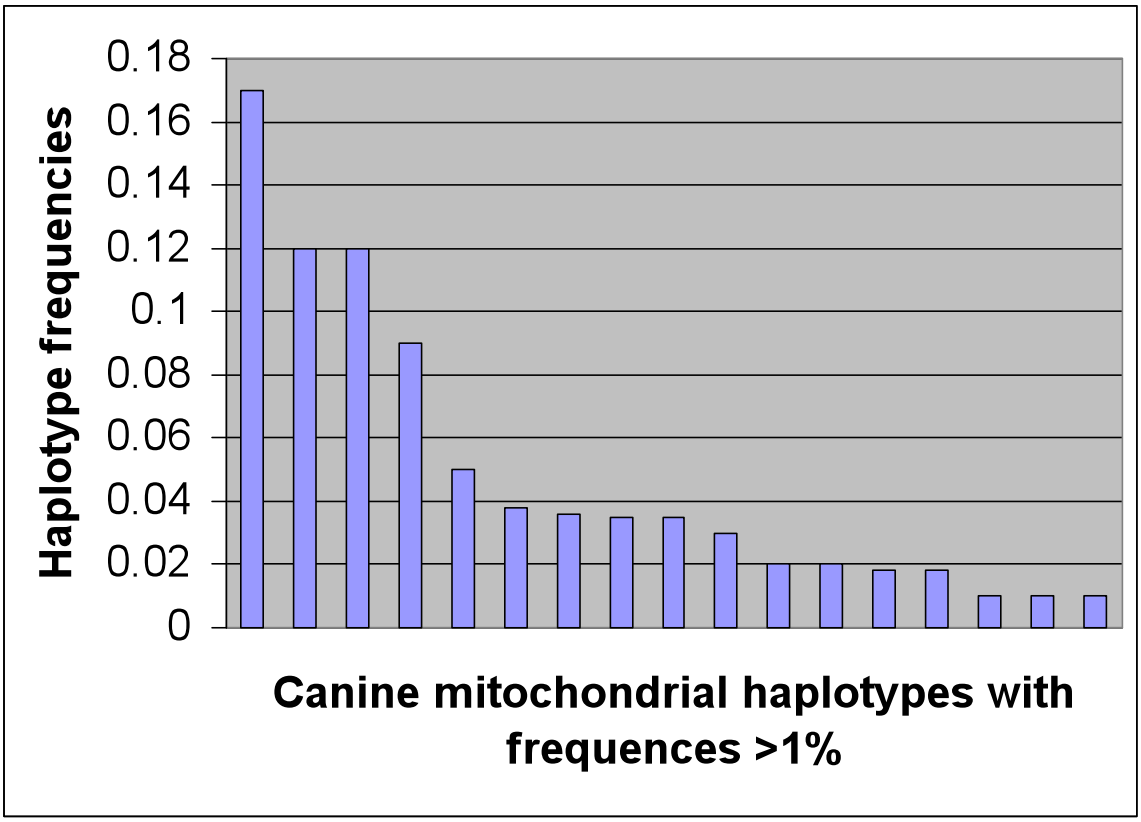

Figure 1. Haplotype Frequencies of Canines. Data from 348 dogs; this study shows that canines display higher frequency within 3 to 4 major haplotypes, which provides little distinguishing information, unless the canine belongs to one of the more rare and unique haplotypes. Reproduced from Halverson & Basten, 2005a, 600-601.

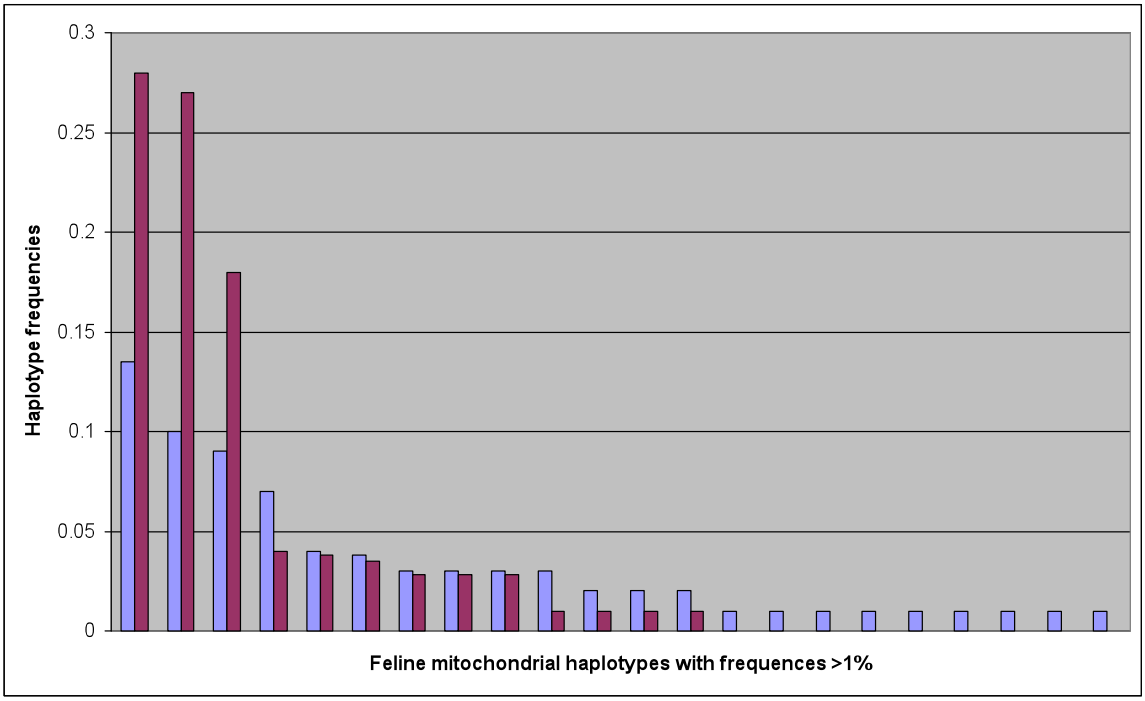

Figure 2. Haplotype Frequences of Felines. Data from 167 cats; this study shows that felines display higher frequency within 3 to 4 major haplotypes, which provides little distinguishing information, unless an animal belongs to one of the more rare and unique haplotypes. Feline DNA varies in the mitochondrial DNA due to a 80 bp tandem repeat region, which is accounted for in the darker bars, and not considered in the lighter bars. Reproduced from Halverson & Basten, 2005a, 600-601.

2.2 Wildlife Identification

Today, most wildlife species identification testing uses mitochondrial DNA sequencing, as this method works well with degraded and processed tissue (Alacs, Georges, FitzSimmons, & Robertson, 2010). The most commonly analyzed areas of the

mitochondria are cytochrome B and cytochrome oxidase 1. Species are identified based on sequence variations of the nucleotides at these genes (Alacs et al., 2010).

3. Practical Forensic Applications

3.1 Trace Casework Analysis

The use of animal hairs, blood, or other trace evidence transferred by Locard's theory of exchange can be tested and compared to show linkage between a suspect, crime scene and victim. In these cases, the presence of traces of a particular animal provides circumstantial evidence that the individual accused was present at the scene of the crime when direct evidence (such as DNA of the suspect) is not available. While extensive research has been performed on fiber transfer to use fiber analysis as a linkage basis in expert testimony, less focus has been offered specifically on animal hair transfer. A study in 1998 by D'Andrea, Fridez, & Coquoz showed that hair transfer is almost unavoidable when an animal is present. This was true even in conditions where an apartment was recently cleaned, in which a victim had only brief exposure to an animal but did not live with or own an animal, and in cases of a short haired animal that was not known to be a heavy shedder (see table 2). The authors noted that most hairs were secondary hairs that are not distinguishable by morphological comparison, which suggests DNA analysis may be the only way to link the hairs to a particular animal. The study also notes that these secondary hairs often have poor root quality, which may not yield enough nuclear DNA for STR testing, indicating that mitochondrial DNA testing would be the predominant testing method applicable to transfer cases (D'Andrea, Fridez, & Coquoz, 1998).

Table 2. Results of Animal Hair Transfer Study. The table shows that in all situations tested, some animal hairs were transferred, while the number of hairs found decreased in the time passed from the event to the collection of evidence. Reproduced from D'Andrea, Fridez, & Coquoz, 1998, 1258.

Type of Offense	Animal Living on Premises	Sampling Carried Out	Number of Hairs found
Burglary	1 Angora cat	Immediately after burglary	311 cat
	1 European cat		101 dog
	1 Poodle dog		
Burglary	1 European-Siamese cat	Immediately after burglary	24 cat
Burglary	1 English Setter dog	Immediately after burglary	300 dog
Burglary	1 English Setter dog	1 hour after burglary	179 dog
Burglary	1 English Setter dog	Only on shoes, 4 hours after burglary	26 dog
Burglary	2 half Angora-European cats	Immediately after burglary	610 cat
Burglary	2 half Angora-European cats	Only on shoes, 4 hours after burglary	109 cat
Assault	1 English Setter dog	Immediately after assault	12 dog
Assault	2 half Angora-European cats	Immediately after assault	255 cat

3.1.1 Case Study: Snowball the Cat

One of the first cases to successfully use pet hair transfer to implicate a suspect is that of a white American shorthair cat (Mennotti-Raymond, David, & O'Brien, 1997). The victim, Shirley Duguay, was murdered and buried in the woods. A man's jacket which was stained by the victim's blood was found at the scene near the body. The jacket also contained white cat hairs. The suspect in the murder investigation lived at home with his parents and their pet cat, Snowball. The cat hairs from Snowball were shown to match one of the 27 hairs found on the jacket, using 10 STR loci for DNA comparison (see table 3). This was compared to a database of 19 cats from the area of the crime, in addition to 9

cats from throughout the U.S. to estimate frequency of the data. As a result, the defendant was convicted, which introduced the use of STR typing of feline DNA into the US legal system.

Table 3. Comparison of Snowball to evidence. The STR match window shows the acceptable allele size difference within which an allele is considered a match. Snowball (the pet cat of the suspect's parents) was a match at all loci to the evidentiary samples from the jacket found at the crime scene. Reproduced from Menotti-Raymond, M.A., David, V.A., & O'Brien, S.J., 1997, 774.

STR Locus	**Allele Size (bp)**		**Allele size difference (bp)**	**STR match window (bp)**
	Snowball	Evidence		
FCA 026	147.83, 143.73	148.11, 143.73	0.28, 0.0	0.37
FCA 043	126.38, 120.52	126.29, 120.50	0.09, 0.02	0.59
FCA 080	259.27, 253.39	259.20, 253.14	0.07, 0.25	0.3
FCA 088A	121.91, 110.50	121.91, 110.50	0.0, 0.0	0.42
FCA 126	143.41, 141,08	143.41, 141.08	0.0, 0.0	0.53
FCA 132	152.73, 150.69	152.73, 150.69	0.0, 0.0	0.27
FCA 149	132.02, 128.05	131.80, 128.07	0.22, 0.02	0.29
FCA 058	229.03	229.24	0.21	0.36
FCA 090	93.54	93.54	0.0	0.46
FCA 096	210.95	211	0.05	0.25

3.1.2 Case Study: State of California v. David Westerfield

The first case to allow canine mitochondrial DNA was the *State of California v. David Westerfield* in 2002. In this case, a seven year-old girl was abducted, murdered,

and her body was found two weeks later. Dog hairs were found in a neighbor's motor home, a quilt, and the dryer lint trap, which were consistent with the family dog (Questgen Forensics, 2011). Mitochondrial DNA analysis was used as evidence, and Westerfield was convicted for the murder.

3.1.3 Crown v. Daniel McGowan

In the case of *Crown v. Daniel McGowan,* the suspect was the owner of a van believed to be used in a case of abduction. Dog hairs from the victim's clothes were compared the suspect's dog. The testing successfully used the Stockmarcks Canine typing kit to compare the evidentiary sample to the reference sample. Testing showed that the animal hairs on the victim did originate from the suspects dog, by transfer to the victim when he was transported in the vehicle (Halverson & Basten, 2005b). The comparison electropherogram showing the consistency can be seen in Figure 3.

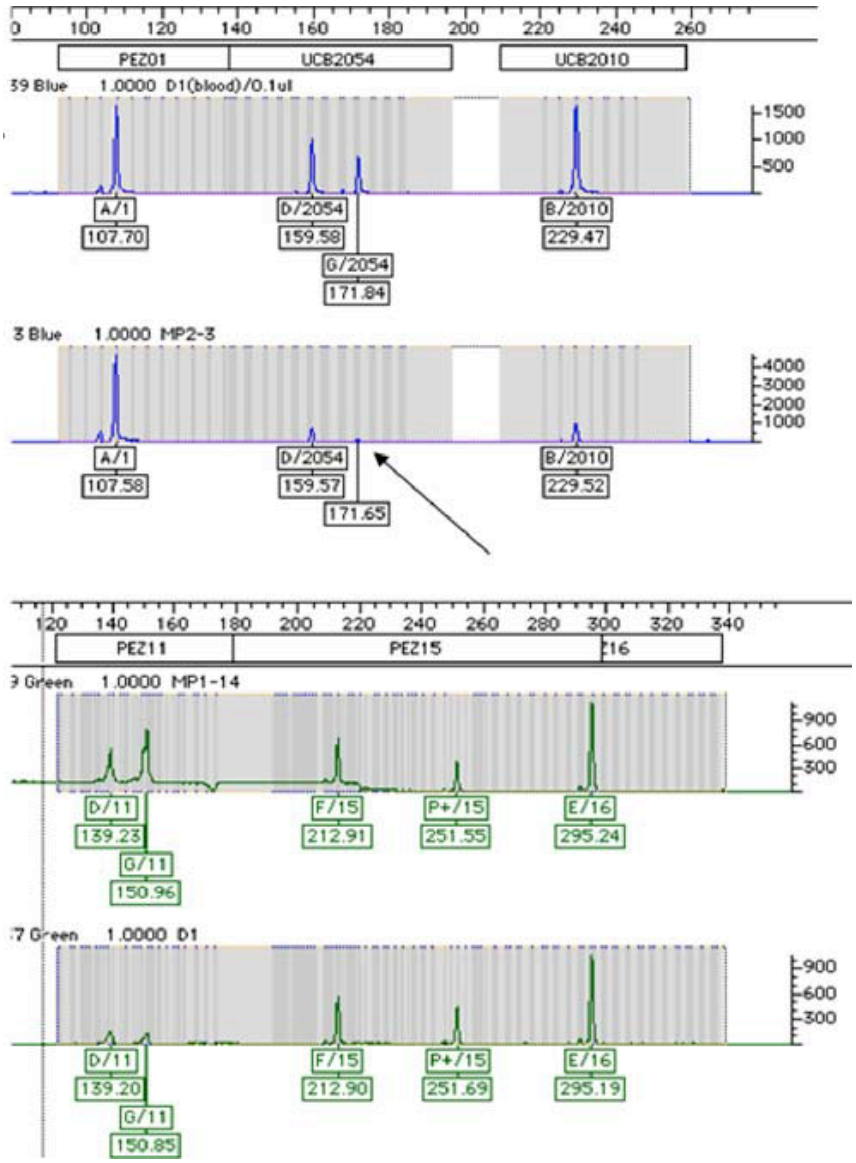

Figure 3. Electropherogram of Dog Hair Comparison. The top row of alleles in each dye lane represents the reference samples from Duchess, the suspect's dog. The bottom row represents the evidentiary sample. Reprinted from Halverson & Basten, 2005b, 11.

3.1.4 Transfer Exclusions

In addition to linking a suspect or victim by trace transfer, there lies additional value in DNA testing to exclude suspects. Savolainen & Lundeberg analyzed a case in which two female victims were found at separate crime scenes, with several dog hairs found either on the bodies or on items at the crime scene (1999). After mitochondrial typing of the hairs, both scenes displayed the same fairly unique variant of canine DNA. Reference hairs were collected from dogs of seven suspects, and all suspects were excluded from the crimes on the basis that their dogs did not match the rare mitochondrial type. The study by Savolainen & Lundeberg looked at another case in which dog hairs were found in the vehicle of a suspect, that were believed to belong to the family dog of a missing female whose body or location had not been found. After typing the hairs in the vehicle compared to the female's dog, the hairs were found to come from two different dogs, and the suspect was excluded (Savolainen & Lundeberg, 1999).

3.2 Animal Centered Investigations

In some cases, an animal may be the offender in an attack on a human victim, or cause further mutilation to a body left at an otherwise human created crime scene. In these cases, identification of the animal is necessary to guide investigators from misinterpreting injuries as part of a violent crime, and for remediation (typically euthanasia) of the correct animal. It is also common for animals to be the victims of a criminal act. Animal cruelty cases require an adequate level of proof to convict an

offender of abuse or neglect, and organized crimes such as dog fighting rings require an investigator to link abuse to multiple possible perpetrators (ASPCA, 2009).

3.2.1 Animal Attacks

Estimates of dog bite wounds range from 3.5 to 4.7 million incidents per year in the U.S. alone (Eichmann, Berger, Reinhold, Lutz, & Parson, 2004). Bite wounds are the predominant injury inflicted, with a majority of attacks occurring on children ages 5 to 9 (Eichmann et al., 2004). Clarke & Vandenberg (2010) were able to use dried saliva from the shirt of a victim of a dog attack to test for the suspected animal. A tape lift from the shirt was compared to a reference sample, and a full canine DNA profile showed a significant likelihood that it was 10 billion times more likely that the suspect dog versus a random dog was the source of the saliva (Clarke &Vandenberg, 2010). As a result, the court attributed costs to the owner, and the dog was put down (Clarke &Vandenberg, 2010). The study by Eichmann et al. showed that obtaining a full or partial DNA profile from animal inflicted wounds is possible, with the indication that blood soaked bandages can be used successfully for typing the attacking canine (see table 4). In addition to testing hair and blood evidence, STR typing of saliva around dog bite wounds has been shown to be successful for canine identification (Eichmann et al., 2004).

Table 4. Dog Bite Case Study Success Rates. The table indicate class B++ for materials heavily soaked in blood, B+ for materials moderately soaked in blood, and B- for materials showing no blood. Of the 52 samples analyzed, fully soaked swabs and bandages yielded full to partial profiles, indicating typing can be performed on canine inflicted injuries with capability to identify the attacking animal. Reproduced from Eichmann et al., 2004, 341.

Collected Material	Class	Full Profiles n (%)	Partial Profiles n (%)	No results n (%)
Swab	B++	13 (31.7%)	2 (4.9%	8 (19.5%)
Bandage	B++	14 (34.1%)	2 (4.9%)	8 (19.5%)
Swab	B+	1 (2.4%)	1 (2.4%)	10 (24.4%)
Bandage	B+	1 (2.4%)	1 (2.4%)	9 (22.0%)
Swab	B-	0	0	6 (14.6%)
Bandage	B-	0	0	6 (14.6%)

3.2.2 Animal Inflicted Artifacts

A unique case showed that availability of molecular testing can be vital to show the animal inflicted nature of injuries. In this case, an 11 year-old boy was anally penetrated by a German sheperd (Weigand, Schmidt, & Kleiber, 1999). DNA testing of the underpants of the child compared to a reference sample collected from the dog showed that the sperm present did originate from the dog (see figure 4). In this case, two males were suspected of abusing the child, and the genetic testing was vital to proving their innocence. The child came forward several weeks later and admitted to manually stimulating the dog, which led to the attack (Weigand et al., 1999). Without genetic testing, these men may have been falsely implicated in sexual abuse of the boy. Similarly,

genetic testing can show animal inflicted injuries on human remains, which may be mistaken for injuries inflicted by the perpetrator of a murder.

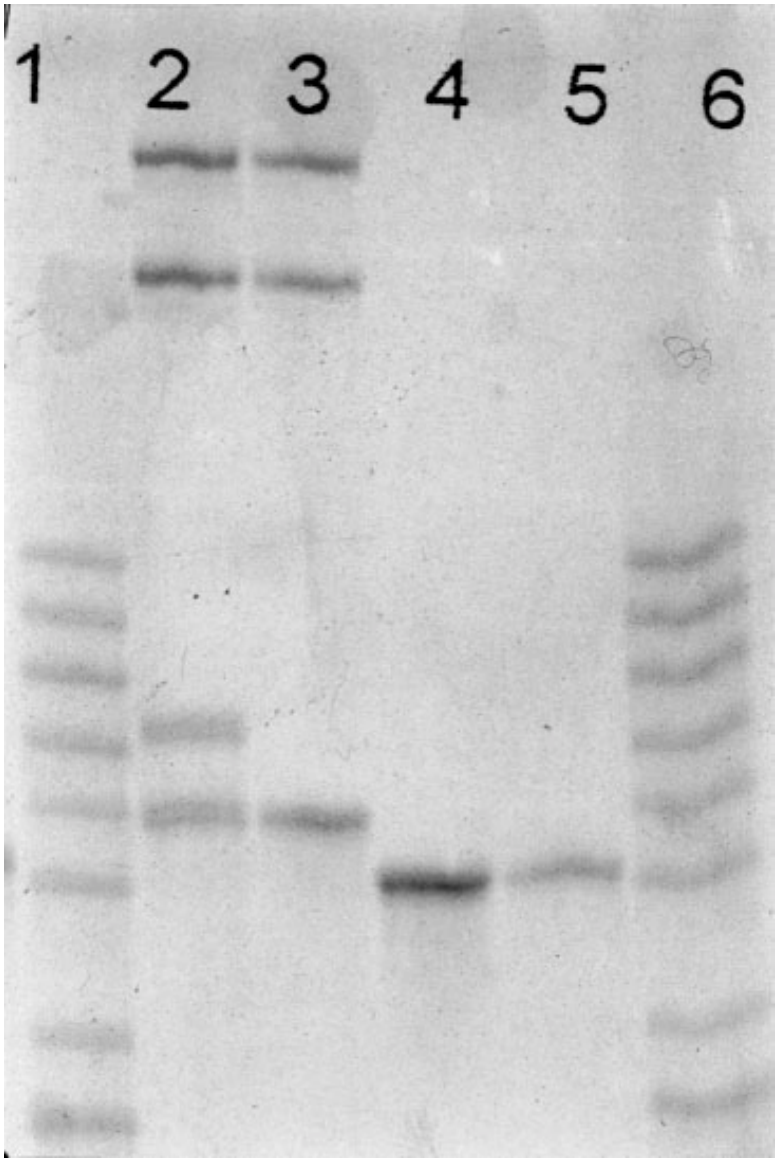

Figure 4. Fragment separation of DNA in German Sheperd case. Lane 4 contains the dog ejaculate DNA, while Lane 5 contains the differential extraction of the sperm component of a sample taken from the boy's underpants. Reprinted from Weigand, Schmidt, & Kleiber, 1999, 325.

3.2.3 Animal Cruelty Cases

Animals are not only centered in investigations as attackers; animal cruelty cases involve human offenders with an animal central as the victim. These cases must be proven through physical evidence, as the victims are a silent witness. Such cases include dog or cock fighting, puppy mills, or individual maltreatment of animals.

The development of Canine CODIS began in 2010, as a joint operation between the American Society for the Prevention of Cruelty to Animals (ASPCA), the Missouri Humane Society, the Louisiana SPCA, and the UC Davis Veterinary Genetics Laboratory. The system is used to collect and type canine samples from animals seized from dog fighting raids in order to compile a genetic database to exchange information in dog fighting investigations (ASPCA, 2011). Blood can be searched against the database found at new sites suspected of dog fighting, and familial mapping assists with linking breeders and fighting rings. The database was developed out of an eight-state raid, which resulted in the seizure of 407 dogs (yielding over 100 puppies after their rescue) as the largest federal dog fighting case in U.S. history (ASPCA, 2009). The ASPCA has also worked with the federal Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) after dog fighting was found in conjunction with a drug and weapons raid in Halifax, Virginia. Animals seized in this raid were also added to the Canine CODIS system, and multiple individuals were convicted in association with dog fighting (ASPCA, 2011).

3.2.4 Exclusion of Animals

A study by Brauner, Reshef and Gorski showed the exclusionary value of DNA testing for animal attacks (2001). A large unidentified dog attacked a six year-old girl,

resulting in severe injuries, including bite wounds to her arms and scalp, and a broken arm. After the perpetrating animal ran away from the scene, a dog in the area was identified based on eyewitness description of the attack. The owners maintained that their dog was not involved, but their dog was impounded for investigation. Hairs transferred during the attack were collected from the victim, and after DNA testing, hairs from the suspected dog did not match the hairs found on the victim. In addition, microscopic amounts of blood found on the dog's coat did not match blood from the victim. As a result, the owners did not face any legal implications to the dog attack, and the dog itself was not put down for the attack.

3.3 Wildlife Protection

Illegal wildlife activity falls into several categories, mainly consisting of poaching or hunting out of season otherwise legally hunted species, trade of live animals for the exotic pet trade, and trade of animal parts of endangered regulated species. Elephant ivory, rhino horn, animal pelts, and bear bile are among some of the most commonly traded items bearing high financial return, as ounce for ounce these items are often more valuable than gold (Linacre & Tobe, 2011). Trade of these items may be in the form of powders, oils, or sculptures that are unidentifiable by expert morphological or microscopic analysis, since the processing of the sample disfigures the natural form. A further complication to these investigations is that identification is key to prosecution, as only specific species are protected under the Endangered Species Act and the Convention for the International Trade of Endangered Species of Flora and Fauna (CITES). Mitochondrial DNA is mainly used for species identification, however many different

loci have been used in identification studies with little standardization across the field (Linacre & Tobe, 2011).

3.3.1 Animal Trade- Rhinoceros Horn

Rhinoceros horn is a product that is illegally traded, predominantly in parts of Asia, as sculptures or powdered into medicines (Hsieh et al., 2003). Linacre et al., (2004) used mitochondrial DNA to successfully identify rhinoceros in various products. Previous studies used the entire cytochrome B region to test for species identification, but this large fragment size did not always provide a result for the degraded DNA in such processed samples (Hsieh et al., 2003). Linacre's testing used a smaller 402 base pair region of cytochrome b in the mitochondrial DNA. The method was able to determine the presence of rhinoceros horn in powders, sculptures, daggers, and mixed powder precriptions (Linacre et al., 2004). The method successfully determined the species identification, even when rhino horn was mixed as a minor component in different ratios with another species (Hsieh et al., 2003). Overall, this shows that DNA testing can be extremely useful for species identification of rhinoceros, which is significant as all four species of rhinoceros are at an endangered status. Similar applications to other species can provide the species identification needed to enforce illegal trade laws and help protect the dwindling numbers of such endangered species.

3.3.2 Animal Trade- Ivory

Wozney & Wilson state that, "the ivory trade is recognized as the single-most important cause of the decline in elephant populations worldwide" (2012, pg 2). This trend is increasing, as 25,000kg of ivory were seized between 2005 and 2006, which

amounted to more than what had been seized in the prior three years combined (Wozney & Wilson, 2012). Tusks are removed from the individual elephant, and hairs and leathers are often dyed making a visual identification difficult to impossible. While genetic sequencing has been used to identify such samples, Wozney & Wilson propose an alternative that is more cost effective, and can identify the presence of low levels of DNA in degraded processed material (2012). Their study showed success using real-time PCR to quickly and cost effectively test for the presence of elephant or wooly mammoth DNA (Wozney & Wilson, 2012). This method could be effective for samples that would not provide quality sequence results, and as a result could not be used to make a species identification (Wozney & Wilson, 2012). The main benefit is the quick testing procedure, in addition to the low cost, which makes the method ideal for forensic laboratories.

3.3.2.1 Tracing Ivory Origins

Wasser et al. (2008) conducted a study to test if DNA could be used to track the origin of the collected contraband samples of elephant ivory. The goal of this study was to assist wildlife investigators to prevent poaching at the source where most animals are hunted. This attempts to deter the illegal activity at its onset, in addition to proving it has occurred once the contraband is found. Wasser et al. estimates that only 10% of illegally traded animal parts are intercepted, which indicates a need to find methods to prevent poaching proactively (2008). This study showed it was possible to use 16 microsatellite loci to type ivory samples collected in two massive seizures (the first seizure consisted of 6.5 tons of ivory seized in Singapore in 2002 and the second seizure consisted of 33.9 tons seized in Hong Kong in 2006). The samples were processed and compared to

reference data from elephant populations to distinguish if the ivory came from savannah or forest elephants (see figure 5). DNA analysis was also able to match ivory seized in Hong Kong to residual chips found in cargo later found in Cameroon, thus linking the operating trade network. This information can be utilized by wildlife investigators to target law enforcement in poaching "hot spots" (Alacs et al., 2010).

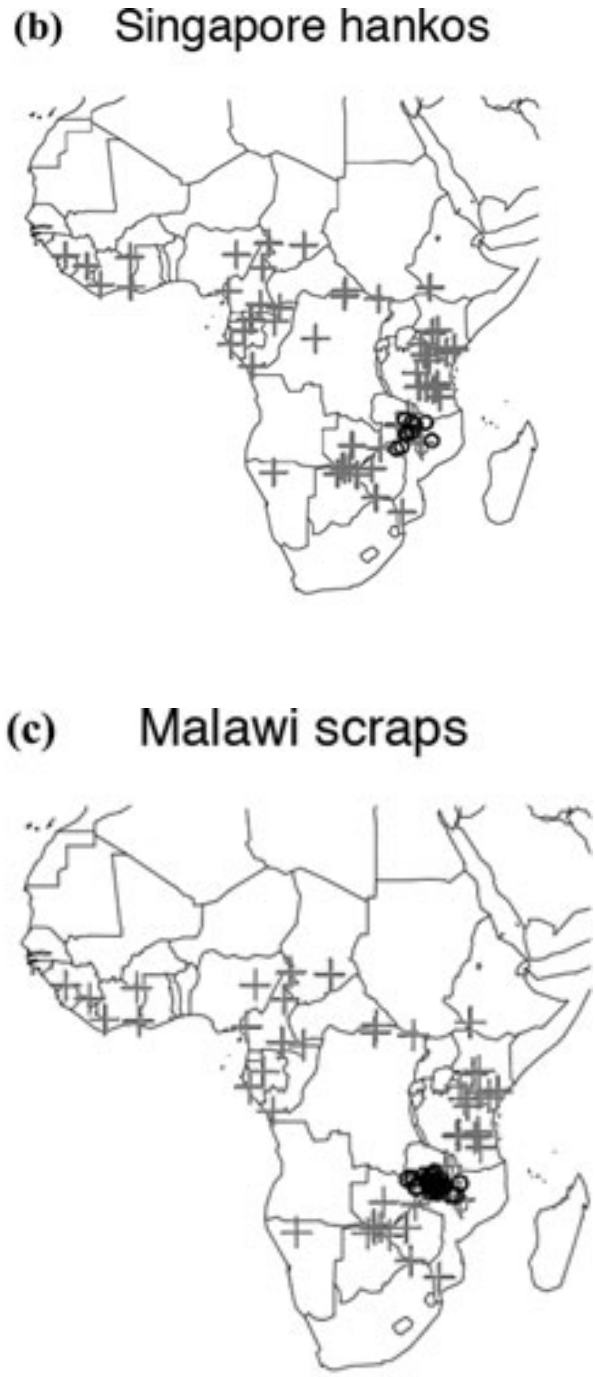

Figure 5. Geographic Assignment of Ivory Samples from Illegal Trade Seizures. By comparing DNA profiles of unknown samples to reference data of elephant populations,

location of the ivory source was successfully assigned for three types of seized ivory. Crosses show the reference locations for assignment of tusks origin. Reprinted from Wasser et al., 2008, 1068.

3.3.3 Animal Consumption

Illegal wildlife trade also includes the consumption of wild game, which has increased to an international trade of bushmeat and fisheries estimated at over $60 billion per year, resulting in the overexploitation and decline of some species (Eaton et al., 2010). This unregulated trade also has human health implications from transfer of zoonotic diseases, in addition to the impact on wildlife (Eaton et al., 2010). There is the additional issue of false labeling of consumer products, used as an attempt to avoid prosecution for illegal harvesting in commercial trade. Poaching remains difficult to prosecute, as physical evidence must be obtained to show that the meat or animal part has actually originated from the protected species. In many cases there may also be extenuating circumstances, such as certain hunting seasons, permitted hunting of a particular sex, or permit allowances for hunting of a certain number of that species (An, Lee, Min, Lee, & Lee, 2007).

3.3.3.1 Case Study: Korea Hunting Management Association

In one exemplary case, a wildlife guard in the Korea Hunting Management Association found roe deer skin, which is a protected species. Law enforcement was able to find a medical prescription at the scene, which lead to the home of the suspect (An et al., 2007). Frozen meat packets were found, but the suspect claimed they were beef and

male pheasants (both which are legal meat sources). However, female pheasant and roe deer are both illegal to hunt, so all collected meats were tested. In addition, several animal hairs from the suspect's car were collected and submitted for testing. Without molecular testing, the sex of the pheasants would have been unidentifiable, as they were all naked and had their organs removed (An et al., 2007). The testing showed that 2 out of 5 of the pheasants were female, and that some of the meat was also from roe deer. As a result, the individual was imprisoned for 8 months, put on probation for 2 years, and was sentenced to 80 hours of community service (An et al., 2007). Without the genetic testing, law enforcement would have had no way to identify the sex of the birds, or the identity of the packaged meats, and this individual would not have been prosecuted.

5. Obstacles Regarding Animal DNA Testing

5.1 Statistics

A significant issue in the use of animal DNA for forensic investigations lies in the use of statistics to indicate the relevance of a typed profile. In human DNA testimony, the comparison of the evidence to the suspect is often provided as a likelihood ratio (the likelihood that the profile did originate from that individual) or as a random match probability (probability of exclusion) (Cassidy & Gonzalez, 2005). However, all humans belong to one species that has been widely researched with compiled database information to provide mathematical relevance to such calculations. In animal applications, the population used to create a database for a species will demonstrate specific allele frequencies. However, animals within the same species may not all derive from the same population, and small or isolated populations (such as endangered species) may display different genetic variation (Cassidy & Gonzalez, 2005). In addition, breeding within small populations such as puppy mills for dog fighting, or captive breeding of endangered animals, can impact the standard assumptions of inheritance used in statistical calculations (Cassidy & Gonzalez, 2005). Human population statistics are based on Hardy-Weinberg equilibria, which is the assumption that alleles are inherited independently (Halverson & Basten, 2005a). Closed breeding populations demonstrate linkage disequilibria, which is a phenomenon in which the species forms a population

substructure in which alleles are not randomly inherited (Halverson & Basten, 2005a). These obstacles require further development of databases with increased population data to better assess inheritance patterns for a particular species, and a more conservative inbreeding coefficient in statistical calculations to account for inbreeding within the closed population. Cassidy & Gonzalez (2005) also recommend not using STR loci that show frequent disequilibria for animal DNA typing, since the linked inheritance of multiple genes causes a lower power of discrimination.

5.2 Species Diversity

Another difference in animal DNA analysis from human analysis is that there are numerous species that may be require testing, some of which may be closely related. Development of primer sequences for each individual species can be costly and requires extensive research, which may not be possible for every specific animal in an individual case (Cassidy & Gonzalez, 2005). Each species may also display variation in the mutation rate for their genetic code, which may cause insertion or deletion of nucleotides and impact the results of an STR typing test (Cassidy & Gonzalez, 2005). The human mutation rates are known and accounted for in statistical calculations, and the same research must be conducted for other species to assess the impact on match probabilities. In human testing, scientists use allelic ladders of commonly occurring alleles to compare the data from a particular sample in order to correctly assign alleles to the individual sample. However, there is no such allelic ladder developed for animal DNA markers, which significantly impacts the repeatability and reliability of allelic assignments made by current animal testing methods (Cassidy & Gonzalez, 2005). This creates difficulty in

comparing results between laboratories, and there is no established standard for allele designations when it comes to animal markers. Development of ladders is costly and technologically demanding, which is part of the hindrance in developing such a tool (Cassidy & Gonzalez, 2005).

5.3 Extraction Methods

Animal DNA typing faces a fundamental problem in the ability to extract the DNA from collected evidence in order to conduct the biological analysis. Often the materials collected as evidence are processed products, such as taxidermy trophy animals, tanned hides, cured meats, or animal parts that have been processed for medicinal use (Cassidy & Gonzalez, 2005). Most extraction methods currently used are intended for tissue samples, blood, or epithelial cells commonly collected in human investigations. Extraction methods will need to be improved and modified to account for the variation in animal sourced evidence, and to improve the purification of possible inhibitors introduced by the processing or manufacturing of such evidence. For example, a study by Eaton et al. (2010) showed that DNA could be successfully typed for multiple old tissue and blood samples stored for over 20 years, but only 1 out of 5 leathered crocodile skins could be identified.

5.4 Genetic Complications

There are some limitations in the basic science behind the mitochondrial DNA sequencing used for species identification. Mitochondrial DNA can show heteroplasmy, which is the phenomena in which one individual displays two or more mitochondrial profiles in different cells throughout the body (Alacs et al., 2010). This phenomena is

well known in human DNA typing, and with detection of such heteroplasmy, this can actually be an asset as it would increase the uniqueness of the individual animal's genetic profile, as long as appropriate measures to detect the heteroplasmy have been researched. Limitations also exist in nuclear DNA typing. A phenomena known as pseudogenes occur when part of the mitochondrial DNA sequence is inserted into the nucleus (Alacs et al., 2010). This insertion can result in change to mutation rates and how the mitochondrial DNA is amplified, which can change how an analyst interprets the data. However, Alacs et al. (2010) points out that these pseudogenes can be detected by sequencing the entire mitochondrial DNA genome, and similar to heteroplasmy, the pseudogenes can provide additional uniqueness to an individual's genetic profile. Alacs et al. also points out that a combination of mitochondrial and nuclear DNA should be used for testing, since mitochondrial DNA may not show recent evolutionary variations, which could distinguish recently evolved species (2010).

Species level identification faces another challenge in the need to expand phylogenetic studies which track the evolutionary ancestry of each species (Alacs et al., 2010). Scientists will need to use a combination of conserved genes that do not mutate at a high rate over time to show early evolutionary taxonomic branching, but also incorporate highly mutating genes to show individualization of a recent species or individual animal (Alacs et al., 2010). The high mutating genes also face the issue of homoplasy, which occurs when mutations revert the gene back to its original state, or when the same mutation occurs in different species lineages (Alacs et al., 2010). These

relationships must be fully studied and understood in order to select the best combination of which genes to incorporate into testing for wildlife profiles.

6. Legal Implications

The widespread applicability of DNA analysis to animal sourced materials indicates the potential for such materials to be presented as forensic evidence. Much of the testing methods applied to animal sourced evidence are derived from human testing. As a result, animal DNA testing draws on the acceptance of human DNA testing as an indication that the methods are acceptable when applied to animal based evidence. Frye standards initially required scientific testimony to be based upon methods that are peer reviewed with general acceptance in the relevant scientific community. Daubert v. Merrell Dow Pharmaceuticals, Inc. established criteria that scientific expert testimony must be relevant, reliable, and based upon use and application of sound scientific methods. This case also called for investigation of potential error rates of a particular method. Rule 702 of the Federal Rules of evidence expands that such methods and principles must be soundly applied to the facts of the case. While human DNA testing has met these requirements (Cassidy & Gonzalez, 2005), it is important to assess whether this acceptance should transfer to animal DNA typing, or if the similarity to human typing methods can suffice as prior acceptance.

6.1 Case Study: Washington v. Tuilefano and Lealuaialii

The case of Washington v. Tuilefano and Lealuaialii demonstrates the pitfalls of relying on such an assumption of acceptance. In this case, gang members murdered a

couple during a home invasion, and shot the couple's dog at close range when he attacked (Questgen, 2011). The suspects were found with blood spatter on their shoes and clothes, and DNA testing of 10 loci did not exclude the dog, and provided a likelihood ratio of $4.8x10^9$ that the blood did derive from that individual canine. (Questgen, 2011). However, this evidence was appealed in 2003, based on a lack of peer review publications on canine STR markers. Since that time, studies have been published specifically on canine markers in response to the demand for peer-reviewed literature on canine specific markers (Halverson & Basten, 2005a). As a result, by 2005, similar canine marker data was accepted during Frye hearings in, "9 assault and homicide trials nationwide and Great Britain" (Halverson & Basten, 2005b, pg 3). This indicates that animal based testing can be accepted, and that novel publications for other species specific testing may be needed to gain more widespread acceptance beyond canine and feline testing.

6.2 Plea Bargains

Cassidy and Gonzalez state that, "To date, relatively few criminal cases involving animal DNA evidence have actually gone to trial in US courts" (2005, pg 1459). They go on to explain that most cases in which the offense is against the animal (for example, illegal hunting or animal cruelty cases) do not carry a harsh enough punishment to result in a trial, and instead most perpetrators accept the fines or other minor punishment associated with a plea. In addition, budgetary restrictions have prevented the processing of animal evidence, unless such evidence is found in more severe cases such as murder investigations or federal offenses (Cassidy & Gonzalez, 2005).

7. Current and Future Research

7.1 Domestic Species

Forensic DNA development for animal based evidence has focused heavily on domestic species. Entire genomes are being sequenced for canine and feline species, to assist research on genome mapping and inheritance (Cassidy & Gonzalez, 2005). Registry databases for compiling profiles have been developed, and have been used for parentage analysis to provide statistical relevance for forensic identifications (Cassidy & Gonzalez, 2005). This data is being used to identify nuclear DNA markers for wildlife testing, that has previously been limited to mainly mitochondrial DNA testing (Alacs et al., 2010). Future research should be conducted to compile more standardized databases for individual species, since no official database is utilized across all forensic laboratories. This would require further investigation into optimal gene locations for testing within each species, to provide a set panel of core loci, such as those used in CODIS for human data banking. This would facilitate the application of commercial multiplex kits containing the selected loci, which would standardize the testing procedures and methods validated for use in forensic cases across all laboratories. Ideally these kits would be tested and reviewed by an agency such as the Scientific Working Group on DNA Analysis Methods (SWGDAM) before widespread use and acceptance.

7.2 DNA Barcoding

One solution to standardizing animal species identification has been the generation of one set primer sequence to test a universal "barcode" across all species (Eaton et al., 2010). A universal sequencing region would be compared to a database of compiled sequence data across multiple species that would be globally available to the forensic community (Eaton et al., 2010). This would be ideal for standardizing testing between laboratories, and for establishing acceptability of animal DNA typing in a legal context. However, the study by Eaton et al. also showed difficulty in obtaining complete amplification of such a sequence for degraded samples, such as samples over 15 years old, or leathered animal skins. The study suggests using multiple barcode regions instead of 1 set region to work around this limitation, and to combine these "mini-barcodes" with the larger universal barcode region (Eaton et al., 2010). Alacs et al. adds that,

> "DNA can be readily extracted from highly processed and degraded products commonly encountered in wildlife trade markets such as cooked and dried meats, claws left on tanned hides, dried shark fins, egg shells, animal hairs, bone, ivory, rhinoceros horns, turtle shells, feathers, and fish scales" (2010, pg 181).

Future research should investigate both improved extraction methods, and mini-barcodes, to see which method yields most information (or if some combination thereof is most beneficial).

The Barcode of Life Data System (BOLD) is an online consortium that combines barcode identification of specimen into a universal database, which is openly accessible for contributions and use in identifications (Ratnasingham & Hebert, 2007). Overall,

Eaton et al. describes the barcoding method as low cost and successful method to differentiate species that could be used by wildlife investigators and conservationists to positively identify meats, eggs, and products of harvested animals (2010). This system is an example of the efforts to standardize genetic typing in the scientific community, which will ease the transition of such technologies to the forensic community. If the barcoding method can be successfully developed to differentiate between species, validation studies will need to occur within the forensic field before adopting the method directly into casework analysis. There may also be an opportunity to facilitate the Barcode of Life database into a CODIS-like format, to allow for searching of evidence against the species population data. This would enable to forensic community to utilize previously acquired data, saving limited time and money.

7.3 Pyrosequencing

Pyrosequencing is a novel DNA sequencing technology which uses, "a series of enzymatic reactions to detect visible light emitted during the synthesis of DNA and enables more rapid screening of samples compared to conventional sequencing methods" (Alacs et al. 2010, pg 183). This technology can sequence short regions of the mitochondrial DNA, to determine the variations in sequence between species (see figure 6). This method has been used to develop a mammalian assay, which could be used by wildlife investigators as a screening assay for species identification prior to conducting individualizing tests on an animal (Karlsson & Holmlund, 2007). Karlsson & Holmlund chose to validate this method because of its, "accuracy, flexibility, parallel processing and that it can be easily automated" (2007, pg 16). Their assay uses the 12S and 16S

ribosomal RNA genes, which showed high variation among mammals (Karlsson & Holmlund, 2007). However, analysis of this pyrosequencing data will require development of reference data to compare results of unidentified samples. The method also will require more research into possible sequence mutations within these gene regions for species tested, since most data currently collected is for human mutation rates (Karlsson & Holmlund, 2007).

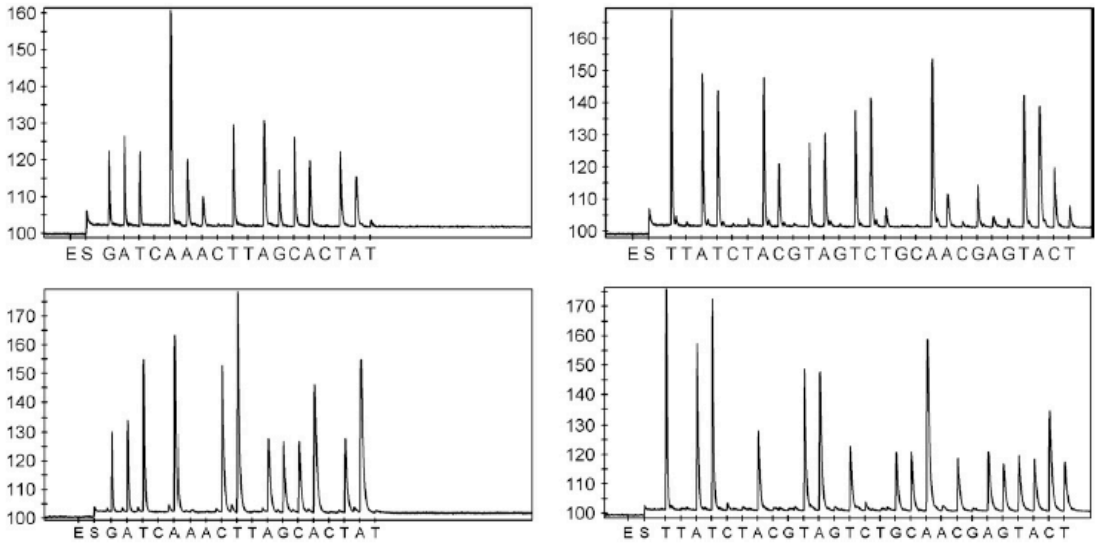

Figure 6. Pyrograms of the 12s and 16s rRNA Regions. The top panel displays pig data compared to the bottom panel of human data, developed by the pyrosequencing technique. Reproduced from Karlsson & Holmlund, 2007, 18.

7.4 PCR-RFLP

An alternative to direct sequencing is discussed by Alacs et al. The PCR-RFLP

method uses traditional polymerase chain reaction (see figure 7), which amplifies the DNA, essentially making hundreds of thousands of copies of the region of interest targeted by specific markers for a given sequence. This is coupled with restriction fragment length polymorphisms (RFLP), as restriction enzymes are used to detect base pair differences within the gene that has been amplified. These enzymes will cut the DNA into fragments at specific sequences. The number and size of the fragments generated will provide the inferred profile based on the base pair composition of that animal at each gene. The banding pattern of these fragments will be unique for each species, and the correct restriction enzymes, as well as which genes to amplify will need to be studied to determine which show most variability between species (Alacs et al., 2010). This method is cheaper than direct sequencing, and uses capillary electrophoresis technology already employed by many forensic laboratories (Alacs et al., 2010).

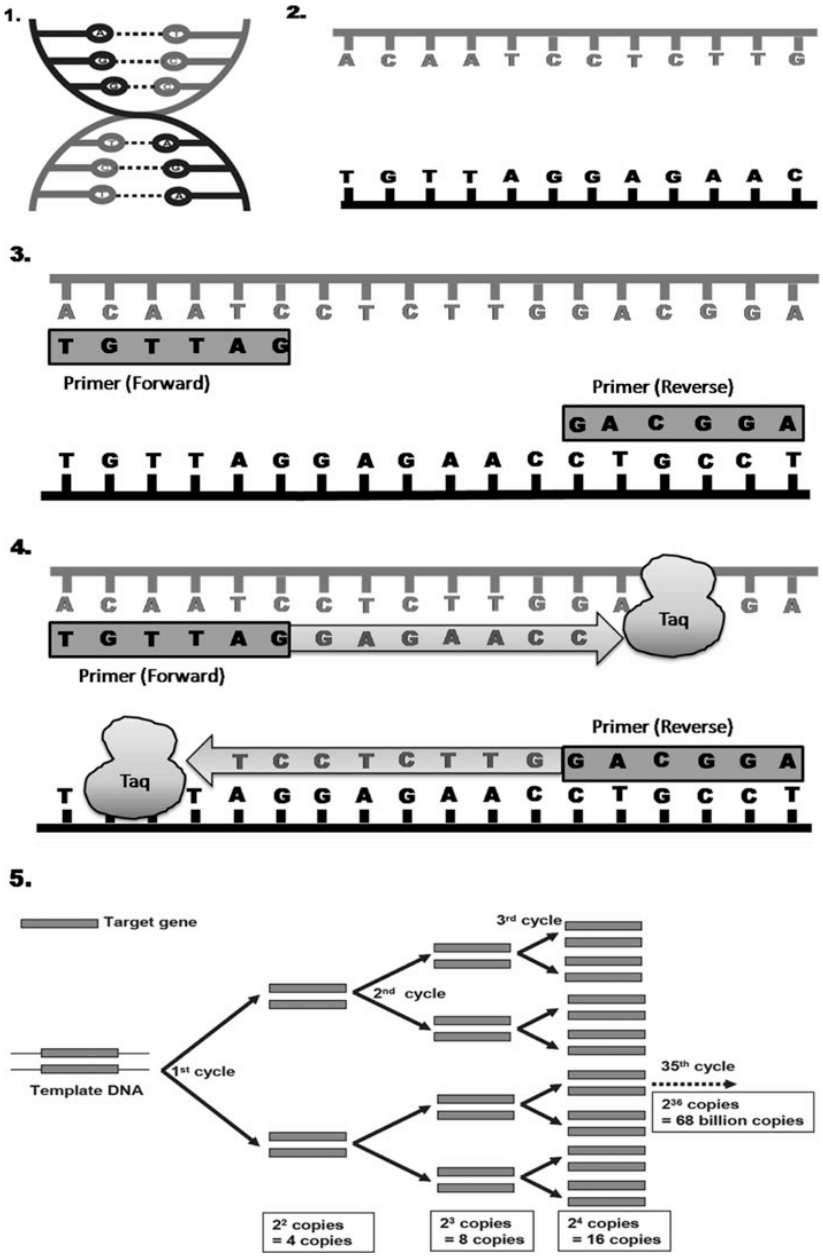

Figure 7. Polymerase Chain Reaction. The diagram shows the process of PCR. In steps 1-3, double stranded DNA is separated into two separate template strands. Targeting primers bind with a segment of each strand, and the Taq enzyme extends the primer sequence. Each cycle doubles the amount of DNA present, creating billions of copies of the DNA as the end product. Reprinted from Alacs et al., 2010, 183.

7.5 AFLP

Amplified Fragment Length Polymorphism (AFLP) is a profiling method, which is a reverse process of PCR-RFLP. In this method, restriction enzymes first cut the DNA into fragments at commonly occurring sites. A portion of the fragments are then amplified with PCR, by using primer sequences which target the regions of interest. This method is ideal because it requires little prior knowledge of the sample origin, but is limited to high quality samples that may not be available when samples are in a trace volume or degraded state (Alacs et al., 2010). This method has been studied and developed into a database format by Hong & Chuah (2003). Their study indicates that this method is, "cheap, easy, and fast, it is a very robust and reliable technique," making it desirable for forensic applications (2003, pg 2). The method has been used for detecting variations between plants, fungi, animals, and bacteria (Hong & Chuah, 2003). This application can also be used to generate more species-specific primer sequences in order to create appropriate STR marker kits for a given species (Alacs et al., 2010).

7.6 SNPs

Single nucleotide polymorphism (SNP) is an alternative variation that can be tested for species identification. This type of profiling is optimal for mixtures, which is highly applicable to traditional medicines that may contain trace amounts of several species (Tobe & Linacre, 2010). This could also apply to animal attacks, in which investigators would expect a mixture of human and animal DNA (Tobe & Linacre, 2010). This typing method can incorporate the universal primer sequences being pursued for wildlife barcoding, and can also include species-specific markers as a screening test. The

added benefit is that multiple SNP regions can be combined into a multiplex to test for several species at once. However, this is also a limitation, since any species that is not specifically included with a species-specific primer set in the multiplex will not be detected, which may not be predictable for samples of unknown origin (Tobe & Linacre, 2010).

7.7 Future Directions

As animal DNA genetic studies continue to be developed, new technologies will assist forensic applications for animal DNA profiling. For example, entire genome sequencing is on the verge of being rapid and affordable, which is ideal for forensic applications. Increased studies on individual species will increase the awareness of the genetic variations unique to that species, and assist in the development of specific primer sequences and primer kits for identification. Establishment of universal barcode regions plays a key role in unifying tested genetic regions, which could increase consistency for forensic comparisons. Overall, research is massively expanding the capabilities to generate identifying information on a species and individual level. The challenge for the forensic community will be to select which technologies and methods are most applicable to legal investigations (see table 5), and to create synergy between the scientific and criminal justice communities in order to cater those applications to forensic testing.

Table 5. Comparison of Animal DNA Typing Methods. The study assesses many of the emerging technologies for animal DNA typing, and constructively outlines the advantages and disadvantages of each, must be weighed in selecting ideal technologies to apply to forensic casework. Reproduced from Alacs et al., 2010, 182.

	Species ID	Regional ID	Population ID	Individual ID	Parentage	Limitations for forensics	Advantages for forensics	Applications to generate baseline genetic data
Mitochondrial gene (mtDNA) sequencing	√√	√√	√	×	√√ maternity	Heteoplasmy	Suitable for trace and degraded DNA	Phylogenetics
					× paternity	Nuclear paralogs	Universal primers available	Phylogeography
						Maternal inheritance		Population genetics
						Single linked genome hence effectively is one single marker		
Nuclear gene (nDNA) sequencing	√√	×	×	×	×	Not suitable for trace or degraded DNA	Recommended for use in combination with mtDNA for species	Phylogenetics
						Universal primers not available for most species	Identification	
							Can detect hybrid individuals	
Pyrosequencing	√√	√ not assessed	√√ for SNP genotyping	√√ for SNP genotyping	√√ for SNP genotyping	Only short fragments of 10 to 500 bp can be sequenced	Enables very rapid high throughput genotyping of short fragments or SNPs	Population genetics for SNP genotyping
Amplified fragment length polymorphism (AFLP)	√	√	√	√	√	Dominant marker, therefore less informative for all applications	No prior genetic knowledge of the organism required	Phylogenetics, phylogeography population genetics. Limited use because of their dominance
						Not suitable for trace or degraded DNA		
Species-specific priming	√√	×	×	×	×	Knowledge of species boundaries required	Rapid screening once developed	None
							Cost effective	
Short tandem repeat (STR). Also called simple tandem repeat (SSR) or microsatellite	×	√√	√√	√√	√√	Allelic dropout can occur when trace or degraded DNA is used	Highly informative marker for many applications	Most commonly used marker for population genetics because of its high information content
						Development time is substantial	Techniques have already been validated for human forensics	
Single nucleotide polymorphism	√√	√√	√	√	√	Approx five times more loci required compared to STRs	Highly reproducible	Use of this marker for phylogenetics, phylogeography and population genetics is still in its infancy
						Currently not available for many species	Rapid screening of samples	

√√ is highly informative, √ informative, × not informative

This research left some question unanswered that will require further research before animal typing can be fully implemented in the forensic arena. For example, domestic species testing has been shown to be relevant, reliable, and accepted in a legal context, but wildlife testing may not be seen in the same perspective, depending on the type of testing utilized. Validation studies are needed to approve any novel methods that are currently being researched, such as barcoding or entire genome sequencing. Cost assessments will need to compare various methods before adopting a widespread adoption of any given technique. For example, some methods can utilize current equipment contained in most labs, while other methods may be more successful in obtaining a DNA profile, but at the expense of requiring entirely new protocols and lab settings. Assessments need to encompass the methods currently in use by forensic laboratories that already conduct animal DNA testing, to ensure there is a compatibility or transition plan for synchronizing consistent and repeatable typing results between labs. Forensic working groups and advisory councils will also need to include structured inclusion of animal DNA typing in their recommendations, along with accreditation standards for labs that choose to implement such techniques.

8. Conclusions

8.1 Discussion

DNA analysis has been a fundamental development in the field of forensic science that has improved the ability of investigators, juries, and judges to understand biological evidence. This research shows that animal-specific DNA is present at crime scenes, and actually plays a central role in a significant portion of crimes at a global level. Animal hairs or tissues may be transferred at the scene of a crime, demonstrating Locard's theory of exchange, thus linking the suspect, victim, and crime scene. A specific animal may be central to an investigation, as either the victim of human neglect or cruelty, or in an attack on a human victim. Entire species of animals are involved in expansive crime organizations, which thrive on the black market in trade of exotic and endangered animal parts. The potential for use of DNA evidence in these crimes has yet to be realized, as the true extent of wildlife crime is immeasurable, but estimated to be a $20 billion dollar a year industry (Alacs et al., 2010). While the extent of animal hairs left at crime scenes is unknown, the sheer number of pets in the U.S. alone shows the potential for such analysis, if crime scene investigators are trained to seek and submit this evidence.

The use of animal DNA evidence has been limited in the legal system for multiple reasons. Crimes directly involving animals offer lesser punishments, and are often settled

out of court with plea bargains. Initial cases that use animal DNA evidence were not accepted by the courts, since this type of testing had not been established to meet all criteria necessary of scientific testimony (although strides have been made to alleviate this limitation). Overall, the use of animal DNA has increased in acceptance, and will continue to grow as typing methods and database information become standardized.

The most limiting factor of animal DNA analysis for a forensic application is the lack of commercial kits for species and individual animal identifications that can form a standard across laboratories. Human DNA typing relies heavily on such multiplex panels, in addition to universally accepted core loci for comparison between individuals to establish a solid foundation of uniformity and consistency between cases. Animal DNA testing is still at a phase of extensive research and development, and determining the best loci and methods of genetic typing that can appropriately display variation between species.

Animal DNA experts will also face the need to educate lawyers, judges, and juries on animal DNA testing, as well as the differences between human and animal DNA profiling. There are many genetic differences that come with testing multiple species, which leaves potential for misinterpretation of testing results if the limitations and requirements of animal testing is not understood. In addition, experts should strive for continuing efforts to develop mainstream databases of population data in order to allow statistical interpretation of results that is based upon thorough and extensively compiled data.

8.2 Impact

8.2.1 Domestic Species Investigations

81.7 million cats are estimated to be present in 37.5 million U.S. households, while the American Kennel Club estimates that U.S. households average 1.7 dogs, and the American Pet Product Manufacturing Association estimates that 59.5% of Americans own at least 1 dog or cat (Tarditi, Grahn, Evans, Kurushima, & Lyons, 2011, and Himmelberger et al., 2008). These numbers indicate that pet hairs should be common and expected findings at crime scenes that may hold valuable information, yet only about 20 investigations used this type of evidence from 1996 to 2008 (Himmelberger et al., 2008). Increased development of standardized animal DNA typing methods will enable laboratory testing of physical evidence that has otherwise been ignored in forensic investigations. Development of a universal database for compiling canine and feline geographic subtypes will better enable DNA evidence to hold statistical significance in terms of what a "match" implies in a specific case for courtroom testimony (Tarditi, et al., 2011). Overall, this data demonstrates enhanced capacity law enforcement can expect for investigations involving trace transfer of pet hairs, in addition to cases that involve animals directly.

8.2.2 Wildlife Investigations

Significant potential lies in wildlife investigations, which regulate the legal trade of wildlife, as well as protection of endangered or protected species. Illegal hunting, smuggling, and breeding of animals is an estimated $20 billion a year industry, of which

less than 10% of activity is estimated to be intercepted (Wasser et al., 2008). This level of crime ranks just under drug trafficking, human trafficking, and arms trafficking, and trends indicate that the level of activity has increased over time. Prevention of illegal wildlife trade is notably significant for environmental protection of endangered species to maintain biodiversity and ecological stability of keystone species. In addition, there are public health risks from unregulated transfer of species across national borders, which may contain zoological pathogens that can be released in their new environment. Finally, disbanding of organized crime rings not only serves the aforementioned purposes, but also provides a lasting impact and overall reduction in future illegal wildlife trade, as well as possible links to other criminal activities. For example, estimates in Brazil show that 40% of drug trafficking shipments are also associated with wildlife trade (Alacs et al., 2010). Alacs et al. also reports that in 1993, one third of cocaine seizures in the U.S. were associated with wildlife imports (2010).

8.2.3 Impact of Research

This research has shown through thorough extensive literature review that animal DNA testing is a powerful and underutilized tool for forensic investigations. The limitations and obstacles to current testing methods have been assessed, and current and future research aiming to overcome these limitations has been reviewed. Recommendations have been made for improving such research to address the needs of the forensic community, with the assumption that forensic laboratories will eventually implement such techniques. The greatest impact of this research is the indication that animal sourced evidence has been, and will continue to be, a valuable information source

in forensic investigations. Furthermore, this research shows that DNA analysis is the best scientific approach to interpreting the information provided by animal-sourced evidence. The potential impact for both domestic and wildlife species investigations provides ample support for the need to continue developing animal DNA testing techniques. Development of forensic animal DNA typing will benefit the criminal justice system in solving crimes that incorporate animal based evidence.

References

References

Alacs, E.A., Georges. A., FitzSimmons, N.N., & Robertson, J. (2010). DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science, Medicine, and Pathology (6)* 180-194.

An, J., Lee, M.-Y., Min, M.-S., Lee, M.-H., & Lee, H. (2007). A molecular genetic approach for species identification of mammals and sex determination of birds in a forensic case of poaching from South Korea. *Forensic Science International, 167,* 59-61.

ASPCA. (2009). Eight-state dog fighting raid- Missouri, Illinois, Iowa, Texas, Oklahoma, Arkansas Nebraska, and Mississippi. Retrieved from http://www.aspca.org/fight-animal-cruelty/aspca-in-action/eight-state-dog-fighting-raid.aspx.

ASPCA. (2011). Halifax County, Virginia- April 2011. Retrieved from http://www.aspca.org/Fight-Animal-Cruelty/aspca-in-action/halifax-county-virginia.

Brauner P, Reshef A, & Gorski A. (2001). DNA profiling of trace evidence—mitigating evidence in a dog biting case. *Journal of Forensic Sciences, 46(5),* 1232-1234.

Butler, J.M., David, V.A., O'Brien, S.J., & Menotti-Raymond, M. (2002). The MeowPlex: A new DNA test using tetranucleotide STR markers for the domestic cat. *Profiles in DNA, 5 (2),* 7-10.

Butler, J.M. (2005). *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers* (2nd Edition). New York: Elsevier Academic Press.

Cassidy, B.G., & Gonzalez, R.A. (2005). DNA testing in animal forensics. *Journal of Wildlife Management, 69(4),* 1454-1462.

Clarke, M., & Vandenberg N. (2010). Dog attack: the application of canine DNA profiling in forensic casework. *Forensic Science, Medicine, & Pathology, 6,* 151-157.

D'Andrea F., Fridez F., & Coquoz R. (1998). Preliminary experiments on the transfer of animal hair during simulated criminal behavior. *Journal of Forensic Sciences*, *43 (6),* 1257-1258.

DeNise, S., Johnston, E., Halverson, J., Marshall, K., Rosenfeld, D., McKenna, S., Sharp, T., & Edwards, J. (2004). Power of exclusion for parentage verification and probability of match for identity in American kennel club breeds using 17 canine microsatellite markers. *Animal Genetics, 35,* 14-17.

Eaton, M.J., Meyers, G.L., Kolokotronis, S.-O., Leslie, M.S., Martin, A.P., & Amato, G. (2010). Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates. *Conservation Genetics, 11,* 1389-1404.

Eichmann, C., Berger, B., Reinhold, M., Lutz, M., & Parson, W. (2004). Canine-specific STR typing of saliva traces on dog bite wounds. *International Journal of Legal Medicine, 118,* 337-342.

Halverson, J.L., & Basten, C. (2005a). Forensic DNA identification of animal-derived trace evidence: Tools for linking victims and suspects. *Croatian Medical Journal, 46,* 598-605.

Halverson, J., & Basten, C. (2005b). A PCR multiplex and database for forensic identification of dogs. *Journal of Forensic Sciences, 50 (2),* 1-12.

Himmelberger, A.L., Spear, T.F., Satkoski, J.A., George, D.A., Garnica, W.T., Malladi, V.S., Smith, D.G., Webb, K.M., Allard, M.W., & Kanthaswamy, S. (2008). Forensic utility of the mitochondrial hyerpvariable region 1 of domestic dogs, in conjunction with breed and geographic information. *Journal of Forensic Sciences, 53 (1),* 81-89.

Hong, Y., & Chuah, A. (2003). A format for databasing and comparison of AFLP fingerprint profiles. *BMC Bioinformatics, 4 (7),* 1-9.

Hsieh, H.-M., Huang, L.-H., Tsai, L.-C., Kuo, Y.-C., Meng, H.-H., Linacre, A., & Lee, J.C.-I. (2003). Species identification of rhinoceros horns using the cytochrome b gene. *Forensic Science International, 136,* 1-11.

Karlsson A.O., & Holmlund G. (2007). Identification of mammal species using species-specific DNA pyrosequencing. *Forensic Science International, 173*, 16-20.

Linacre, A., & Tobe, S.S. (2011). An overview to the investigative approach to species testing in wildlife forensic science. *Investigative Genetics, 2 (2),* 1-9.

Linacre, A., Hsieh, H.-M., Huang, L.-H., Tsai, L.-C., Kuo, Y.-C., Meng, H.-H., & Lee, J.C.-I. (2004). Identification of rhino from 'medicinal' powders and sculptures. *International Congress Series, 1261*, 595.

Menotti-Raymond, M., David, V.A., & O'Brien, S.J. (1997). Pet cat hair implicates murder suspect. *Nature, 386*, 774.

Questgen Forensics. (2011). Casework: 1998-2005. 1-8. Retrieved from http://www.zoogendna.com/uploads/casework98-09.pdf.

Ratnasingham, S., & Hebert, P.D.N. (2007). BOLD: The barcode of life data system (www.barcodinglife.org). *Molecular Ecology Notes, 7,* 355-364.

Savolainen P., & Lundeberg J. (1999). Forensic evidence based on mtDNA from dog and wolf hairs. *Journal of Forensic Sciences, 44(1),* 77 – 81.

Tarditi, C.R., Grahn, R.A., Evans, J.J., Kurushima, J.D., & Lyons, L.A. (2011). Mitochondrial DNA sequencing of cat hair: An informative forensic tool. *Journal of Forensic Sciences, 56 (S1),* S36-S46.

Tobe, S.S., & Linacre, A. (2010). DNA typing in wildlife crime: recent developments in species identification. *Forensic Science, Medicine, and Pathology, 6,* 195-206.

Wasser, S.K., Clark, W.J., Drori, O., Kisamo, E.S., Mailand, C., Mutayoba, B., & Stephens, M. (2008). Combating the illegal trade in African elephant ivory with DNA forensics. *Conservation Biology, 22 (4),* 1065-1071.

Wiegand, P., Schmidt, V., & Kleiber, M. (1999). German sheperd dog is suspected of sexually abusing a child. *International Journal of Legal Medicine, 112,* 324-325.

Wozney, K.M., & Wilson, P.J. (2012). Real-time PCR detection and quantification of elephantid DNA: Species identification for highly processed samples associated with ivory trade. *Forensic Science International, in press*, 1-7.

Curriculum Vitae

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