served. The ubiquitous presence of a shoulder peak at d= 1.9, at $47^0$ (corresponding to the 312 hkl lattice plane) is observed for antler material but is absent in ivory. The twin peak of 211 and 112 in ivory samples are well-separated giving rise to a plateau, however, the same is indistinguishable in case of antler. The 210 peaks are more prominent in antler samples than in ivory. The background line between the peaks 213 and 004/411 is concave upward in case of antler, whereas, for ivory it is like a descending slope.

In addition to this, there exist differences in terms of other parameters like crystallinity, crystallite size, state of strain and cell volume parameters that may be useful in distinguishing antler, ivory and bone materials of different species. A fake Rhino horn was also tested and it was found that it matches with the hydroxy-apatite pattern. The value of the calculated crystallite size was smaller for ivory as compared to antler. Upon plotting the angle versus relative intensity, the highest peak could be used to differentiate between all hydroxy apatite and non-hydroxy apatite minerals. Scatter plot between maximum and minimum background intensity could differentiate among ivory, rhino horn and antler. Cell parameter and cell volume of ivory and antler were useful for distinguishing these two items. The 'a' cell parameter of ivory ranged from 9.45-9.53 whereas for antler it ranged from 11.62-11.85. Crystallite cell volumes of ivory were smaller in comparison to antler.

Thus, by using this technique, which is non-destructive and requires small sample amounts, it is possible to differentiate between wildlife trade items and lead for proper implementation of Indian wildlife (protection) Act and CITES.

**Keywords**: Horn characterization, X-Ray Diffraction, Wildlife protection

**WIL-FP-07**

**The Wildlife Forensics DNA Facility: A new Initiative of Wildlife Institute of India**

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Mandates of the Wildlife Forensics Cell of Wildlife Institute of India are to standardize techniques for identification of species from various parts and products of different species and provide support to enforcement agencies for implementation of the *Indian Wildlife (Protection)* act 1972. As part of a five year WII-USFWS project a major emphasis has been on identification of species based on morphometry techniques and four manuals are in the process of finalization viz. "Identification of species from hair", "Characterization of species from canines", "Identification of species from claws and beaks" and "Tibetan antelope - Trade and Wildlife forensic techniques for identifying Shahtoosh hair".

One of the major problems for dealing with tissue samples sent to us, was that most of the samples were not properly preserved and it was not possible to use normal electrophoretic techniques such as IEF. Therefore, a manual was prepared on protocols for tissue sample preservation, viz. "A field guide for collecting tissue samples for Wildlife Forensics analysis." Dealing with pending tissue cases (n= 129), it has been decided to establish a Wildlife Forensics DNA facility to standardize techniques for identifying species from various animal parts. Pending cases are for tissue, processed skins, claws, canines, nails, whiskers, bones, bear bile's, musk pod, blood, meat, hair, blood stains and meat preserved in formalin, salt solution etc. We shall address the problem of standardizing protocols for extracting DNA from Wildlife Forensics materials that are highly degraded and developing markers using molecular techniques such as RAPD, PCR-RFLP, AP-PCR, Southern blotting and specific probes.

We also intend to identify the source of origin of tiger, leopard and elephant from parts and products. Four protocols were tested by us for 30 meat samples. 10% of these samples contained very good quality DNA that was used for RAPD amplification. 70% of the samples contained degraded DNA and 20% of the samples yielded very poor quality DNA that needed to be extracted again by modifying protocols. We also tested DNA protocols for other biological samples such as skin (n = 6), hairs (n = 10), bear bile (n = 4), musk pod (n = 4), antler (n = 1), ivory (n = 2) and blood (n = 5). As DNA molecules are highly stable under extreme conditions and exhibit high polymorphism, they can play a major role as molecular markers for rapid detection of Wildlife species and their products as well as the level of diversity among them. We intend to analyze one mitochondrial protein coding gene sequence (cytochrome b), two mitochondrial ribosomal RNA gene sequences (12s RNA and 16s RNA) and in nuclear DNA one un-translated region (UTR) of SON DNA binding protein gene sequence. Collaboration with zoos at Delhi, Kanpur, Chennai and Mysore has allowed us to procure reference tissue samples of around 75 species. Major constraints are funds and space for developing the Wildlife Forensics facility.

**Keywords**: Wildlife trade, DNA extraction, PCR-RFLP, RAPD

**WIL-TO-01**

**Species Identification of Animals With Matrix-Assisted-Laser-Desorption/Ionization-Time-Of-Flight Mass Spectrometry (Maldi-Tof Ms) Using Keratin Structures (Siam). A New Method for Quality Control and Animal Protection**

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Species Identification of Animals with Matrix-Assisted-Laser-Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-ToF MS) using Keratin Structures (SIAM). A new Method for Quality Control and Animal Protection Klaus Hollemeyer *(T), Wolfgang Altmeyer(#), and Elmar Heinzle (T) (T) Biochemical Engineering Institute, Im Stadtwald, Saarland University, D-66123 Saarbrücken, Germany, and (#) GENE-FACTS GbR, Science Park Saar, Stuhlsatzenweg 69, D-66123 Saarbrücken, Germany For the quality control of feathers, down and hair it is necessary to identify the tested material properly. This is because of the commercial value of some high priced raw material as well as for exclusive ready-to-use products and because of falsifications with cheap substitutes.

A further important aspect is the need to exclude material from endangered species from illegal trading often being incorrect declared or smuggled. So far the identification of feathers, down and hair is mainly performed with visual and microscopic methods. These are often time consuming and need the experience of experts. Even then up to 35% of all down can not be identified undoubtedly for example. Because of the biochemical and physical properties of these materials some of the classic identification methods fail like Fatty-Acid-Methyl-Ester analysis (FAME) for fat containing samples, Enzyme-Linked-Immuno-Assay (ELISA) for soluble antigen-antibody-reactions or the Polymerase-Chain-Reaction (PCR) using amplifiable DNA- or RNA- sequences. The latter method is hardly usable for tinted or chemically processed materials. Two-Dimension-Gel-Electrophoresis (2-D) followed by an amino acid sequence analysis is not economic and not for high throughput. The request for quantification of mixed samples can hardly be performed using these methods.

To overcome these drawbacks we recently developed the new SIAM method for the identification of the origin of feathers, down and hair and for the quantification of mixed samples exclusively using the almost insoluble proteins of these keratin structures. After a thiol reduction step samples of reference material are enzymatically cleaved by trypsin, a specific cleaving endoproteinase, No prior solubilisation or isolation steps for the structure proteins were

performed. The resulting pool of cleavage peptides originating from the pool of digested structure proteins is measured with MALDI-ToF MS leading to a mass spectrum with non-specific and specific peptides patterns for the reference species. After data processing and discarding of non-specific peptides partly originating from trypsin autoproteolysis a set of semi-specific peptides (SEMP's) is calculated, consisting of two groups: the unique species-specific peptides (USSP's) being unique for the actual species and the more often peaks (MFP's) occurring in up to 80% of all tested animals. Until now feathers and down from 20 avian species and about 40 furs and hair samples from different mammals are tested. Each of the spectra showed specific SEMP's. For identification of a native species only patterns of USSP's are used, while for the identification of a tinted or bleached sample or for the determination of zoological relationship between several species, the MFP's are also needed. For quantification purposes of binary samples MFP's being unique only for the two tested species are sufficient. The ratio of the relative intensities of two or more peaks, each specific for one of the two species, is linear to the ratio of the weight content of these species.

Originally developed for the discrimination of feathers and down of goose and duck in commercial samples for the bed linen industry, the range of applications have largely increased additionally analysing hair. Not only for industrial quality control purposes of raw material and ready-to-use products originating from wool, but also for the identification of illegal feathers, furs and wool of endangered animals this method is predestinated. For this purpose we are creating two peptide mass libraries of poultry and fur bearing animals making it possible to discriminate legal and illegal animal product trade by a simple comparison of an actual suspicious spectrum data set with those of the libraries. A further application may be the identification of grinded up rhinoceros horns in mixtures with other materials in the classic Chinese pharmacy. In principle all materials consisting of keratin structures are suitable for this new method. The new method produced a worldwide interest after the publication. Especially the potential use for detecting illegal furs, wool and feathers make this method interesting for the authorities. We are cooperating with the manufacturers of MALDI-ToF MS systems to create a compact system including sample preparation devices, automatically performed measurement, data processing, comparison with library data and interpreting the results. This would make the SIAM method usable also for on-site findings by semi-skilled authorities at airports or harbours.

**Keywords**: Keratin, MALDI-ToF MS, Animal Protection

### WIL-TO-02
### Using DNA Markers and Population Genetic Principles in Wildlife Forensics: An Overview

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The use of genetic methods in forensics is going through a period of rapid progress, thanks to the development of molecular techniques, which allow analyzing minute DNA traces obtained from almost any kind of biological samples, with levels of precision, repeatability and reliability that were until recently unthinkable . The principal aim of forensic genetic testing is to verify the hypothesis that a specific DNA fingerprinting is univocally associated to a particular individual, or that the DNA fingerprinting of an offspring is derived from the DNA fingerprinting of the two putative parents. Genetic variation is usually extensive within and between individual genotypes, as well as within and among populations, thus offering a multitude of DNA markers to be used for forensic individualization. A unique advantage of the DNA markers is that the results of laboratory testing (i.e., DNA fingerprints, haplotype and allele frequencies, molecular sexing) can be interpreted in the context of the mechanisms of biological heredity, that is the transmission of inherited traits through the generations across individuals and populations. Moreover, data on DNA markers can be interpreted using population genetic models, and evaluated using expectations from the theory of probability.

Molecular data and population genetic analyses, which are extensively used in human forensics, are being increasingly applied also to wildlife forensics. In my paper I will present an overview of the main molecular procedures that are currently used to generate molecular markers in wildlife forensics, and their applications to species, population and individual identification. The Polymerase Chain Reaction (PCR) allows highly specific in vitro amplification of selected target DNA sequences, starting also from minute and degraded biological traces. In this way it is possible to obtain analyzable DNA also if extracted from manipulated organic products (i.e., wood, ivory, products that are used in traditional medicine, cooked or canned or salted aliments, and so on). It also possible to obtain DNA samples and genetic information from wild-living populations without the necessity to capture the individuals, that is by non-invasive sampling. In these cases, DNA can be usually extracted from shed hairs or feather roots, or from faeces, urines, blood droplets and so on. The developing field of non-invasive genetics is offering valuable tools also to wildlife forensic genetics. Information on DNA markers arising from population and conservation genetic studies can be obtained from the scientific literature, or downloaded from the publicly available molecular genetic data banks. Often genetic markers, which were developed in domestic or wild species, can be used in other closely related wild species for forensic purposes. In these cases where markers are not available, molecular techniques now offer improved, and often relatively inexpensive methods to develop specific markers (i.e., anonymous markers detected by AFLP methods, or fast procedures to isolate microsatellites from specific DNA libraries).

Common problems in wildlife DNA forensics are: the lack of baseline population data, to be used as reference for statistical evaluation of DNA evidences, and the often unknown genealogical structure and inbreeding levels of captive reared animal stocks. Solutions to these two problems call for collaboration between population, conservation and forensic geneticists. Collaborative projects should be aimed to develop integrated DNA data banks of wild- and captive-living populations. The forthcoming developments of novel laboratory (i.e., DNA microchips) and improved statistical methods (i.e., Bayesian population structure and admixture analyses, and individual assignment procedures) will make collaborations of forensic and population genetics even more fruitful in the near future.

Wildlife forensic genetics is assuming a relevant role in the enforcement of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the main international agreement to regulate the trade and captive reproduction of endangered plant and animal species. Molecular techniques allowing for the identification of the species that are used in commercial plant or animal products, or for the assessment of reproduction in captivity, will predictably be increasingly used by national authorities within the CITES framework.

**Keywords**: Wildlife forensics, DNA methods, Population genetics

### WIL-TO-03
### A High-Density Multiplex PCR Design for Efficient Identification of Shark Body Parts: Application to CITES/IUCN Species of Concern

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As a result of overfishing, the population status of many of the world's commercially fished sharks has become an issue of in-