CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

Fifty-seventh meeting of the Standing Committee
Geneva (Switzerland), 14-18 July 2008

THE USE OF DNA TECHNIQUES FOR TRACKING LEGAL AND ILLEGAL IVORY TRADE

1. The attached document has been submitted by Kenya.

Department of Biology

Samuel K. Wasser. Ph.D.
P.O. Box 351800
University of Washington
Seattle WA 98195-1800
wassers@u.washington.edu
(206) 543-1669
FAX (206) 543-3041

Endowed Chair in Conservation Biology *Director, Center for Conservation Biology*

Monday 14 July 2008

The use of DNA techniques for tracking legal and illegal ivory trade

Illegal trade: identifying poaching hotspots for preventative law enforcement

International wildlife crime is escalating in this climate of global trade. We contend that the most effective way to contain the supply side of this illegal trade is to determine where the wildlife is being removed and focus enforcement on these source countries. This approach obstructs the trade before it enters into an increasingly complex web of international criminal activity that is extremely expensive and difficult to police, and few criminals are ever prosecuted. It allows authorities to direct law enforcement to poaching hot spots, prevents countries from denying their poaching problems at home and potentially stops trade before the wildlife is actually killed.

Forensics tools have been limited in their ability to determine product origin because their information typically begins only at the point of shipment. My Center has developed highly effective genetic methods to assign poached ivory to its geographic origin(s). However, these methods are applicable to policing trade in a wide variety of wildlife products. We are collaborating with the Interpol Working Group on Wildlife Crime and the Lusaka Agreement Task Force to identify poaching hot spots in Africa as well as strategies being used by large crime syndicates involved in the illegal ivory trade.

Findings

We applied this method to two large shipments of contraband African elephant ivory seized in Singapore/Malawi and Hong Kong/Cameroon (Wasser et al. 2008). Despite these two sets of seizures coming from opposite ends of Africa, both traffickers appeared to be using similar strategies. Both crime syndicates were targeting specific populations for intense exploitation. Target populations were hit hard and fast, presumably to satisfy specific purchase orders from their buyer(s). This contradicted the more common belief that traffickers were employing a decentralized plan of assembling large consignments by opportunistically procuring ivory stockpiles as they became available across Africa. It also meant that focusing law enforcement on identified hot-spots should prove a viable anti-poaching strategy.

Equally of interest in both cases, the ivory was smuggled internally in multiple shipments, through an intermediate country (Malawi for ivory from Zambia, and Cameroon for ivory from Gabon), prior to being shipped to Asia. This is a risk reduction strategy; it minimizes the time the ivory is in the dealer's possession and reduces the likelihood that a poacher caught in one country will identify a buyer in another country. These patterns would have been virtually impossible to uncover using non-genetic forensics tools, where information typically begins at the point of international export. In fact, focusing attention on the exporting country could actually distract authorities from locating the country where the ivory is actually being poached.

We currently face a sophisticated infrastructure laid by wildlife crime syndicates, connecting supply with practically unlimited demand. Although any long-term solution will need to include public awareness to curb demand, DNA assignment analyses offers one of the first reliable tools to focus enforcement on source countries, helping to prevent this trade at the supply end, where its containment is most urgent.

METHODS:

Acquiring the DNA

We have relied on colleagues and government agencies to help us collect dung samples from elephants across the African continent. Using DNA extracted from the elephant's intestinal mucosal cells, sloughed off in the millions in each gram of feces, we have compiled a geographic-specific map of elephant allele frequencies for 16 separate microsatellite DNA loci from across Africa (Wasser et al 2004, 2007). Alleles typically refer to alternate forms of a gene, which is a functional sequence of DNA at a specific location (locus) on a given chromosome. Microsatellite DNA is non-functional, as far as we know (i.e., does not code for proteins), and is found throughout the organism's genome. Its alleles consist of strands of repetitive nucleotide sequences (each repeat is typically 2-4 base pairs long) that have a high mutation rate. Most of this resultant allelic variation is retained because mutants do not appear to impact the organism's survival. The more that mutations accumulate in populations separated over space and time, the more genetically distinct they become from one another. This makes the highly variable microsatellite DNA ideal for discriminating between disparate populations, particularly when this information is combined across 16 different loci.

DNA is also found in elephant tusks. It is present throughout the tusk but is especially concentrated on the layers just below the surface. My lab was the first to extract DNA from ivory, taking advantage of a pulverization technique used in dental forensics. We place a piece of DNA the size of the first digit of one's smallest finger into a polycarbonate tube along with a magnet. Stainless steel plugs are placed in each end of the tube, which is then dropped into a well of liquid nitrogen and sealed off in a machine called a freezer mill. The liquid nitrogen freezes the ivory to a chillingly brittle -240 °C and then the freezer mill shifts a magnetic field back and forth over the tube, causing the magnet to smash the ivory against the stainless steel plugs, pulverizing it into a fine powder. The freezing temperature maintains the integrity of the DNA throughout this process, which is then easily extracted from the ivory powder. The next step is to compare the alleles in the ivory samples to those comprising the dung-based allele frequency map, across the 16 different loci, providing the information needed to assign a geographic origin to the ivory. We use some very sophisticated statistics to do that.

Using DNA to assign sample origins

The statistical method uses the Smoothed Continuous Assignment Technique (Wasser et al. 2004, 2007), implemented in software SCAT (http://stephenslab.uchicago.edu/software.html), developed by my colleague Matthew Stephens at the University of Chicago. Relying on the fact that populations close to one another tend to be genetically more similar than populations that are more distant, SCAT uses a smoothing technique to generate continuous allele frequencies across the entire elephant range (including intervening areas without reference samples) from the reference allele frequencies we acquired from dung samples collected across Africa. We do this separately for forest and savanna elephants owing to extensive genetic differentiation between these two newly acknowledged species. This enables us to determine the range of plausible locations for each ivory sample by comparing its observed alleles with the geographic map of continuous allele frequencies from that sample's respective habitat (assigned as forest or savanna from its DNA, prior to conducting the larger analysis). The breakthrough of this approach is that for the first time it enables us to assign a tusk to any location in Africa. Assignments based on previous methods were restricted to only those areas where reference samples were previously collected.

Legal trade: potential use of DNA sampling

Since DNA sampling, or genotyping, provides a unique fingerprint of the tusk, this can provide a highly reliable tool for monitoring the legal trade. To do this, countries would simply need to sample the tusks in their stockpiles by removing a small piece (~16 cm^2) of ivory from the base on each tusk (see Annex). These samples could then be genotyped and a DNA reference library created or stored to be genotyped later, if needed. (Storing the tusks for subsequent genotyping would be less expensive. However, it would be important that these be stored in a common location that would not be compromised and could be accessed readily when needed. Delaying genotyping would also add considerable time to the verification process of an ivory sample when needed. If stockpiles were already genotyped, it would be

relatively quick and easy to genotype ivory at the time of sale and check their genotypes against the DNA reference library.)

Genotyping the stockpiled tusks could be used to:

1. Verify that ivory being legally sold were part of the original stockpile (and not ivory illegally introduced to replace previously stockpiled ivory that had been illegally sold). This method could also be used to determine the source of illegally sold, stockpiled ivory. These findings would require a 1:1 genotype match of any given ivory sample to any sample already in the reference database.

2. Track chain of custody in legal transactions.

3. Assign origin to the stockpile to determine if all ivory did indeed come from the host country, and, if not, where the outliers were derived from. (This analysis would be analogous to that done in our lab to assign origin to a seizure. It would, however, have some margin of error for those source populations that have historically experienced a high degree of immigration and emigration.)

References

Wasser, S.K., W.J. Clark, O. Drori, E.S. Kisamo, C. Mailand, B. Mutayoba, and M. Stephens. 2008. Combating the illegal African elephants ivory trade using DNA forensics. Conservation Biology, in press.

Wasser S.K., C. Mailand, R. Booth, B. Mutayoba, E. Kisamo, B. Clark and M. Stephens. 2007. Using ivory to track the origin of the largest ivory seizure since the 1989 trade ban. Proceedings of the National Academy of Sciences of the United States **104**: 4228-4233.

Wasser S.K., A.M. Shedlock, K. Comstock, E.A. Ostrander, B. Mutayoba and M. Stephens. 2004. Assigning elephant DNA to geographic region of origin: Applications to the ivory trade. Proceedings of the National Academy of Sciences of the United States **101**: 14847-14852.

Annex

Tusk Sampling Protocol

1. Everyone handling the tusks should wear rubber or latex gloves at all times and ensure that all necessary precautions are taken throughout the whole process.

2. If possible, arrange all tusks that were acquired together (e.g., by the same culling, seizure, or other activity) into a single group. Take an inventory of all the ivory, and put permanent identification marks on each piece, which should match with all samples taken. Label each piece consecutively by grouping and number per group. Thus, the second sample from group 1 would be labeled: 1-2. The fourth sample from group 8 would be 8-4.

3. Try to match each tusk to its pair (by shape, size and color).

4. Select only one tusk per pair (although some tusks may not have a pair) and saw a piece ~4 cm X 4 cm from the base of each of tusk (where the tusk connects to the skull), using a fine toothed saw blade (if possible). Please wear rubber gloves, changed with each sample. If you wish to use the same saw blade for multiple samples, please wipe the blade clean between samples with a 10% bleach solution (1 part bleach, 9 parts water). If the tusk is paper thin at the end, please start cutting the tusk a little higher up where the ivory is at least 2-3 mm thick at the thinnest part. If you do not have a saw, you can try to break off a piece of the ivory with a hammer, pliers or some other implement. However, remember to clean the tool between tusks.

5. Place each 4 cm X 4 cm sample in its own zip-loc bag. Using a water-proof pen, clearly write the permanent ID number on the outside of the bag that you wrote on the outside of the tusk before processing. Be sure to make a final list of the samples sent, and include a COPY of this list in the shipment.

Figures: from Wasser et al 2008.
Figure 1

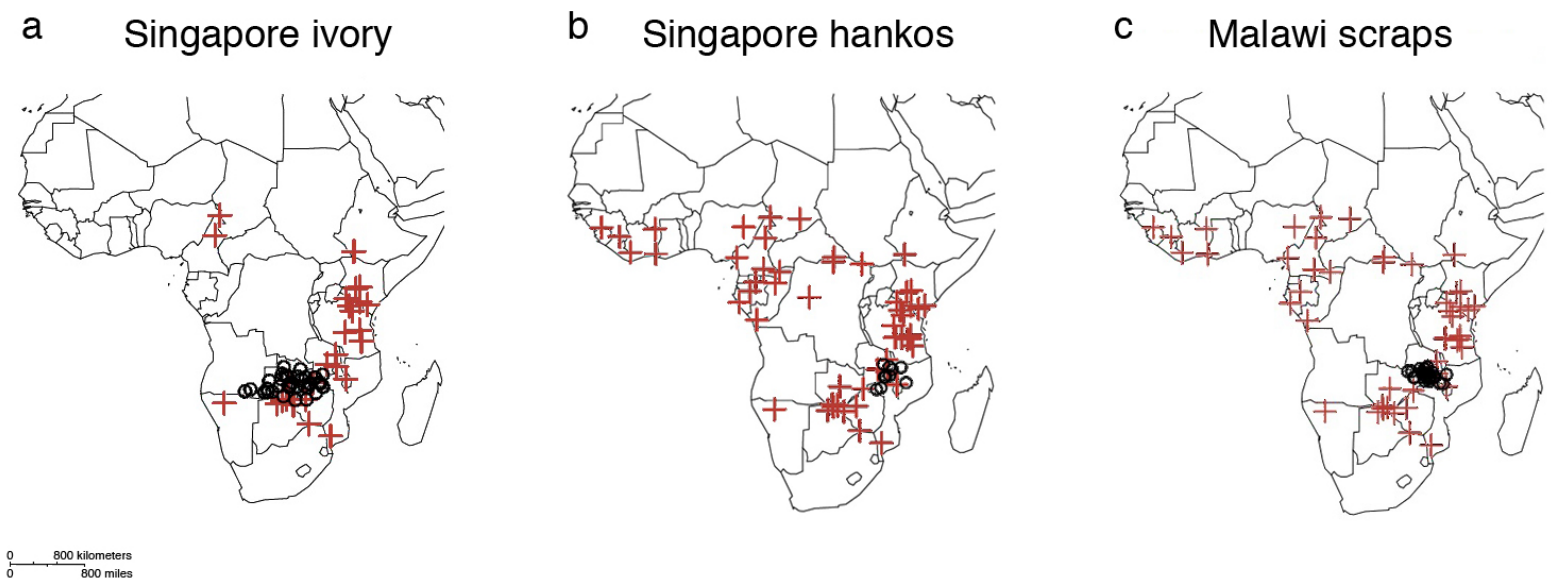

Figure 1. Assignment results of: (a) 37 tusks, (b) 12 hankos, and (c) 40 hanko shells from the Singapore and Malawi seizures. Circles represent estimated origins of individual samples. Crosses indicate locations of reference samples used to make these assignments. Crosses from Figure 1a show only savanna reference locations used in the assignment of those tusks (Wasser et al 2007). The remaining figures show all savanna and forest reference locations used to assign origin to savanna or forest elephants, respectively (see Methods). The composite of genetic and forensic evidence strongly suggest that the tusks, hankos and hanko shells all have similar origins. These results also support the hypothesis that the hankos were carved from small to medium sized tusks (likely obtained from younger elephants in the poached population) that would otherwise catch a low price on the international ivory market.

Figure 2

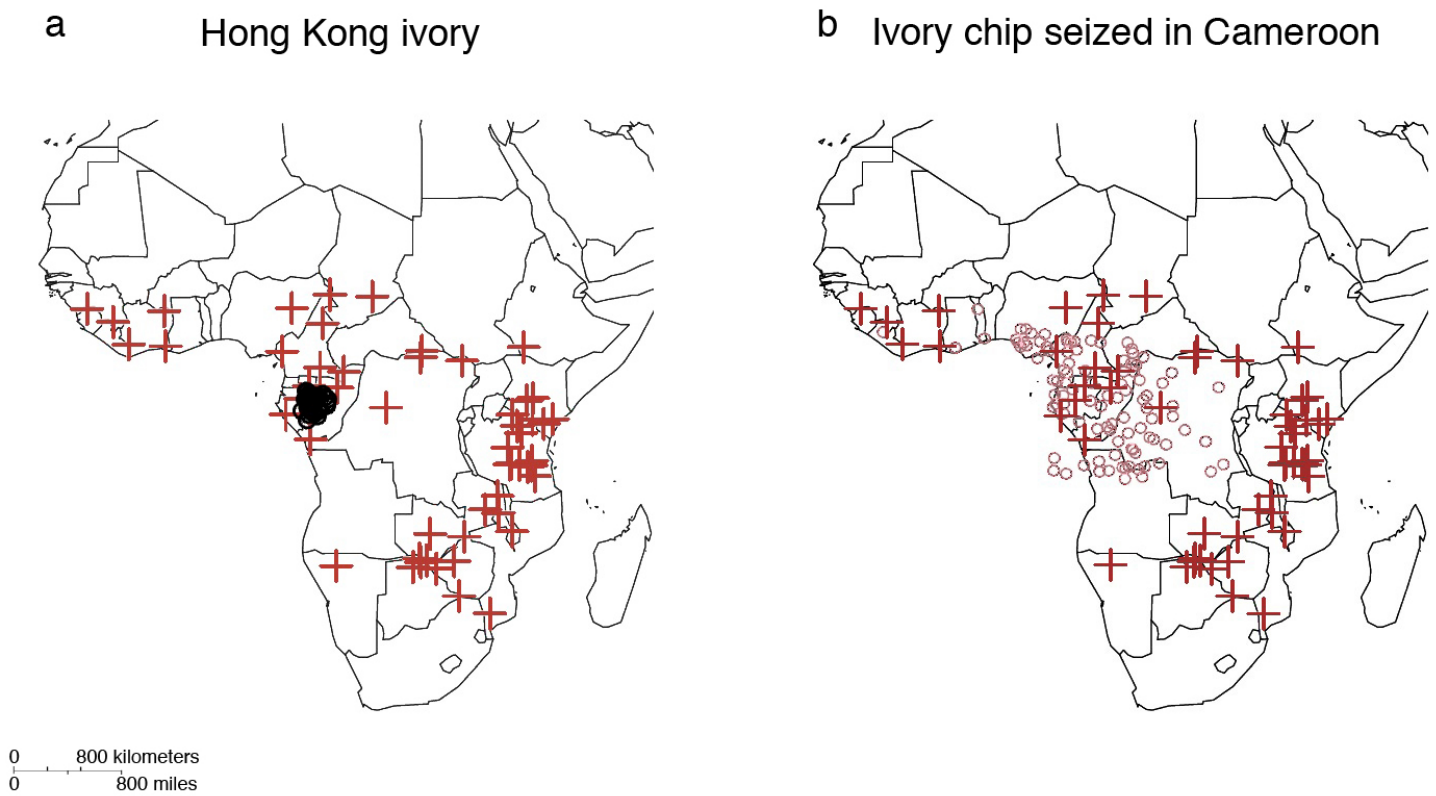

Figure 2. Assignment results of (a) 40 tusks from the Hong Kong seizure, and (b) the ivory chips remaining from a single tusk seized in an analogous container on its return to Cameroon from Hong Kong. Each circle in Figure 2A represents the estimated origin of an individual tusk. By contrast, each circle in Figure 2B represents one of 100 random draws from the set of all possible locations for just one sample—the single ivory chip—weighted according to their probabilities, using a uniform prior (Wasser et al. 2004). The spread of these 100 random draws reflects the assignment confidence for this one ivory chip sample. Crosses are described in Figure 1.