Contributed Paper

Combating the Illegal Trade in African Elephant Ivory with DNA Forensics

SAMUEL K. WASSER, *‡‡ WILLIAM JOSEPH CLARK, † OFIR DRORI, ‡ EMILY STEPHEN KISAMO, \S CELIA MAILAND, * BENEZETH MUTAYOBA, ** AND MATTHEW STEPHENS††

*Center for Conservation Biology, Department of Biology, University of Washington, Seattle, WA 98195-1800, U.S.A. †Interpol Working Group on Wildlife Crime and Department of Law Enforcement, Israel Nature and Parks Authority, Jerusalem 95463, Israel

‡Last Great Ape Organization, Vallee Nlongkak, Younde, Cameroon

§Lusaka Agreement Task Force, P.O. Box 3533, Nairobi, Kenya

**Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania

†Department of Human Genetics and Statistics, University of Chicago, Chicago, IL 60637, U.S.A.

Abstract: International wildlife crime is burgeoning in this climate of global trade. We contend that the most effective way to contain this illegal trade is to determine where the wildlife is being removed. This allows authorities to direct law enforcement to poaching bot spots, potentially stops trade before the wildlife is actually killed, prevents countries from denying their poaching problems at home, and thwarts trade before it enters into an increasingly complex web of international criminal activity. Forensic tools bave been limited in their ability to determine product origin because the information they can provide typically begins only at the point of shipment. DNA assignment analyses can determine product origin, but its use bas been limited by the inability to assign samples to locations where reference samples do not exist. We applied new DNA assignment methods that can determine the geographic origin(s) of wildlife products from anywhere within its range. We used these methods to examine the geographic origin(s) of 2 strings of seizures involving large volumes of elephant ivory, 1 string seized in Singapore and Malawi and the other in Hong Kong and Cameroon. These ivory traffickers may comprise 2 of the largest poaching rings in Africa. In both cases all ivory seized in the string had common origins, which indicates that crime syndicates are targeting specific populations for intense exploitation. This result contradicts the dominant belief that dealers are using a decentralized plan of procuring ivory stocks as they became available across Africa. Large quantities of ivory were then moved, in multiple shipments, through an intermediate country prior to shipment to Asia, as a risk-reduction strategy that distances the dealer from the poaching locale. These smuggling strategies could not have been detected by forensic information, which typically begins only at the shipping source.

Keywords: African elephant, DNA, assignment tests, ivory trade, poaching, wildlife trafficking

Combate del Comercio Ilegal de Marfil de Elefante Africano con ADN Forense

Resumen: El crimen de vida silvestre internacional está floreciendo en este tiempo de comercio global. Sostenemos que la forma más efectiva de contener este comercio ilegal es determinar dónde está siendo removida la vida silvestre. Esto permite que las autoridades dirijan el cumplimiento de la ley a los sitios candentes de la caza furtiva, potencialmente detiene el comercio antes de la vida silvestre sea cazada, evita que los países nieguen sus problemas de cacería furtiva y combate al comercio antes de que entre a una red de actividades criminales cada vez más compleja. Las berramientas forenses ban estado limitadas en su capacidad para determinar el origen del producto porque la información que pueden proporcionar típicamente comienza en el punto de embarque. Las pruebas de asignación de ADN pueden determinar el origen del producto, pero su utilización ba sido limitada por la incapacidad de asignar muestras a localidades donde las muestras de referencia no existen. Aplicamos métodos de asignación de ADN que pueden determinar el(los) origen(es) de productos de vida silvestre de cualquier sitio dentro de su rango de distribución. Utilizamos estos métodos para examinar el(los) origen(es) de dos cadenas de confiscaciones de grandes volúmenes de marfil de elefante, una cadena confiscada en Singapur y Malawi y la otra en Hong Kong y Camerún. Estos traficantes de marfil pueden dos de grupos de traficantes más grandes de África. En ambos casos, el marfil confiscado tenía orígenes comunes, lo que indica que lo grupos mafiosos están apuntando a poblaciones específicas para explotarlas intensivamente. Este resultado contradice la creencia dominante de que los traficantes utilizan un plan descentralizado para procurarse reservas de marfil a medida que se bacen disponibles. Entonces, grandes cantidades de marfil fueron movilizadas, en múltiples embarques, por medio de un país intermediario antes de ser embarcadas a Asia, una estrategia para reducir riesgos que aleja del sitio de la cacería furtiva al distribuidor. Estas estrategias de contrabando no podrían baber sido detectadas por información forense, que típicamente comienza en el sitio de embarque.

Palabras Clave: ADN, comercio de marfil, elefante africano, pruebas de asignación, tráfico de vida silvestre

Introduction

The illegal wildlife trade is booming around the world (Clark 2008; McMurray 2008). Illegal trade in wildlife products has reached over \$20 billion a year (Interpol 2008). China is the largest market, with the United States and Japan running close seconds (McMurray 2008). The illegal trade has become a high-profit enterprise with exceptionally low risks. Prosecutions of illegal wildlife traffickers are relatively rare, largely because law enforcement officers, prosecutors, and judicial systems typically consider such crime a low priority. Not surprisingly, evidence now indicates that organized crime has become heavily involved in illegal wildlife trafficking (Clark 2008). There has been a conspicuous increase in frequency of seizures of large consignments of contraband, including coral, snake skins, conch shells, ivory, the Tibetan antelope chiru or shahtoosh, abalone, and other wildlife products, each recently characterized by authorities as the "largest of this type in history" (Clark 2008). Wildlife traffickers are exploiting the globalization and liberalization of legal international trade and taking advantage of the most modern commercial and technological developments, both of which make illegal trade in wildlife products nearly impossible to track once the product has left the source country. Even the Internet is now being used as a vehicle for illegal international trade in wildlife. Large volumes of wildlife are being traded, and less scrupulous merchants are capitalizing on inadequate or nonexistent regulations (Clark 2008). To make matters worse, illegal trade is primarily exploiting wildlife that is already at risk. The situation has become urgent.

These trends in wildlife trafficking are typified by resurgence in the illegal trade of African elephant ivory. Over 25,000 kg of ivory were seized between August 2005 and August 2006, which is far more than the total volume of ivory seized in the prior 3 years combined (Wasser et al. 2007). The wholesale price of high-quality ivory 2004 to US\$850/kg in 2006. Osaka Customs recently appraised their August 2006 ivory seizure of 2.8 tons at 1 billion Japanese yen (about US\$10 million), worth a retail price of US\$3570/kg (Mainichi Daily News 2007). Forest police in Guangxi Zhuang Autonomous Region seized 790 kg of ivory in southern China in March 2008 and estimated the (retail) value at 36 million yuan (US\$5.1 million; retail price of US\$6500/kg) (China News 2008). These increases in ivory prices are consistent with other durable commodities, such as gold and silver, which people are acquiring for investment. Customs generally assumes that a 10% seizure rate for "general goods" contraband is successful. General goods includes contraband such as bootlegged DVDs and CDs

quadrupled around this same period, from US\$200/kg in

"general goods" contraband is successful. General goods includes contraband such as bootlegged DVDs and CDs, counterfeit electronics, and other prohibited items. It excludes specific contrabands such as drugs and explosives, which have higher seizure rates because of special detection equipment, intelligence gathering units, and targeting analysis (Clark 2008). With a 10% seizure rate for ivory (Wasser et al. 2007), which is likely conservative given the suspected number of unseized shipments in the 2 cases we investigated, the 25,000 kg of ivory seized by authorities between August 2005 and August 2006 actually corresponds to approximately 250,000 kg of smuggled ivory or 38,000 poached elephants. This could represent an annual offtake of 8% of the remaining 470,000 elephants in Africa (Blanc et al. 2007), which is already higher than the 7.4% annual offtake that occurred over the 10 years prior to the 1989 ivory ban (Douglas-Hamilton 1988; Said et al. 1995). At this rate, elephants, a keystone species across sub-Saharan Africa, could be virtually extinct across most of their range by 2020. Much of this illegal trade is being driven by rapidly growing markets in China and the United States, along with the persistent markets in Japan (Courable et al. 2003; Martin 2007; Clark 2008). At the same time, discussion surrounding a sustainable, legal trade in ivory dominates the Convention on International Trade in Endangered Species (CITES) meetings and is prominent in the news media (e.g., Loder 2008).

We contend that a key strategy for containing this illegal trade is to determine where the wildlife is being removed and direct law enforcement to these areas. Focusing law enforcement on areas where poaching is most concentrated potentially thwarts the trade before it enters into an increasingly complex web of international criminal activity that is extraordinarily expensive and difficult to track. This approach can also prevent countries from denying their poaching problems at home and may ultimately stop the trade before the wildlife is actually killed. Unfortunately, forensics investigators have typically been unable to determine the geographic origin(s) of poached ivory because their information generally begins at the point of shipping. Yet, ivory may or may not be exported from the same country in which it was poached. Here we show that DNA assignment methods can potentially address these problems by assigning geographic origin to seized wildlife products from anywhere within the species' range (Wasser et al. 2004, 2007). We used these methods to examine the geographic origin(s) of 2 seizures involving large volumes of ivory from African elephants, 1 seized in Singapore and Malawi and the other in Hong Kong and Cameroon.

Methods

Sample Origins

In June 2002, 6.5 tons of ivory-purported to be stone sculptures-were seized in Singapore after being shipped from Malawi via Mozambique and South Africa (EIA 2002; Wasser et al. 2007). This was the largest seizure since the 1989 ivory ban and the second largest in the history of the trade. The shipment included 532 large tusks, averaging 11 kg. Small- and medium-sized tusks were conspicuously absent. Wasser et al. (2007) used DNA analysis to determine that the tusks in this seizure originated in an area in Zambia. The shipment also contained 42,000 cylindrical signature seals, called *bankos* or chops, each 6 cm in length and 14-18 mm in diameter. Wasser et al. (2007) did not analyze the hankos because the DNA was nearly impossible to amplify at the time. Four months prior to the Singapore seizure, authorities raided a licensed ivory factory in Lilongwe, Malawi. Over 100 ivory scraps were confiscated in this raid. The bored holes in those scraps matched the sizes of the hankos. We use the term banko shells to describe these ivory scraps. We used DNA analyses to examine the geographic origins of the hankos seized in Singapore and hanko shells seized in Malawi to determine whether the tusks (Wasser et al. 2007), hankos, and hanko shells all came from elephants poached from the same population.

In May 2006, 3.9 tons of ivory tusks were seized in Hong Kong. When Hong Kong authorities x-rayed the container at the port, they found the ivory in a compartment at the back of the container, behind an internally constructed false wall. The container's main cargo was forest timber. One month later Cameroon authorities searched 2 similar containers returning from Hong Kong, owned by the same company, carrying used tires for resale. These also had false compartments, but were empty except for a few ivory chips recovered from the floor of the closed false compartment. The chips were presumed to be representative of the ivory previously transported in that container. A flour sack from Gabon was also on the floor in the false compartment of one of these containers. We used DNA analyses to also establish common origins of these tusks seized in Hong Kong and ivory chips seized in Cameroon.

Sample Selection

We used a stratified method to collect samples of ivory from hankos from Singapore and hanko shells from Malawi and tusks seized in Hong Kong and ivory chips seized in Cameroon (Wasser et al. 2007). Whenever possible, we located and set aside one Hong Kong tusk in each pair to avoid sampling the same animal twice. We lumped the single tusks into groups by external features such as color (as if buried in the same soil) or common markings (e.g., many tusks in the Singapore seizure had "Yokahama" written on them). Tusks were then sampled proportionately from each of these groups. This assured that all groups were represented and maximized the likelihood of selecting tusks from multiple locations to be genotyped. The hankos were wrapped in packages of 150, 120, and 100 pieces, depending on their respective diameters (14, 15, 18 mm). We sampled 1 hanko from each of 100 randomly chosen packets. Hanko shells (n =100) were randomly sampled from the contraband seized at the Malawi carving factory.

DNA Extraction and Analyses

Ivory was extracted (Mailand & Wasser 2007) from all samples, amplified for 16 microsatellite DNA loci, and alleles called with the methods described in Wasser et al. (2004, 2007). We used microsatellite DNA loci for these assignments because they tend to be hypervariable due to a high mutation rate.

We made geographical assignments by comparing the ivory genotypes at all 16 loci to over 600 reference DNA samples extracted from tissue and scat samples collected from elephants in known savanna and forest habitat locations across sub-Saharan Africa (Fig. 1b). The reference data were from Wasser et al. (2004, 2007), augmented with over 75 samples from Gabon, Congo-Brazzaville, and Democratic Republic of Congo.



Figure 1. Geographic origin assignments of (a) 37 tusks, (b) 12 bankos, and (c) 40 banko shells (leftover scraps from which bankos were punched) from the Singapore and Malawi seizures of ivory (circles, estimated origins of individual samples; crosses, locations of reference samples used to assign sample origins). Crosses in (a) show only savanna elephant reference locations used in the assignment of those tusks (Wasser et al 2007). Figures (b) and (c) show all savanna and forest reference locations used to assign origin to savanna or forest elephants, respectively (see Methods). The geographic origins assigned to the bankos (Fig. 1b) and banko shells (Fig. 1c) in the Singapore and Malawi seizures, respectively, overlap the origins of the tusks (Fig. 1a) in the Singapore seizure, all being consistent with a relatively restricted southern Africa origin centered on Zambia.

Each sample of unknown origin was assigned, on the basis of its DNA, as being from either forest or savanna elephants (Wasser et al. 2004). Only samples that met our criterion of having 7 or more amplified loci with 2 confirmed alleles (Wasser et al. 2004, 2007) were included in our analyses. Because of the extent of genetic differentiation between forest and savanna elephants (Roca et al. 2001; Comstock et al. 2002), this assignment can generally be done with very high confidence (Wasser et al. 2004).

We then performed more refined geographic assignments for each individual with the "smoothed continuous assignment technique" (Wasser et al. 2004), implemented in SCAT software (http://stephenslab.uchicago. edu/software.html). This software uses allele frequencies from reference samples and spatial smoothing methods to generate a geographic map of allele-frequency variation across the entire elephant range (including intervening areas without reference samples). This method relies on the fact that populations close to one another tend to be genetically more similar than populations that are more distant. The smoothing was performed separately for reference samples from savanna and forest habitats. The SCAT software takes the smoothed reference maps of allele frequency variation and uses a Markov-chain Monte Carlo (MCMC) algorithm to sample from the posterior distribution for the geographic origin of each DNA sample to be assigned on the assumption there is a uniform prior on this origin. In essence, the approach compares observed alleles with the geographic map of allele frequencies from that sample's respective habitat (forest or savanna) to determine the range of plausible locations

for each sample. The advantage of SCAT over traditional assignment tests (e.g., Paetkau et al. 1995) is that it allows tusks to be assigned to any location in Africa, not just to those areas where reference samples are available.

Once each sample was analyzed individually, we used an approach described in Wasser et al. (2007) to analyze groups of samples jointly. The idea is to exploit the fact that the combined data from a group of samples contains substantially more information about the origin of that group of samples than is contained in any single individual sample. The approach assumes that the group of samples originated from some unknown geographic region, R (which may or may not be a contiguous region), and attempts to estimate R. Formally, we used Voronoi tessellation to specify a prior distribution on R and then an MCMC algorithm to sample from the posterior distribution of R given the observed genotype data. The Voronoi prior is flexible, allowing for the possibilities that the samples being analyzed arose from a relatively restricted geographic origin or from multiple geographic regions spread across the continent. This approach provides an estimate for R, the region of origin for the entire group of samples, and produces a refined estimate of the geographic origin of each individual sample. Wasser et al. (2007) showed that these refined estimates can substantially improve assignment accuracy, particularly if the samples originated from a relatively restricted region.

We report assignments obtained from this latter approach (the Voronoi prior) for each group of multiple samples of ivory from a single seizure. For the single ivory chip, we present results for the uniform prior only (Fig. 2b) because this chip was seized alone.



Figure 2. Geographic origin assignments of ivory from (a) 40 tusks from the Hong Kong seizure and (b) chips remaining from a single tusk seized in an analogous container on its return to Cameroon from Hong Kong. Circles in (a) represent the estimated origin of an individual tusk. By contrast, each circle in (b) represents 1 of 100 random draws from the set of all possible locations for just one sample—the single ivory chip—each location being weighted according to the probability that the sample arose from that location, assuming a uniform prior (Wasser et al. 2004). The spread of these 100 points reflects the assignment confidence for this one ivory-chip sample. Crosses are described in Fig. 1.

Results

Alleles obtained from the Singapore and Malawi seizures compared with reference allele-frequency distributions for forest versus savanna elephants indicated that all of these samples were from savanna elephants. The origins assigned to the hankos (Fig. 1b) and hanko shells (Fig. 1c) investigated in the Singapore and Malawi seizures, respectively, overlapped origins previously described for the tusks (Fig. 1a) in the Singapore seizure. The tusks originated from a relatively restricted part of southern Africa, centered on Zambia (Fig. 1a). The origins of hankos (Fig. 1b) and hanko shells (Fig. 1c) closely overlapped one another and that of the tusks, although the former 2 were centered slightly more to the northeast of the tusks. The DNA from the hankos was difficult to amplify, restricting us to 12 samples that met our criterion of having 7 or more loci with 2 confirmed alleles (Wasser et al. 2004, 2007). Nevertheless, the hanko shells had high amplification success.

The Hong Kong tusks were from forest elephants. The tusks were assigned to a tightly clustered area centered on southern Gabon, near the Congo-Brazzaville border to the east (Fig. 2a). Ivory chips from only 1 of the 2 samples seized in Cameroon were large enough to yield amplifiable DNA at \geq 7 loci. The DNA from that sample (Fig. 2b) was also from a forest elephant, with an assigned origin consistent with those of ivory in the Hong Kong seizure. The assignment data for the chip are not presented in the same was as the data from the tusks in Fig. 2a. Because

the ivory chips came from a single sample, each circle in Fig. 2b represents 1 of 100 random draws from the set of all possible locations for the single ivory chip. The clustering of these 100 random draws illustrates the confidence surrounding assignment of this single sample. The more tightly clustered the 100 random draws, the higher the confidence of that assignment. In this case the points are somewhat widely scattered, reflecting the difficulty of precise assignment of a single sample. Over 60% of tusks in the Hong Kong seizure showed a similarly wide scatter over this same area when analyzed as individual samples in this way (data not shown). Nonetheless, the scatter of points clearly overlapped the area to which the Hong Kong seizure was assigned, which suggests that the DNA data were consistent with the chip and tusks sharing a common origin.

Discussion

Authorities have had a difficult time suppressing the resurgence in illegal wildlife trade, partly because nearly all forensics information has begun only at the shipping location. Very little was known of the geographic origin of the ivory we investigated in the present seizures. The DNA analyses we conducted verified the savanna (Zambia centered) and forest elephant (Gabon centered) origins of all products in these respective ivory seizures. Linking the origin of the hankos to the tusks in the Singapore seizure could also explain the absence of small to medium tusks in the Singapore seizure and hence the atypically large weight of tusks in this seizure. The smaller tusks were likely carved into hankos, making the overall consignment more appealing to its intended Japanese market (EIA 2002), whose buyers prefer large tusks.

Showing that all seized ivory products in each series had common origin further suggests that both crime syndicates were targeting specific populations for intense exploitation, hitting them hard and fast to satisfy the demands of a particular consignment. They then may or may not shift to some other place for the next consignment. These findings contradict the previous dominant hypothesis that dealers used a decentralized plan of opportunistically procuring ivory stocks as they became available across Africa. The syndicates then smuggled large quantities of ivory in multiple shipments through an intermediate country of export prior to shipment to the Far East. This risk-reduction strategy minimizes the time the ivory is in the dealer's possession and distances the dealer from the country where the elephants were poached; a poacher caught in one country is less likely to identify a dealer in another country. Such operations appear to have recurred many times in particular regions over several years. These patterns would have been nearly impossible to uncover without genetic forensics tools. We now are confident that some major poaching syndicates continue to work a particular region for extended periods. Thus, focusing law enforcement on identified regions should provide a viable strategy. We also know that focusing attention on the country of export could distract authorities from identifying the country where the ivory is actually being poached.

The scale of operations in these 2 cases is indicative of sophisticated crime syndicates with expertise in commodity trade, finance, and other commercial disciplines (Courable et al. 2003; Wasser et al. 2007; Clark 2008). The raid on the Malawi ivory-carving factory uncovered detailed documentation linking the Singapore and Malawi seizures to an ivory trafficking operation that included 19 similar shipments over the preceding 8 years; 9 of these occurred in the preceding 3 years and included the 6.5 tons of ivory in the Singapore seizure (EIA 2002). If each of these 19 shipments from this factory contained 6 tons of ivory, this would represent over 110 tons of ivory, corresponding to 17,000 adult elephants killed (on the basis of CITES estimates of 6.6 kg ivory/elephant, 1.8 tusks per individual, and tusk weight of 3.68 kg).

Forensic evidence from the Hong Kong and Cameroon seizures also pointed to a massive, highly organized trafficking operation. The serial numbers on the container seized in Hong Kong and the containers seized on their return to Cameroon each had been changed at least twice, suggesting that each container had been shipped with contraband 3 or more times. That could represent 36 tons of ivory shipped within the past 2.5 years, from 5500 poached adult elephants. A partial customs registry revealed 12 additional shipments from the same company in similar containers with the same volume and type codes and shipping and receiving destinations, which potentially doubles the above tonnage of smuggled ivory and adult elephants killed over this period.

The sizes of these 2 poaching syndicates reflect a growing trend, whereby wildlife traffickers are becoming far more sophisticated, exploiting the globalization and liberalization of legal international trade to get their contraband into the high-paying markets of industrialized countries. The increasing layers of criminality added as one proceeds from the supply end suggests that it should be most feasible and economical to localize and control this traffic in the countries of origin, as determined by DNA analyses. The same is true of other wildlife trafficking, all of which is exhibiting unprecedented growth.

As with all wildlife products, DNA analyses of the product can help inform authorities how crime syndicates are operating and where to concentrate law-enforcement efforts to stop poaching at its source. Identifying the origin of poached products additionally forces countries to take responsibility for the illegal killing of their wildlife by exposing them to CITES and other internationally enforced sanctions. Cutting off the supply can also help thwart the long history of ineffective prosecution of illegal wildlife traffickers in consumer countries (e.g., Stiles & Martin 2001; Courable et al. 2003; Clark 2008). Perhaps most important, stopping poaching at its source would keep the remaining wildlife from being killed in the first place. This alone warrants placing greater effort on the first link in this long chain-a point made even more pressing in light of remaining elephants that are projected to be killed to support this trade (Blake et al. 2007; Wasser et al. 2007).

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