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SHORT COMMUNICATION

Male biased sex ratio of poached elephants is negatively related to poaching intensity over time

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Abstract Poaching and habitat loss has caused a massive decline in the number and range of African elephants over the past few decades, with the greatest post ivory ban losses occurring since 2006. Poaching targets the largest individuals for their greater tusk sizes. This should also bias poaching towards males. We hypothesized that elephant sex ratios from heavily poached populations will initially be strongly male biased, but become progressively less male biased in heavily poached areas over time. This will occur because large, older female matriarchs will eventually provide the next source of large tusks once the largest males are killed. In this paper, we examined the sex ratio of ivory samples from very large ivory seizures (0.5−6.5 tons) made between 2002 and 2013. Origins of these large seizures were independently determined and in most cases, consisted of individuals poached in the same or nearby location. Our results indicate a male-biased sex ratio among these large ivory seizures. The earliest and largest seizure (6.5 tons seized in 2002) from Zambia was 4−6 times more male biased than later seizures. However, male bias progressively declined in samples that originated from that same, heavily poached area over time, indicating a rise in female targets. These trends are likely to have negative implications on elephant recovery given the importance of female matriarchs to elephant social structure and population growth.

Keywords African elephants · Ivory · Sex ratio · Female matriarch · Elephant recovery

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Introduction

African elephants (*Loxodonta africana*) dramatic decreased in numbers and range over the past two centuries. Populations were estimated to approach 5 million individuals around two centuries ago, declining to 1.3 million in 1979 and roughly 400,000 by 2010 (Douglas-Hamilton 1987; Blanc 2007; UNEP et al. 2013). Forest elephants alone declined by 60 % over the past decade (Maisels et al. 2013). Most of this decline was due to a combination of habitat loss and poaching (Douglas-Hamilton 1987; Milner-Gulland and Beddington 1993; Blanc 2007; Burn et al. 2011; Maisels et al. 2013; Underwood et al. 2013).

Poaching for ivory concentrated on solitary adult males for their larger tusks (Gobush et al. 2008). Female matriarchs also have large tusks and range in more conspicuous family groups, often with smaller home ranges, making them more accessible compared to solitary males (Poole 1989). Some heavily poached populations also indicate a disproportionately high number of poached females (Idhe 1991). We accordingly hypothesized that poachers will increasingly target large, older female matriarchs over time because selective poaching of large, solitary males will increase the relative size and accessibility of female tusks.

We used genetic sexing tools (Ahlering et al. 2011) to sex a representative portion of tusks from nine large ivory seizures that occurred between 2002 and 2013. The origins of each of these seizures were previously determined by DNA assignment (Wasser et al. 2007, 2008, 2009). We predicted that the sex ratio of poached elephants would become progressively less male biased over time, both within and between populations. Such selective loss of female matriarchs can have long-term impacts on behavior, social structure and population growth that significantly

impact elephant recovery (Gobush et al. 2008, 2009; Gobush and Wasser 2009; Archie and Chiyo 2012).

Methods

Samples collection

We analyzed a total of 961 ivory samples from nine seizures (ranging from 0.5 to 6.5 tons), made between 2002 and 2013, and one sampling of all carcasses from a single poaching incident in 2013. Seizures were made in Singapore, Hong Kong, Taiwan, and Kenya, but determined by DNA assignment to originate from Zambia (Wasser et al. 2007), Gabon (Wasser et al. 2008), and Tanzania (Wasser et al. 2009). Seven of the nine seizures were from savannah elephants (*Loxodonta africana africana*). One 2006 seizure was from forest elephants (*Loxodonta africana cyclotis*). The 2013 carcasses and seizure were both mixtures of forest and savanna elephants. The 2002 Singapore seizure consisted on 512 tusks and 42,000 signature seals (hankos). The tusks and hankos were determined to have come from the same population, suggesting that the smaller tusks were cut into hankos to increase their market value (Wasser et al. 2008). However, the hanko sex ratio was calculated separately from the whole tusks because it was impossible to determine how many individuals were killed to provide the 42,000 hankos.

Sex was assigned to all tusks previously analyzed for origin assignment. The original sampling of tusks was conducted to maximize the representativeness of each seizure (Wasser et al. 2007). Each tusk was size-matched to its likely pair, with one tusk removed from each pair; individual uniqueness was subsequently confirmed based on 16 microsatellite loci (Wasser et al. 2004). Remaining tusks were next grouped based on similarities in external markings and then randomly sampled from those groups to maximize coverage of potential sampling locations, assuming similar marked tusks came from similar areas (Wasser et al. 2007, 2008).

A 3 cm × 3 cm and ~1 cm thick piece was sawed at the location closest to where it connects to the skull and placed in a uniquely labeled vial. The saw blade was disinfected with a 10 % bleach solution between sub-sampling. All samples were then shipped to our laboratory at the University of Washington in full compliance with CITES and USDA regulations.

DNA extraction

The DNA extractions were performed as described in Mailand and Wasser (2007). Small pieces of ivory were cut from each sample, sterilized in 5 % bleach to remove any DNA on the surface from prior handling, then rinsed thoroughly with sterile water, and air-dried in a sterile fume hood. Samples were then pulverized into powder, decalcified in an EDTA solution and the DNA extracted using Qiagen Tissue DNA kits. Two extraction blank controls were included in each set of 22 samples. PCR was performed for 16 microsatellite loci for individual assignment, as described in Wasser et al. (2004, 2007, 2008).

Molecular sexing

We used the sexing markers described in Ahlering et al. (2011). Two Y-chromosome markers (SRY and AMELY) and one X-chromosome marker (PLP) were multiplexed together labeled with FAM, HEX and NED.

The three primers were standardized separately and then together as a multiplex system using control tissue and fecal DNA samples of African elephants of known sex. Each sample was amplified in 10 µl volumes with 5 µl of Qiagen multiplex mix (Qiagen), 4 µM of BSA, 0.2 µM of primers and 3 µl of fecal/ivory DNA extract (1 µl for tissue DNA). The PCR conditions included an initial denaturation at 95 °C for 15 min, followed by 55 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 45 s, and a final extension of 72 °C for 30 min. Multiple negative controls and one male and female positive control were included in each set of reactions (six negative controls/plate). Post-PCR 1 µl of product was mixed with formamide and run on an ABI 3100 genetic analyzer with ROX400 size standard (Applied Biosystems).

Product sizes were visualized with the program GENEMARKER and analyzed by creating an allelic bin specific for elephant sexing markers. The entire procedure was repeated three additional times to ensure data quality. Only samples with more than two (for males) or three (for females) identical results were used to assign sex. The sex ratio of the assigned samples from each seizure was calculated separately.

Results

A total of 961 ivory samples were used in this study, collected from 11 different groups (nine seizures and one set of carcasses). DNA from all samples were previously PCR amplified for 16 microsatellite loci. Only 64 % ($n = 612$) of these samples were considered to be high quality (i.e., successfully amplified at ≥7 loci. The remaining 349 samples were assumed to be of relatively poor DNA quality. Overall success rate for elephant sex determination from all the samples in this study was 65.5 % ($n = 632$). Success rate for sex identification from good and poor samples were 76 and 48 %, respectively (Table 1).

Table 1 Sexing success rate from different types of seized ivory samples

Sample type	Sample information	
	Samples with microsatellite data	Samples without microsatellite data
Ivory		
Total	600	302
Male	282	90
Female	171	44
Hanko		
Total	12	47
Male	7	22
Female	3	13

Sex was unambiguously determined for a total of 401 males (63.4 %) and 231 females (36.6 %). Sex ratios variation ranged from 0.2 to 13 (M/F) across the 11 groups (Table 2). The sex ratio of tusks in the 2002 seizure was markedly higher (13) compared to all other seizures (2.33–1.37). However, the hankos in the 2002 seizure had a markedly lower sex ratio (1.81) compared to the tusks in that seizure, consistent with the expectation that they were cut from smaller individuals (i.e., a mix of individuals of each sex). The sex ratio of ivory derived from that same population in 2006 was lowest in our sample (0.86). No other patterns were found in the data.

Discussion

This study included all seizures examined by the Center for Conservation Biology since 2002. All of these seizures were subsampled in a manner that aimed to maximize the inclusion of samples from multiple locations (Wasser et al. 2007, 2008), which likely also produced a representative sex ratio estimate from these seizures. We also took precautions to minimize allelic drop-out, extracting each sample twice, conducting two PCRs per extract, with each Y-allele having to be observed at least twice to confirm a male and the X-allele observed at least three times in isolation to confirm a female. The particular method we used (Ahlering et al. 2011) further supported these efforts, showing high amplification success even for low quality samples (with a 66 % success rate in this study). This current system has two Y and one X markers, increasing detection of males when allelic dropout in Y-chromosome is likely. Adding another X marker may similarly increase detection of females.

We detected an overall sex ratio skew in favor of males, with several important distinctions. The heaviest male bias (13:1) occurred in the earliest and largest seizure of tusks, which occurred in 2002, compared to a sex ratio ranging between 2.33 and 1.37 from 2006 to 2013. While the tusks in that seizure were all notably large (mean tusk size = 12 kg), the seizure also included 42,000 signature seals, suspected to be carved from smaller tusks to increase their value since the signature seals and tusks in that

Table 2 Seizure information and sex ratio (M/F) across samples across seizures

Country of seizure	Year seized	Assigned location	No. of samples	Success rate (%)	Sex ratio (M/F)
Singapore (ivory)	2002	Zambia	64	43.8	13
Singapore (hankos)[a]	2002	Zambia	61	73.8	1.81
Singapore	2006	Zambia	55	50.9	0.86
Hong Kong	2006	Gabon	145	69.6	1.51
Hong Kong	2006	Tanzania Mozambique	150	67.1	1.77
Taiwan	2006	Tanzania Mozambique	80	75	2.33
Taiwan	2006	Tanzania Mozambique	58	72.4	1.33
Kenya	2011	Tanzania	220	62.6	1.83
Kenya	2011	Tanzania	77	67.5	1.47
Uganda	2013	DRC	10	60	0.20
DRC[b]	2013	DRC/Uganda	35	85.7	2.33

[a] This was from a large sample of signature seals in the 2002 Singapore seizure but was analyzed separately because they were suspected to be cut from smaller (younger) individuals

[b] This was from a poached sample of elephants in Garamba National Park; all carcasses in the poached sample were genotyped

seizure were all assigned to the same location (Wasser et al. 2008). The significantly lower sex ratio (1.8:1) of the signature seals supports that hypothesis as it indicates that many more females were represented in the hanko samples compared the tusks in the 2002 seizure. The size of the 2002 seizure, coupled with evidence from subsequent seizures, suggests that this population was heavily exploited by poachers between 2000 and 2006. The total weight of the 2002 seizure was 6.5 tons, making it the largest ivory seizure on record since the 1989 ivory ban (Wasser et al. 2007). A six-ton seizure made in the Philippines in 2005 was also suspected to have originated from that same population based on numerous shipping similarities (William Clark, personal communication), but could not be analyzed because most of the tusks were subsequently stolen from the customs guarded warehouse (Martin et al. 2011). However, the 2006 Singapore seizure we analyzed was genetically assigned to that same area (S. Wasser, unpublished data). That seizure had the lowest sex ratio (0.87:1) detected in any of the seizures examined. Thus, the sheer volume of these seizures assigned or implicated to be of Zambian origin suggests an enormous amount of poaching was occurring in Zambia between 2000 and 2006, and this trend appears to be reflected by the change in sex ratio of the poached ivory. Poaching selectively targeted males for their larger tusk size. However, an apparent sustained period of heavy poaching was reflected by an increase in the percentage of female matriarchs poached over time. This trend might also indicate that the most recent seizures of Zambian ivory were from more recently poached elephants. It might similarly be possible to use the sex ratio of future confiscated ivory seizures to assess the poaching pressure experienced by the affected population, provided tusk origin can also be determined.

Genetic tools can provide valuable information on poaching dynamics to better inform conservation and management efforts. In particular, the long life expectancy, slow-reproduction rate and strong social bonding among African elephants can affect their social structure and may produce alternative and detrimental behavioral and social tactics. Targeting matriarchs can disrupt social structure, leading to long-term negative impacts that reduce competitive ability, cooperation, reproductive success and population growth (Gobush et al. 2008, 2009; Archie et al. 2006; Wittemyer et al. 2011). Heavy poaching of males can also produce inbreeding impacts by allowing younger males to become the dominant breeding bull earlier, increasing their tenure and hence likelihood of eventually inbreeding (Ishengoma et al. 2008; see also Ramakrishnan et al. 1998; Sukumar et al. 1998 for Asian elephants). Combining DNA assignment of population origin and sex ratio can thus provide valuable tools for tracking, understanding and hopefully mitigating long-term poaching trends of elephants across Africa.

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