

Research Article

Non-invasive genotyping of Sumatran elephants: implications for conservation

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Abstract

Reliable baseline information necessary for the monitoring and conservation of Sumatran elephants is scarce. We here combine non-invasive molecular genetics methods and capture-recapture modeling to estimate elephant population size, distribution, sex ratio, and age structure for the Bukit Tigapuluh landscape in Sumatra, Indonesia. Two separate subpopulations were found, for which we estimated a population size of 99 (95% CI = [86, 125], PCCL = 38.59%) and 44 elephants (95% CI = [37, 56], PCCL = 43.18%), respectively. Low elephant densities are likely the result of patchy habitat usage and anthropogenically increased mortality, the latter assumption being supported by strong skews in both sex ratio and age structure as well as direct evidence of elephant killing. Still, the Bukit Tigapuluh landscape currently holds the largest known population of elephants in central Sumatra, representing one of the most important areas for their conservation in Indonesia. Conservation of both the elephant population and their habitat in this region should thus be of high priority. We identified several threats to the population, including (i) the risk of inbreeding and subsequent loss of genetic diversity, (ii) illegal elephant killing, and (iii) the lack of protected habitat. In order to overcome these challenges we suggest: (i) the implementation of a meta-population management program, (ii) monitoring and safeguarding elephants and improving law enforcement, and (iii) providing sufficient safe habitat to mitigate human-elephant-conflict (HEC) and ensure elephant survival.

Keywords: *Elephas maximus sumatranus*, capture-recapture modeling, abundance estimation, sex ratio, age structure, Bukit Tigapuluh Landscape.

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Introduction

The Sumatran elephant (*Elephas maximus sumatranus*) is one of three currently recognized subspecies of Asian elephants [1,2] and is both genetically [3] and anatomically different from other Asian elephants [1]. The critically endangered status of the Sumatran elephant is primarily due to massive habitat destruction, which has resulted in the local extinction of the species in at least 15 areas of Sumatra and continues to threaten most of the remaining wild populations [4].

Based on data compiled by the Indonesian Elephant Conservation Forum (Forum Konservasi Gajah Indonesia, FKGI) in 2007, the estimated population size was 2,400 to 2,800 animals [5]. That number may have dropped to below 2,000 individuals in 2014, but accurate information is scarce (head of FKGI Krismanko Padang, pers. communication). The lack of reliable elephant population size estimates for Indonesia was already noted a decade ago [6] and it appears that not much new data have been produced since then. Certainly, information alone cannot save elephants from extinction, but national and local conservation strategies based on incomplete and/or outdated data would likely fail as scarce conservation resources would not be adequately allocated and important conservation needs and opportunities may be overlooked.

Sound scientific information on elephant populations in Indonesia is not easily gathered, as the dense and tangled vegetation rarely allows for direct sightings, complicating the scientific sampling framework. Therefore, substantial effort was made to develop methods that overcome sampling issues for elusive animals (see [7] for review). For elephants, dung-count based distance sampling is a preferred method that can be used in a variety of habitats [8] including the Sumatran and Bornean jungle [9,10]. However, the use of dung-counts has been shown to lack precision when applied to very small elephant populations that live in low densities [11], as they do in Indonesia.

A promising alternative to dung counts is the sampling of DNA from fresh dung for individual identification using methods similar to those applied in human forensics [12]. If these samples are taken within a capture-recapture sampling framework, reliable population size estimates become possible even for small groups where individuals have never been sighted [13–15]. In addition to abundance estimates, important information on population structure can be obtained using DNA-based sex determination [16,17] and measurements of elephant dung bolus dimensions for age class estimation [8,18]. While not successfully applied to Sumatran elephant populations before, this non-

invasive sampling method has provided reliable population size estimates in mainland Asia and Africa [19–23].

Our study was carried out in the Bukit Tigapuluh Landscape in Central Sumatra and is part of an island-wide attempt by both government and non-government members of the FKI to provide crucial baseline data for population monitoring and conservation planning. We combined non-invasive molecular genetics methods and capture-recapture modeling to estimate population size, distribution, sex ratio, and age structure of a local elephant population.

Methods

Research location

The Bukit Tigapuluh landscape is located approximately in the geographical centre of the Indonesian island of Sumatra ($1^{\circ}4'27.72''S$, $102^{\circ}30'43.89''E$), stretching over two provinces (Riau and Jambi) and covering more than 3,200 km² of land including the 1,440 km² Bukit Tigapuluh National Park (Fig. 1). The climate is tropical with high rainfall and warm temperatures year round. The original vegetation type, the extremely species-rich dipterocarp rainforest [24] is now largely limited to the rugged centre of the landscape and surrounded by a patchwork of various land-use types, including oil palm and rubber tree plantations, pulpwood plantations, surface coal mining areas, small settlements, private farmland, and non-active former logging areas now partly covered with secondary forest (Frankfurt Zoological Society, unpublished data 2005-2011).

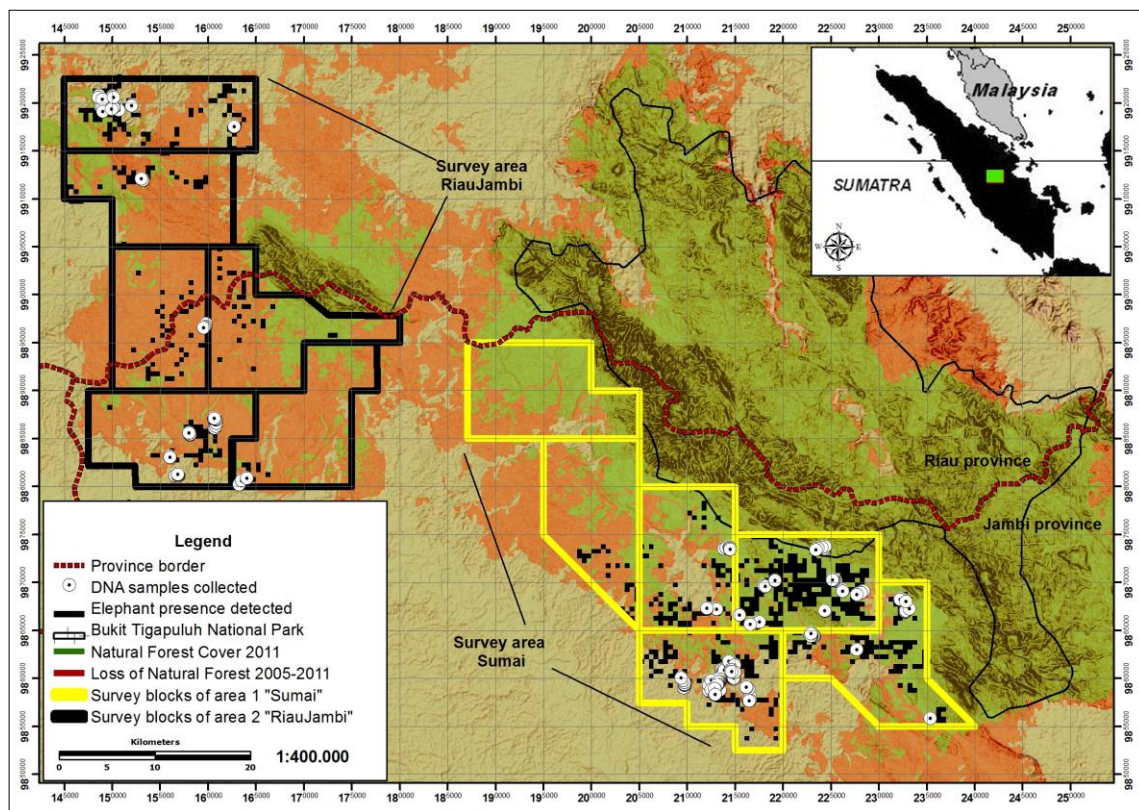


Fig. 1. Map* of the Bukit Tigapuluh Landscape in Sumatra, Indonesia. Shown are the survey blocks of survey area "Sumai" and survey area "RiauJambi", elephant presence (small black squares), and the locations of DNA samples collected (black & white dots). *Map data sources: forest cover = Frankfurt Zoological Society, unpublished data 2005-2014; provincial borders = BAKOSURTANAL, Bogor 2008.

Field Survey

The study area consisted of two 900 km² survey areas, “Sumai” and “RiauJambi”, which together cover all areas used by elephants in 2011 (Fig. 1). The borders of these survey areas were determined based on elephant distribution data collected between 2005 and 2011 ([25,26], Frankfurt Zoological Society, unpublished survey & patrol data 2005-2011) and a three-month pre-survey of all potential elephant habitat not recently surveyed.

Both survey areas contained six equally sized survey blocks, which were subdivided into six 25 km² sampling blocks. This design allowed all resident elephants to potentially be sampled, as the home range of Sumatran elephants is substantially larger than the sampling blocks [27,28]. Each survey area was surveyed three times by six field teams within discrete time intervals of a minimum of 12 survey days, separated by a break of ten days. Each time the area was surveyed corresponds to one sampling occasion. During each sampling occasion survey teams searched for fresh elephant dung in each sampling block for two full days. The landscape-wide survey was completed within six months, from May to October 2011, with total survey time not exceeding three months for each survey area.

Fresh elephant dung (\leq one day old) was sampled by collecting approximately five grams of dung from the outermost layer of intact dung boli into a sampling tube filled with ethanol absolute (MERCK, Frankfurt, Germany). Recent (up to seven days old) dung was only sampled in the absence of fresh dung piles. All samples were stored at ambient temperature in the field and at -20°C in the laboratory. Dung bolus circumference was measured for up to three boli of each dung pile sampled, using a soft measuring tape and following established procedures [8]. The location of the sample was recorded using a hand-held GPS (Garmin GPSMap 76CSX).

Laboratory Analysis

Genomic DNA was extracted using the QIAamp[®] DNA Stool Mini Kit (QIAGEN, Hilden, Germany) following a modified protocol [29]. The extraction was performed in a biosafety cabinet exclusively used for this purpose. Preparation of Polymerase Chain Reaction (PCR) mixture for amplifications was carried out in a DNA-free PCR hood, while the addition of DNA template was completed in a separate UV-sterilized hood in order to prevent contamination. A GeneAmp[®] PCR System 9700 thermo cycler (Perkins Elmer/ Applied Biosystems, Singapore) was used for amplification. Each panel included a negative (no-template) control to detect reagent and sample contamination and a positive control (DNA extracted from blood samples collected from Sumatran elephants by the author) to ensure consistent and comparable results.

For sex determination, two short Y-specific fragments (SRY1 and AMELY2) and one longer X-specific fragment (PLP1) [17] were amplified in multiplex PCRs. PCR was conducted in a 15 μ L reaction containing 1 U AmpliTaq Gold[®] 360 DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 1X AmpliTaq Gold[®] 360 Buffer (Applied Biosystems, Foster City, CA, USA), 0.08 mM of each dNTP, 0.4 μ M of each primer, 2 mM MgCl₂, 0.3 μ L 360 GC Enhancer, 150 ng – 250 ng of extracted DNA template, and PCR-grade water. Upon electrophoresis on a 2% agarose gel, females showed a single band (the 191 bp long PLP1 fragment) while three bands (the 191 bp long PLP1, the 122 bp long AMELY2, and the 71 bp long SRY1 fragments) indicated a male. Females were confirmed a minimum of three times and males at least twice in order to reduce the risk of false sexing.

Individuals were identified using a set of fourteen microsatellite loci developed for Asian and/or African elephants: EMU07, EMU04, EMU14, EMU10, EMU03, EMU17, EMU13, EMU12 [30], FH60 and FH103 [31], LafMS04, LafMS04, LafMS05 [32], and FH94R [33]. The multiplex PCR amplification was conducted in two separate panels, each reaction containing 1 \times multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 1 \times Q-Solution (QIAGEN, Hilden, Germany), 0.05-0.3 μ M of fluorescently labeled forward primer mix, 0.05-0.3 μ M of reverse primer mix, a minimum of 100 ng of DNA template (DNA

concentration was adjusted for each sample individually), and PCR-grade water to reach a total volume of 10 μ L. PCR was carried out using the following conditions: 95°C for 15 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 90 sec, extension at 72°C for 60 sec, and a final extension step at 60°C for 30 min. Capillary electrophoresis was performed in an Applied Biosystem® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), each run containing a mix of 1 μ L of PCR product, 9.3 μ L of HiDi Formamide, and 0.2 μ L of GeneScan™ 500 LIZ® molecular size marker (Applied Biosystems, Foster City, CA). Genotypes were scored using GeneMapper®V.4.0 software (Applied Biosystems, Foster City, CA, USA). For each locus, heterozygote results were confirmed twice and homozygote results were confirmed a minimum of three times.

Data analysis and modeling

Genotype data from repeated multiplex PCRs were compiled in MICROSOFT EXCEL (2007) to obtain a consensus microsatellite dataset. Consensus genotypes were compared with GENALEX [34], an add-on software for MICROSOFT EXCEL. We tested for Hardy-Weinberg equilibrium (HWE) using GENEPOP on the web [35] and tested for null alleles using CERVUS v.3.0 [36]. To evaluate the power of the markers used for individual identification, both the probability of identity (P_{ID}) and the P_{ID} for siblings, $P_{ID(sib)}$ were calculated using GENALEX.

Samples were considered to have originated from the same individual if they i) had similar bolus circumferences, ii) were identified as originating from the same sex, and iii) had the same genotype profile (with one mismatching allele allowed). Individuals identified the first time were considered “captures” and subsequent encounters of the same individual on a different sampling occasion were considered a “recapture.” Minimum population size was determined by the sum of unique genotypes.

Total abundance was estimated in a capture-recapture modeling framework using MARK v.7.1 [37,38]. We fitted four different closed population estimation models to the data: M(0), M(t), M(h), and M(th) (*sensu* [38]). The behavior model M(b) and its combinations with other models were not applicable because we sampled dung.

A low number of sampling occasions in combination with few identified individuals can substantially decrease estimation power of unknown parameters, especially when heterogeneity models are applied (Brian D. Gerber, personal communication). In order to obtain reliable results for the limited dataset of the RiauJambi area, we merged the data from both survey areas to obtain a larger dataset for the estimation of capture and re-capture probabilities, and then analyzed abundance separately [39]. Model averaging was applied to obtain consensus estimates with asymmetrical confidence intervals [40] for each survey area with models contributing according to their second order Akaike Information Criterion (AICc) weights [41]. To allow a comparison of the precision of our results with those of other surveys, we calculated PCCL, the ratio of the confidence limit (CL) to the population estimate (N) [42]. Abundance estimates were converted into density estimates by dividing population size by the area of survey blocks occupied by elephants in 2011 for each of the two survey areas.

Age was estimated using the classification system developed for dung counts [43]. We distinguished three age classes [19]: class 1: 0-5 years old = neonates & juveniles with mean bolus circumference (mbc) \leq 30 cm, class 2: 5-15 years old = subadults with 30 cm < mbc \leq 42 cm, and class 3: older than 15 years = adults with mbc > 42 cm. For repeatedly sampled individuals with dung dimensions close to age class boundaries, the average bolus circumference calculated for all samples from that individual was used for analysis.

To evaluate whether the physical separation of the two subpopulations had already caused genotypic differentiation, we applied the software STRUCTURE v.2.3.4 [44]. We chose the ‘correlated allele model’ because it is better suited to detect subtle population structures [45]. We applied *K*-values from 1 to

7. The required allele frequency distribution parameter λ was estimated for $K = 1$ (10 runs), as suggested [45]. Its value of 0.958 was subsequently used in all other K -likelihood estimations. To determine the appropriate burn-in and run lengths for accurate parameter estimates, we set $K = 1$ and watched for the likelihoods to converge under various burn-in and run lengths. The final burn-in and run lengths of Markov chains were then 50,000 and 100,000, respectively. To avoid results based on local maxima and to verify the consistency of estimates across runs, we reformed 10 independent runs for each K and its associated parameter set. After completion of all runs we submitted a compressed results file to the web-based software STRUCTURE HARVESTER v.0.6.94 [46]. This software analyzes the results by the ΔK -approach, which exploits the rate of change in the log probability of data between successive K values to determine the most likely K [47].

Results

Indirect signs of elephants such as dung and footprints were detected in all but one survey block, but recent elephant activity (and thus fresh dung) was limited to nine out of the 12 blocks (Fig. 1). A total of 398 samples were collected. The survey area Sumai yielded 270 samples, more than twice as many as the 128 samples from the RiauJambi survey area. The majority of samples ($n=326$, 81.9%) were taken from fresh dung piles (Table 1). After merging replicates (repeated sampling from the same dung pile) and excluding samples with incomplete information, a total of 357 samples remained for DNA extraction and analysis.

Table 1. Number of dung samples collected in 2011, amplification success, and individual elephants captured and re-captured for survey area Sumai and RiauJambi, Bukit Tigapuluh landscape, Indonesia.

Study area	Item	Sampling occasion 1	Sampling occasion 2	Sampling occasion 3	Total
Sumai	Fresh/recent dung piles sampled	94/17	108/2	39/10	241/29
	Samples amplified at min. 13 loci	68	74	48	190
	Individuals captured/re-captured	43/0	22/9	9/25	74/34
RiauJambi	Fresh/recent dung piles sampled	39/0	38/23	8/20	85/43
	Samples amplified at min. 13 loci	22	57	8	87
	Individuals captured/re-captured	14/0	16/9	0/5	30/14

Elephant DNA was successfully amplified from 294 samples (82.35%), but only 277 samples (77.59%) yielded consistent results, of which 272 (76.2%) were successfully sexed. Locus EMU04 was monomorph and thus excluded from further analysis. The observed heterozygosity (H_{obs}) was low to medium and similar to the heterozygosity expected (H_{exp}) under HWE. All loci were at HWE, and there was no evidence for the presence of null alleles (Table 2). We observed up to five different alleles per locus and the presence of two private alleles for each survey area: a160 (EMU12) and a123 (EMU17) in Sumai samples, and a152 (FH103) and a218 (FH94R) in samples from RiauJambi. P_{ID} was 2.96E-07 and $P_{ID(sib)}$ was 8.72E-04, indicating that our set of primers was well able to discriminate between closely related individuals with negligible risk of shadow effects (*sensu* [48]).

We identified 104 individuals, of which 74 originated from Sumai and 30 from the Riau-Jambi area (Table 1). Of these, 26 were classified as juveniles, 44 as subadults, 33 as adults, and one individual remained unclassified due to insufficient dung measurements. Molecular sexing revealed the presence of 73 females and 29 males in our sample. For two elephants sex could not be identified reliably (Table 3). While there were almost twice as many male calves than females, the sex ratio in older age classes was substantially biased towards females, with only three adult bulls detected by our survey at the landscape level.

Table 2. Information on the fluorescent label used (Label), multiplex panel affiliation (Panel), allele size range (ASR), number of alleles (N_A), observed heterozygosity (H_{obs}), expected heterozygosity under HWE (H_{exp}), Hardy-Weinberg-Equilibrium p-value (P (HWE)), and probable null allele frequencies (F_{null}) for 13 microsatellite loci used for non-invasive genotyping of Sumatran elephants in Bukit Tigapuluh, Indonesia.

No	Locus	Label	Panel	ASR (bp)	N_A	H_{obs}	H_{exp}	P (HWE)	F_{null}
1	EMU03	PET	1	138-145	4	0.62	0.63	0.76	0.00
2	EMU07	FAM	1	103-123	5	0.75	0.75	0.88	0.00
3	EMU10	PET	1	95-97	2	0.39	0.45	0.21	0.07
4	EMU14	NED	1	128-134	3	0.61	0.67	0.48	0.05
5	FH60	FAM	1	154-156	2	0.15	0.16	0.55	0.01
6	LafMS04	VIC	1	144-147	2	0.19	0.19	1.00	-0.01
7	EMU12	PET	2	143-160	3	0.18	0.18	1.00	0.00
8	EMU13	NED	2	102-106	2	0.42	0.47	0.40	0.05
9	EMU17	FAM	2	119-129	4	0.58	0.66	0.19	0.07
10	FH103	FAM	2	149-158	4	0.57	0.55	0.86	-0.01
11	FH94R	VIC	2	216-224	4	0.61	0.59	0.84	-0.03
12	LafMS02	VIC	2	131-137	3	0.47	0.56	0.13	0.09
13	LafMS05	NED	2	152-156	2	0.43	0.50	0.23	0.07

Table 3: Age class and sex of successfully genotyped Sumatran elephants for Sumai and RiauJambi area, Bukit Tigapuluh landscape, Indonesia.

Study area	Age class	Female	Male	n.d.	Total
Sumai	Juvenile	4	13	0	17
	Subadult	26	7	1	34
	Adult	20	3	0	23
	n.d.	0	0	0	0
	Total	50	23	1	74
RiauJambi	Juvenile	5	4	0	9
	Subadult	8	1	1	10
	Adult	10	0	0	10
	n.d.	0	1	0	1
	Total	23	6	1	30

n.d.: not determined

Total population size estimates differed only slightly among models for both study sites. This and the generally small difference in AIC values suggested robust results. The simple, less parameterized models M(0) and M(t) performed better than those including heterogeneity (Table 4). The results of model averaging suggested that 99 ± 9.30 (SE) elephants (95% CI = [86, 125]; PCCL = 38.59%) were present in the Sumai area and 44 ± 4.62 (SE) elephants (95% CI = [37, 56]; PCCL = 43.18%) were present in the RiauJambi area in 2011 (Table 4). Based on the total area of survey blocks used by elephants in

2011, our abundance estimates translate into an estimated density of roughly 0.05 and 0.13 elephant per km² for RiauJambi and the Sumai area, respectively.

The STRUCTURE analysis indicated that the most likely population structure comprised the presence of two genotypic clusters (Fig. 2). For $K = 2$ the mean $\ln P(K)$ was -2193.17 (s.d. = 3.81), while for neighboring $K = 1$ and $K = 3$ the values were -2220.46 (s.d. = 0.419) and -2242.17 (s.d.= 20.637), respectively. This was further strengthened by ΔK which was highest for $K = 2$ ($\Delta K = 220.026$), and fell from $\Delta K = 5.129$ for $K = 3$ down to $\Delta K = 0.21$ for $K = 6$. The genotypic clusters, however, were not fully congruent with geographic distribution, as only 22 out of 30 individuals from RiauJambi had a probability of $Q > 0.5$ of belonging to a common cluster. Among those were 17 individuals with Q -values higher than 0.85, indicating true genotypic cluster assignment. All 74 elephants from Sumai had a higher than 50% probability of belonging to the other cluster (together with 8 elephants from RiauJambi). However, among the Sumai elephants only 17 individuals had cluster assignment values of $Q > 0.85$. The majority of elephants had admixed genotypes.

Table 4. Sumatran elephant population size estimates (N) based on four different capture-recapture models (*sensu* [38]) and model averaging using MARK.

Survey area	Model	$\Delta AICc$	AICc weight	N	SE	CI(lo)	CI(up)	PCCL (%)
Sumai	M(0)	0.00	0.53	99	9.37	87	125	38.74
	M(t)	0.75	0.36	99	9.16	86	124	38.25
	M(h)	4.13	0.07	99	9.37	87	125	38.74
	M(t+h)	4.96	0.04	99	9.16	86	124	38.25
	Average	-	-	99	9.30	86	125	38.59
RiauJambi	M(t)	0.00	0.70	44	4.62	37	56	42.97
	M(0)	2.58	0.19	45	4.76	38	57	43.57
	M(t+h)	4.15	0.09	44	4.62	37	56	42.97
	M(h)	6.67	0.02	45	4.76	38	57	43.57
	Average	-	-	44	4.66	37	56	43.18

AICc: for finite sample size corrected Akaike Information Criterion, SE: standard error, CI(lo): lower confidence limit, CI(up): upper confidence limit, PCCL: ratio of the confidence limit to the population estimate.

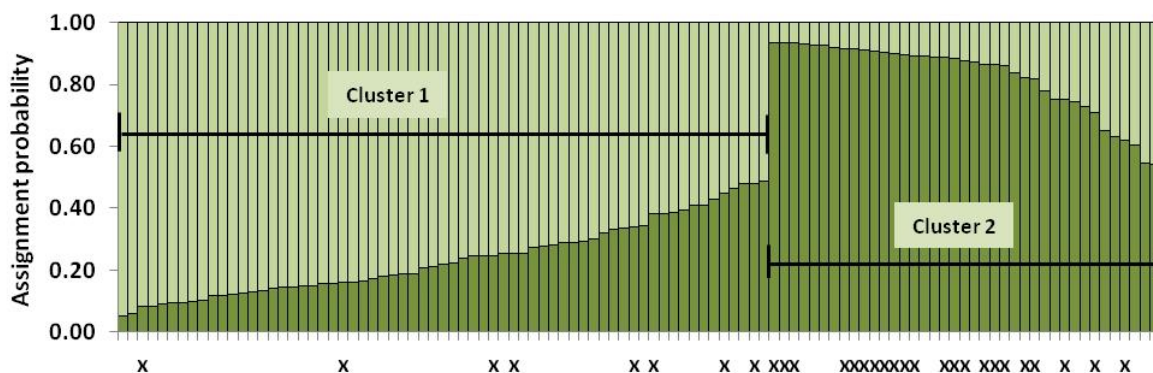


Fig. 2. Estimated population structure of Sumatran elephants in Bukit Tigapuluh, Indonesia, from Bayesian STRUCTURE analyses [44]. The most likely population structure consisted of two genotypic clusters. Bars represent individuals, sorted according to their probability to belong to either cluster 1 or 2. Cluster membership proportions are visualized by colored fractions (cluster 1: lime green, cluster 2: green). Individuals sampled in RiauJambi survey area are marked with an “x”.



Fig. 3. A group of Sumatran elephants searching for food in a pulpwood concession in survey area RiauJambi (A); part of a larger elephant herd drinking and taking a bath in survey area Sumai (B); forest destruction, one of the main problems for elephant conservation in Bukit Tigapuluh (C); postmortem of a poisoned adult female elephant (D). Photo credit: Frankfurt Zoological Society / Alexander Moßbrucker & Albert Tetanus.

Discussion

Accuracy and precision of the population size estimates

Population size estimates for the Bukit Tigapuluh Landscape (including both the Sumai and RiauJambi areas) range from 300-400 animals in 1984 [49] to only 50 animals in 2007 (as mapped in [50]). However, with the exception of a dung count census from 2009 [29] that estimated 117 elephants for the Sumai area (in older literature referred to as “Semambu”) and 47 elephants for the RiauJambi area, available estimates are either outdated, entirely based on guesswork, or both, and therefore do not likely reflect the current population size. While our abundance estimates were very similar to those of the dung count from 2009 [29], we achieved a much smaller PCCL, which supports the conclusion of others [19] that non-invasively collected DNA-based capture-recapture methods are as reliable and more precise than dung counts. Being the most recent and most precise study, our estimates of 99 elephants (95% CI = [86, 125], PCCL = 38.59%) for the Sumai area and of 44 elephants (95% CI = [37, 56], PCCL = 43.18%) for the RiauJambi area should be used for conservation planning and as a baseline for monitoring.

Fragmentation and genotypic structure

An approximately 30km wide corridor where no elephant presence has been found divides the Sumai and RiauJambi populations. The absence of evidence for any individual exchange between these study areas suggests that the elephants in RiauJambi and Sumai represent separate subpopulations. The

genotypic structuring clearly indicated the progressed genotypic isolation of the two subpopulations. The high number of genotypically admixed individuals depicts the situation of the past when gene-flow was undisturbed. However, the now elephant-free corridor between the two areas will likely enforce the 'isolation by distance' process.

Elephant Density and Habitat Condition

Our study revealed an extremely low population density in Bukit Tigapuluh compared to areas of India or Sri Lanka, where 10 to 40 times more elephants inhabit similarly sized areas (*e.g.*, [51,52]), which is not surprising as tropical forests are known for their small carrying capacity [53]. However, the overall density for RiauJambi (0.05 elephants per km²) is extremely low, even for Sumatra, where mean densities of 0.15 and 0.18 elephants per km² and locally up to 0.57 individuals per km² had been estimated previously [10]. Moreover, both our estimates were substantially smaller than those reported for the majority of areas surveyed in Borneo [9].

Preliminary observations made during the field survey suggested that low overall densities may have been caused by the limited availability of habitat, as both populations were using much smaller areas than the perimeter of their distribution would allow. Satellite imagery revealed a steep decline in natural forest cover during the years preceding the survey, with an estimated 2,150.5 km² of natural forest (43% of the total forest) converted between 2005 and 2011 (Frankfurt Zoological Society, unpublished data 2005-2011). Much of this conversion took place within the elephant's range, leaving a heavily fragmented habitat (Fig. 1). The effective, usable area may therefore be considerably smaller than the total area of the 11 survey blocks where elephants were detected in 2011, as much of this area was occupied by both legal and illegal fields and plantations (personal observation), areas where food is limited (*e.g.*, in case of monoculture pulpwood plantations) or where elephants are not tolerated (*e.g.*, in oil palm plantations). In addition, increased anthropogenically caused mortality is likely to have contributed to the low overall densities observed. Evidence for the killing of elephants in the past is outlined below.

Age Structure, Sex Ratio and Evidence for Illegal Killing of Elephants

Asian elephants are generally long-lived and slow-reproducing animals that if undisturbed, enjoy relatively high survival rates well into old age after a few slightly more risky postnatal years [53]. In natural populations we therefore expect to find a greater number of adult animals than subadults and juveniles, as observed, *inter alia*, by Kumara et al. [51] and Easa & Balakrishnan [54] in India. Our sample, however, revealed a distorted age structure for Bukit Tigapuluh, with almost equal numbers of individuals in all age classes for the RiauJambi area and a clear dominance of animals younger than 15 years in the Sumai area. Interestingly, the only available study for other areas in Sumatra reported a similarly young population, which at that time seemed to be dominated by subadult individuals, noting that calves may have been underrepresented in the sample [18]. These unexpected findings were not discussed [18], but computer simulations indicated that populations suffering from increased adult mortality show a relatively increased number of calves, which will subsequently result in a general age distribution shift towards younger classes [53]. Thus, a young population might not necessarily mean recovery, but could instead be a sign of substantial losses in older age classes, as we suspect to be the case for the Bukit Tigapuluh population.

Strong skews in the sex ratio lend further support to this scenario. Statistically, elephants are born at equal sex ratios, but males naturally suffer from somewhat increased mortality rates, which explains the slightly female-biased sex ratios in older age classes observed in many populations [53]. For example, in Rajaji National Park, India, as well as in Ruhuna National Park, Sri Lanka, populations had approximately twice as many adult females as adult males [55, 56], and an adult sex ratio of 1:0.84 (female:male) was estimated for Uda Walawe National Park in Sri Lanka [52]. Natural populations are also partly stochastic systems, with small populations being more affected by random effects than

larger ones [53]. However, it is unlikely that the extreme distortions observed in our study could have been caused solely by chance or natural difference in mortality, but may be due to frequent killing of elephants in the past. This assumption is supported by anecdotal evidence for heavy ivory poaching and poisoning of elephants in response to crop raids. Forest police investigation results compiled by the author prove that humans killed 17 elephants between 2008 and 2014. Although sound direct evidence is lacking, it is likely that elephant killing was far more common prior to 2008, as during this time the region was mostly uncontrolled and unmonitored. We therefore assume that anthropogenically increased mortality has shaped the population structure in Bukit Tigapuluh, as it has for other elephant populations in Asia [53,57,58].

Implications for Conservation

The Bukit Tigapuluh landscape supports the largest known elephant population in Central Sumatra and is therefore among the most important areas for elephant conservation in Indonesia. The protection of the Bukit Tigapuluh elephants (Fig. 3) and their habitat should consequently be of high priority. While potentially viable populations of Sumatran elephants are known to exist in Lampung and are suspected in Aceh province, preliminary unpublished information gathered at a workshop of the FKGI held in Bogor in 2014 suggests that most of the other populations are extremely small. Although these small populations may harbor alleles that are important for the survival of the subspecies, their long-term survival is unlikely. Other than Bukit Tigapuluh, it is likely that only one other landscape in Central Sumatra, Tesso Nilo in Riau province, still holds more than 100 animals. Further study is needed to determine whether any additional viable populations exist that are as yet undetected.

Our study revealed two specific conservation issues:

- The effective population size is reduced by fragmentation into two isolated subpopulations, increasing the risk of inbreeding and loss of local adaptation by genetic drift.
- Population viability is compromised by the distorted demographic structure.

Although the aforementioned conservation issues must be addressed, the fundamental challenges for the survival of the Sumatran elephants in Bukit Tigapuluh are the destruction of their habitat and the impact of illegal killing on their numbers. Habitat destruction and the killing of elephants (Fig. 3) must be addressed as a priority if the subspecies is to survive.

While it is beyond the scope of this study to provide site-specific solutions for the conservation issues discussed, we offer some thoughts about a suitable conservation strategy for the region:

- As no other elephant population appears to be close enough to allow genetic exchange with Bukit Tigapuluh, a meta-population management program that includes an occasional re-stocking of individuals from other populations is required. In addition, exchange between subpopulations could be facilitated by increasing the permeability of the separating corridor in order to allow for natural dispersal.
- The poaching of elephants and their killing due to Human-Elephant Conflict (HEC) are thought to be the two primary causes of elephant deaths. Incidences of poaching could be reduced to some extent by the close monitoring of animals at risk, which may be technically feasible if field teams supported by satellite and radio telemetry are deployed (see [27] for a first trial in the region). However, to be effective, prevention may require deterrence in the form of serious law enforcement. In spite of a long history of illegal elephant killings in Bukit Tigapuluh (first reported in 1995 by [49]), convictions are extremely rare, and improvements to investigations and legal proceedings are likely required.
- Several HEC mitigation methods were tested in the Bukit Tigapuluh region and found to be suitable [26]. However, these methods can only lead to the effective protection of elephants if the habitat is safe and large enough to allow elephants to satisfy their basic needs away from

settlements, fields, and plantations. Allocating and protecting sufficient elephant habitat is therefore a high priority for HEC mitigation.

- As most elephants roam outside protected areas, present conservation efforts need to be adapted to broaden areas of protection. Landscape species such as elephants may need much larger conservation areas with different characteristics from those already allocated. The coexistence of elephants and humans on intensively used agriculture land may not be possible, but extensive productive forest concessions surrounding the Bukit Tigapuluh National Park provide potentially safe habitat. Within these concessions, wildlife-friendly management beyond statutory conservation commitments could transform much of the landscape into suitable elephant habitat without the need to remove all commercial interests. In addition, ecosystem restoration concessions [59] provide an opportunity to restore and protect the archetype forest ecosystem in parts of the production forest, and therefore represent a highly valuable land use model for areas inhabited by elephants and other (critically) endangered species.

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References

- [1] Shoshani, J. and Eisenberg, J. F. 1982. *Elephas maximus*. *Mamm. Species* 182:1–8.
- [2] Choudhury, A. *et al.* 2008. *Elephas maximus*. *The IUCN Red List of Threatened Species*. www.iucnredlist.org, date consulted March 15 2015.
- [3] Fleischer, R., Perry, E., Muralidharan, K., Stevens, E. and Wemmer, C. 2001. Phylogeography of the Asian elephant (*Elephas maximus*) based on mitochondrial DNA. *Evolution (N. Y.)* 55:1882–1892.
- [4] Gopala, A. *et al.* 2011. *Elephas maximus ssp. Sumatranus*. *The IUCN Red List of Threatened Species*. www.iucnredlist.org, date consulted March 15 2015.
- [5] Azmi, W. and Gunaryadi, D. 2011. Current Status of Asian Elephants in Indonesia. *Gajah* 35:55–61.
- [6] Blake, S. and Hedges, S. 2004. Sinking the flagship: The case of forest elephants in Asia and Africa. *Conserv. Biol.* 18:1191–1202.
- [7] Thompson, W. L. Ed. 2004. *Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters*. Washington: Island Press.
- [8] Hedges, S. and Lawson, D. Eds. 2006. *Dung-based Population Survey Standards for the MIKE Programme*. CITES MIKE Programme, Nairobi, Kenya.
- [9] Alfred, R., Ahmad, A. H., Payne, J., William, C. and Ambu, L. 2010. Density and Population Estimation of the Bornean Elephants (*Elephas maximus borneensis*) in Sabah Borneo. *Online J. Biol. Sci.* 10:92–102.
- [10] Hedges, S., Tyson, M. J., Sitompul, A. F., Kinnaird, M. F. and Gunaryadi, D. 2005. Distribution, status, and conservation needs of Asian elephants (*Elephas maximus*) in Lampung Province, Sumatra, Indonesia. *Biol. Conserv.* 124:35–48.

- [11] Barnes, R. F. W. 2002. The problem of precision and trend detection posed by small elephant populations in West Africa. *Afr. J. Ecol.* 40:179–185.
- [12] Fernando, P. 2003. Reliable Noninvasive Genotyping: Fantasy or Reality? *J. Hered.* 94:115–123.
- [13] Waits, L. P. 2004. Using Noninvasive Genetic Sampling to Detect and Estimate Abundance of Rare Wildlife Species. In: *Sampling Rare or Elusive Species*. Thompson, W. L. (Ed.), pp.211–228. Island Press, Washington.
- [14] Lukacs, P. M. and Burnham, K. P. 2005. Review of capture-recapture methods applicable to noninvasive genetic sampling. *Mol. Ecol.* 14:3909–19.
- [15] Karanth, K. U., Nichols, J. D. and Hedges, S. 2012. Estimating Abundance and Other Demographic Parameters in Elephant Populations Using Capture-Recapture Sampling: Statistical Concepts. In: *Monitoring Elephant Populations and Assessing Threats. A Manual for Researchers, Managers and Conservationists*. Hedges, S. (Ed.), pp.112-136. Universities Press Private Limited, Hyderabad.
- [16] Vidya, T. N. C. Application of molecular sexing to free-ranging Asian elephant (*Elephas maximus*) populations in southern India. *Res. Commun.* 85:1074–1077.
- [17] Ahlering, M. A., Hailer, F., Roberts, M. T. and Foley, C. 2011. A simple and accurate method to sex savannah, forest and Asian elephants using noninvasive sampling techniques. *Mol. Ecol. Resour.* 11:831–834.
- [18] Reilly, J. 2002. Growth in the Sumatran elephant (*Elephas maximus sumatranus*) and age estimation based on dung diameter. *J. Zool.* 258:205–213.
- [19] Hedges, S., Johnson, A., Ahlering, M., Tyson, M. and Eggert, L. S. 2013. Accuracy, precision, and cost-effectiveness of conventional dung density and fecal DNA based survey methods to estimate Asian elephant (*Elephas maximus*) population size and structure. *Biol. Conserv.* 159:101–108.
- [20] Gray, T. N. E., Vidya, T. N. C., Potdar, S., Bharti, D. K. and Sovanna, P. 2014. Population size estimation of an Asian elephant population in eastern Cambodia through non-invasive mark-recapture sampling. *Conserv. Genet.* 15:803–810.
- [21] Chakraborty, S., Boominathan, D., Desai, A. a. and Vidya, T. N. C. 2014. Using genetic analysis to estimate population size, sex ratio, and social organization in an Asian elephant population in conflict with humans in Alur, southern India. *Conserv. Genet.* 15:897–907.
- [22] Eggert, L. S., Eggert, J. A. and Woodruff, D. S. E. 2003. Estimating population sizes for elusive animals: The forest elephants of Kakum National Park, Ghana. *Mol. Ecol.* 12:1389–1402.
- [23] Eggert, L. S., Patterson, G. and Maldonado, E. 2007. The Knysna elephants : a population study conducted using faecal DNA. *Afr. J. Ecol.* 46:19–23.
- [24] Whitten, T., Damanik, S. J., Anwar, J., and N., H., Eds. 2000. *The Ecology of Sumatra*. Periplus Editions Ltd.
- [25] Moßbrucker, A. M. 2009. *Zum Status des Sumatra Elefanten (Elephas maximus sumatranus) im Landschaftsraum Bukit Tigapuluh, Sumatra, Indonesien : Abundanz Altersstruktur und Gefährdung*. Diploma thesis, Freiburg University, Germany.
- [26] Moßbrucker, A. M. 2013. *Towards a Peaceful Coexistence between Men and Elephants in Bukit Tigapuluh, Indonesia (Phase I)*. Final Report to USFWS. Frankfurt Zoological Society, Jambi, Indonesia.
- [27] Moßbrucker, A. M. 2013. *Saving the Elephants of Bukit Tigapuluh (Sumatra) with GPS and GIS*. Final Report to USFWS. Frankfurt Zoological Society, Jambi, Indonesia.
- [28] Sitompul, A. F., Griffin, C. R. and Fuller, T. K. 2013. Sumatran elephant ranging behavior in a fragmented rainforest landscape. *Int. J. Biodivers. Conserv.* 5:66–72.
- [29] Archie, E. A., Moss, C. J. and Alberts, S. C. 2003. Characterization of tetranucleotide microsatellite loci in the African Savannah Elephant (*Loxodonta africana africana*). *Mol. Ecol. Notes* 3:244–246.
- [30] Kongrit, C. et al. 2008. Isolation and characterization of dinucleotide microsatellite loci in the Asian elephant (*Elephas maximus*). *Mol. Ecol. Resour.* 8:175–177.
- [31] Comstock, K. E., Wasser, S. K. and Ostrander, E. A. 2000. Polymorphic microsatellite DNA loci identified in the African elephant (*Loxodonta africana*). *Mol. Ecol.* 90:993–1011.

- [32] Nyakaana, S. and Arctander, P. 1998. Isolation and characterization of microsatellite loci in the African elephant, *Loxodonta africana*. *Mol. Ecol.* 7:1436–1437.
- [33] Eggert, L. S., Ahlering, M. and Manka, S. 2008. Lessons from genetic census of elephants. In: *Proceedings of the 2007 international elephant conservation and research symposium*. Olson, D. (Ed.), pp.157–164. International Elephant Foundation.
- [34] Peakall, R. and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *BIOINFORMATICS* 28:2537–2539.
- [35] Raymond, M. and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86:248–249.
- [36] Kalinowski, S. T., Taper, M. L. and Marshall, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16:1099–1106.
- [37] White, G. C. and Burnham, K. P. Program MARK: survival estimation from populations of marked animals. *Bird Study* 46, 120–139 (1999).
- [38] White, G. C. 2007. Closed population estimation models and their extensions in Program MARK. *Environ. Ecol. Stat.* 15:89–99.
- [39] Gerber, B. D., Ivan, J. S. and Burnham, K. P. 2014. Estimating the abundance of rare and elusive carnivores from photographic-sampling data when the population size is very small. *Popul. Ecol.* 56:463–470.
- [40] Cooch, E. G. and White, G. C. 2014. *Program MARK - A Gentle Introduction, 13th edition*. Available online at <http://www.phidot.org/software/mark/docs/book/>. Accessed March 2015.
- [41] Burnham, K. P. and Anderson, D. R. 2002. *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*. Springer-Verlag, New York.
- [42] Barnes, R. F. W. 2001. How reliable are dung counts for estimating elephant numbers? *Afr. J. Ecol.* 39:1–9.
- [43] Tyson, M., Hedges, S. and Sitompul, A. F. 2002. *WCS-Indonesia Sumatran Elephant Project: Six month report for January-June, 2002*. Wildlife Conservation Society, Jakarta, Indonesia.
- [44] Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- [45] Pritchard, J. K., Wen, X. and Falush, D. 2010. Documentation for structure software : Version 2.3., available online at <http://pritch.bsd.uchicago.edu/structure.html>, consulted March 15 2015.
- [46] Earl, D. A. and vonHoldt, B. M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4:359–361.
- [47] Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* 14:2611–2620.
- [48] Mills, L. S., Citta, J. J., Lair, K. P., Schwartz, M. K. and Tallmon, D. A. 2000. Estimating Animal Abundance Using Noninvasive DNA Sampling: Promise and Pitfalls. *Ecol. Appl.* 10:283–294.
- [49] Blouch, R. A. and Simbolon, K. 1995. *Elephants In Northern Sumatra*. A World Wildlife Fund Report, Bogor, Indonesia.
- [50] Uryu, Y. et al. 2008. *Deforestation, Forest Degradation, Biodiversity Loss and CO2 Emissions in Riau, Sumatra, Indonesia*. WWF Indonesia Technical Report, Jakarta, Indonesia.
- [51] Kumara, H. N., Rathnakumar, S. and Kumar, M. A. 2012. Estimating Asian elephant, *Elephas maximus*, density through distance sampling in the tropical forests of Biligiri Rangaswamy Temple Tiger Reserve, India. *Trop. Conserv. Sci.* 5:163–172.
- [52] De Silva, S., Ranjeewa, A. D. G. and Weerakoon, D. 2011. Demography of Asian elephants (*Elephas maximus*) at Uda Walawe National Park, Sri Lanka based on identified individuals. *Biol. Conserv.* 144:1742–1752.
- [53] Sukumar, R. 2003. *The Living Elephants: Evolutionary Ecology, Behavior, and Conservation*. Oxford University Press, Oxford.

- [54] Easa, P. S. and Balakrishnan, M. 1995. The population density and structure of asian elephants in Parambikulam Wildlife Sanctuary, Kerala, India. *Bombay Nat. Hist. Soc.* 92:225–229.
- [55] Williams, A. C., Johnsingh, A. J. T. and Krausman, P. R. 2007. Population Estimation And Demography Of The Rajaji National Park Elephants, North-West India. *J. Bombay Nat. Hist. Soc.* 104:145–152.
- [56] Katugaha, H., de Silva, M. and Santiapillai, C. 1999. A long-term study on the dynamics of the elephant (*Elephas maximus*) population in Ruhuna National Park, Sri Lanka. *Biol. Conserv.* 89:51–59.
- [57] Sukumar, R. 1989. *The Asian Elephant: ecology and management*. Cambridge University Press, Cambridge.
- [58] De Silva, S. *et al.* 2013. Demographic variables for wild Asian elephants using longitudinal observations. *PLoS One* 8:1-10.
- [59] Menteri Kehutanan 2004. *Peraturan Menteri Kehutanan Nomor : SK.159 / Menhut-II / 2004 - Restorasi Ekosistem Di Kawasan Hutan Produksi*.