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Evaluating the effect of forest loss and agricultural expansion on Sumatran tigers from scat surveys

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ABSTRACT

Sumatran tigers (*Panthera tigris sumatrae*) are a critically endangered carnivore restricted to the island of Sumatra, and like many other large mammals on the Indonesian archipelago, they are threatened by high levels of poaching and widespread habitat degradation. Here, we conduct the first range-wide assessment of Sumatran tiger genetics using scat surveys and show that the wild population retains levels of genetic heterozygosity comparable to mainland tigers. However, the population also exhibits signs of subdivision due to the unprecedented rates of deforestation and land conversion in the last 30–40 years. The fact that this subspecies retains such levels of heterozygosity despite high rates of habitat loss and increasing isolation suggests a form of genetic extinction debt with an elevated risk of extinction if no action is taken within the next 30–100 years (see Kenney et al., 2014). However, the inherent time delay in extinction debt provides opportunities for conservation if habitat quality can be improved and connections between existing population fragments can be made. Our study highlights the importance of genetic studies for providing baseline information to improve the population management of highly threatened carnivore species. Mitigating further habitat degradation and expansion of oil palm and other cash crops in this region would improve the viability not only of Sumatran tiger populations, but of other threatened large mammal species as well.

1. Introduction

Sumatra supports a disproportionately high level of global biodiversity. There are 5 bioregions on the island (freshwater swamp, lowland rainforest, montane rainforest, peat swamp, and tropical pine forest), that support up to 200 species of mammals and 580 species of birds, including some that are extinct or virtually so elsewhere in Indonesia, such as the rhino, elephant, and tiger (Whitten et al., 2000; Wikramanayake et al., 2002). Much of this biodiversity is at risk due to vast areas of primary forest (up to 0.38 million hectares per year) being cleared for timber products or converted to other land uses such as agriculture (e.g. coffee, rubber), oil palm, and *Acacia mangium* tree plantations (Margono et al., 2012; Sodhi et al., 2004; Stibig et al., 2014).

Much of the land clearance began in southern Sumatra in the 1970s when the Indonesian government introduced a transmigration scheme to relocate people from other islands in the archipelago (Imbernon, 1999). It is now home to nearly 51 million people spread across 10 provinces (BPS Statistics Indonesia, 2016), and it is estimated that between 1969 and 1993 up to 8 million people relocated and cleared 1.7 million hectares of lowland forest for settlements and agricultural smallholdings (Barber and Schweithelm, 2000; Gaveau et al., 2009a). Much of this degraded forest was converted to industrial timber estates and oil palm plantations in the early 2000s, and with no more accessible lowland forest in south Sumatra, attention has now turned to the peat swamp forests of east Sumatra (Margono et al., 2014).

It is estimated that ~70% of Sumatra's primary lowland forest has already been lost and this trend is set to continue as Indonesia aims to meet much of the global demand for palm oil, pulp, and timber products (Geist and Lambin, 2002; Kinnaird et al., 2003; Suyadi, 2010). With net returns of up to $13,000 per hectare of tropical timber or oil palm there are many commercial barriers to conserving the remaining primary habitat (Wilcove et al., 2013).

Tiger conservation, like that of rhinos and elephants, poses a difficult challenge in this context as they require a large amount of space, have a tendency towards conflict with people in secondary forest or at protected area boundaries, and are under constant threat from poaching due to their commercial value (Linkie et al., 2018). The main remaining populations of these species are therefore located in a few large protected areas of primary lowland or montane forest (Wibisono et al.,

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2011).

Current estimates put the global tiger population at 3000–4000 individuals. Sumatra is one of three regions combined (including India and Russia) containing ~80% of remaining tiger habitat with a Sumatran population of ~500 tigers (Tilson et al., 1993; Linkie et al., 2008a; Goodrich et al., 2015). The Sumatran tiger (*Panthera tigris sumatrae*) is recognized as a distinct subspecies due to its unique location, genetics, and morphological differences (Cracraft et al., 1998; Kitchener, 1999; Hendrickson et al., 2000; Luo et al., 2004; Kitchener and Yamaguchi, 2010; Wilting et al., 2015). It also represents the last remaining population of Sunda tigers since the Java and Bali subspecies are now extinct (Xue et al., 2015).

Continued land conversion across the tiger's range has created a patchwork of primary forest (lowland, montane or peat swamp), secondary forest, and human disturbance that prompted the creation of Tiger Conservation Landscapes (TCLs), and more recently Source Sites, which overlap with the distribution of highly threatened species such as the Sumatran rhino, Asian elephant, and Sumatran orangutan (Sanderson et al., 2006; Walston et al., 2010; Wich et al., 2016). Although tigers can inhabit a broad range of forest types, abundance or occupancy rates are highest in areas of low human presence and infrastructure (Carroll and Miquelle, 2006; Johnson et al., 2006; Harihar and Pandav, 2012; Sunarto et al., 2012; Hebblewhite et al., 2014). Previous studies have shown that tigers mostly require a suitable prey base and good ground cover for hunting to persist, even in degraded forest (Linkie et al., 2008b; Smith, 2009; Sunarto et al., 2012). Designation of these large conservation areas was therefore intended to protect sufficient habitat and prey, free from human threats, to maintain self-sustaining tiger populations. Sumatra holds 12 TCLs and 4 Source Sites covering up to 88,000 km^2 (Wibisono and Pusparini, 2010), and these largely overlap with protected area boundaries. Here we use genetic data obtained from an island-wide scat survey to explore how disruption of the once contiguous forest on Sumatra has affected this last Sunda tiger subspecies.

2. Material and methods

2.1. Sample collection

Fecal samples (scats) were collected from nine different field sites across Sumatra (Fig. 1a, Table A1). Samples were collected during dedicated scat collection surveys or opportunistically during population monitoring studies prior to this study. Fresh samples were also obtained from a facility holding wild tigers captured following conflict with rural communities. Scat surveys were conducted in a range of habitat types (montane, lowland, and production forests), and sampling transects followed animal trails and logging routes in high tiger density areas identified from camera trap survey data (unpublished results). Field teams covered one transect per day and each route was sampled just once with teams instructed to collect all fecal samples likely to have been deposited by a tiger based on size and appearance. Each sampling period lasted for an average of 2 weeks. We also tested the use of a detection dog in 3 sites (Way Kambas NP, Kerinci Seblat NP, and Batang Hari protection forest) using a 2-year old, male, Labrador Retriever from Bogor, West Java. The dog was trained over 3 weeks by an experienced dog handler to recognize the scent of tiger scats using samples from captive individuals. Dog surveys were conducted alongside the field teams with 20-minute work periods alternating with 10-minute rest breaks.

2.2. Laboratory methods

Each sample was initially preserved with silica gel beads in the field then transferred to ≥96% ethanol once received in the laboratory. Extractions were performed using 2–3 mm scrapings taken from the outer surface of each scat. The QIAamp DNA stool mini kit (Qiagen) was used for all extractions with some modifications (Table A2). A NanoDrop spectrophotometer (Thermo Scientific) was then used to quantify the DNA concentration for each sample. A tiger-specific Cytochrome b primer (Wetton et al., 2004) was used to identify positive tiger samples. Two PCRs were performed for each sample to confirm a positive result, indicated by a single PCR product of ~165 bp. PCRs were performed in 10 µl reaction volumes containing 5 µl Qiagen Multiplex PCR mix, 0.3 µM forward and reverse primers, 0.2 µl (10 mg ml^{-1}) BSA, and 1.2 µl fecal DNA. PCR cycling conditions were as described by Driscoll et al. (2009) and PCR products were visualized on a 2% agarose gel with 1% ethidium bromide. Sex identification was performed using a felid-specific zinc finger primer pair (Pilgrim et al., 2005). Sex was determined by a single PCR product for females (~163 bp) and 2 products for males (~160 and 163 bp). PCR reactions were performed using a 10 µl reaction volume containing 5 µl Qiagen Multiplex PCR mix, 0.3 µM fluorescent labelled forward primer, 0.3 µM reverse primer, 0.5 µl (10 mg ml^{-1}) BSA, and 3 µl fecal DNA. PCR cycling conditions were: 95 °C for 15 min, 45 cycles of [94 °C for 30 s, 56 °C for 1 min, and 72 °C for 30 s], followed by 72 °C for 10 min. Fragment sizes were determined by capillary electrophoresis on an ABI 3130 genetic analyzer (Applied Biosystems).

Genotyping was performed using 24 fluorescent labelled microsatellite loci (Luo et al., 2004; Table A3). Loci were amplified in pairs in 10 µl reaction volumes containing 5 µl Qiagen Multiplex PCR master mix, 0.2 µM forward and reverse primers, 0.5 µl (10 mg ml^{-1}) BSA, and 2 µl fecal DNA. PCR conditions were 95 °C for 15 min, 20 cycles of [94 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s], followed by 35 cycles of [89 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s], then a final extension step of 60 °C for 90 min. Microsatellite allele sizes were determined with GeneMarker software (SoftGenetics LLC) and allele bins for each locus were confirmed with Tandem v1.08 (Matschiner and Salzburger, 2009). Consensus multilocus genotypes were generated using a multi-tubes approach (Taberlet et al., 1996). An allele had to appear twice to be accepted as a true allele; a heterozygote genotype was provisionally accepted after 3 positive PCRs and a homozygote provisionally accepted after 7 positive PCRs. Shaza (Macbeth et al., 2011) was then used to determine the number of unique genotypes, while genotyping error rates and probability of identity (PI_{SIB}) were estimated with Gimlet v1.3.3, Micro-checker v2.3.3, Pedant v1.0, and MicroDrop (Johnson and Haydon, 2007; Valière, 2002; van Oosterhout et al., 2004; Wang et al., 2012). Shaza uses a likelihood test to distinguish between 3 different types of genotype match: (i) false matches in which different individuals have the same genotype (shadows), (ii) false non-matches that represent the same individual with different genotypes due to genotyping error, and (iii) phantoms that are true matches rejected because of insufficient power. However, Shaza is not able to distinguish duplicated genotypes (i.e. potential recaptures of the same individual) from related individuals, so we used Colony v2.0.1.1 (Jones and Wang, 2010) to estimate the pairwise probability of individuals being full- or half-sibs.

2.3. Population genetics

Genepop v4.0 (Raymond and Rousset, 1995) was used to test for Hardy-Weinberg equilibrium. Observed and expected heterozygosity were estimated using GenAlEx v6.4 (Peakall and Smouse, 2006). Unbiased expected heterozygosity was also calculated to account for small samples sizes at each locus. Rare alleles with a frequency < 0.05 were also removed from the dataset to minimize the impact of genotyping errors and to obtain a conservative measure of diversity. Effective population size was estimated with NeEstimator v2 (Do et al., 2014) using a linkage disequilibrium method accounting for sampling error and with minimal allele frequencies set to > 0.05. We tested for isolation-by-distance using a regression between Rousset's genetic differentiation measure $a(r)$ and the logarithm of least cost distances $\ln(r)$ as implemented in SPAGeDi v1.3 (Hardy and Vekemans, 2002). Least cost