



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 km² (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_{SB}) 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 sample 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

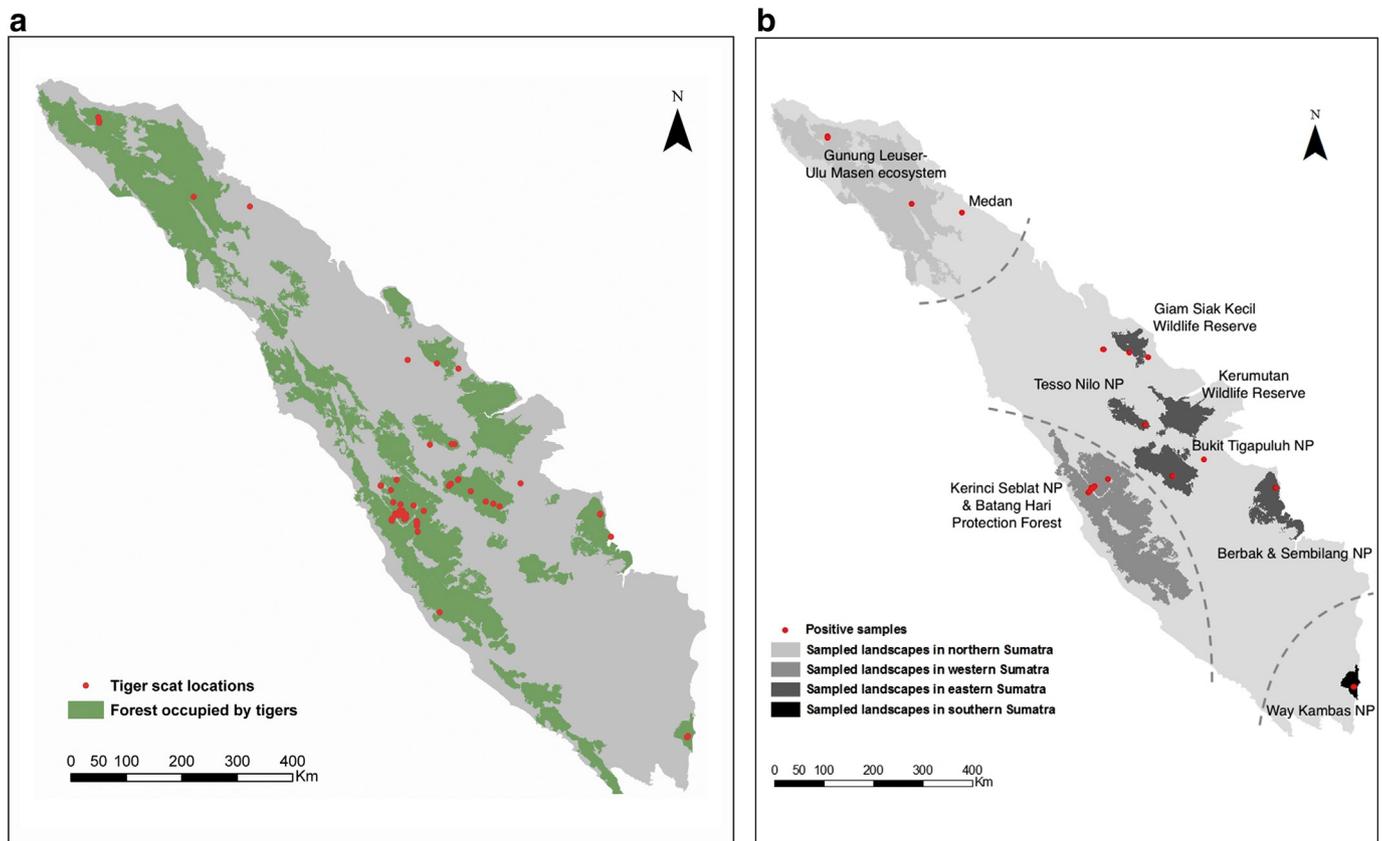


Fig. 1. a. Map showing the remaining Sumatran forest habitat that is occupied by tigers (data from Wibisono and Puspardini, 2010). Locations where fecal samples were collected are indicated by the red points.

b. Regional subdivision of the Tiger Conservation Landscapes and protected areas sampled during this study. The Northern group includes the Ulu Masen ecosystem; the Western group includes Kerinci Seblat NP and Batang Hari protection forest; the Eastern group includes Tesso Nilo NP, Kerumutan wildlife reserve, Bukit Tigapuluh NP, and Berbak NP; and the Southern group includes Way Kambas NP. Locations of the tiger positive samples are represented by the red points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

distances were estimated between individual sample locations using human footprint data from the Last of the Wild v2 (Sanderson et al., 2002) as our landscape map. Distances were computed in ArcView 3.1 with the Pathmatrix v1.1 extension (Ray, 2005). The inverse of the regression slope was then used to estimate neighborhood size, a measure of effective population size based on the distribution of individuals within a given area (Wright, 1946; Rousset, 2000). We also used GenAlEx to test for spatial autocorrelation using 50 km distance classes up to a total distance of 1550 km using 9999 random permutations and 10,000 bootstraps (Peakall et al., 2003).

2.4. Population structure

We defined four separate regions to coincide with the current designation of Tiger Conservation Landscapes and associated protected areas: 1. North - Ulu Masen/Gunung Leuser ecosystem, 2. West - Kerinci Seblat NP and Batang Hari protection forest, 3. East - Tesso Nilo NP, Bukit Tigapuluh NP, Kerumutan wildlife reserve, and Berbak NP, and 4. South - Way Kambas NP (Fig. 1b). Regional differentiation was tested using pairwise values of θ_w (Weir and Cockerham, 1984) computed in Genepop and a locus-by-locus AMOVA implemented in Arlequin v3.1 (Excoffier et al., 1992, 2005) using 19 microsatellite loci and 16,000 permutations. We then used BayesAss v1.2 (Wilson and Rannala, 2003) to estimate recent rates of gene flow between the four defined regions (North, East, West, and South).

Analysis of population structure with no a priori grouping was performed using Structure v2.3.3 (Pritchard et al., 2000), Tess v2.3.1 (François et al., 2006), and Geneland (Guillot et al., 2005) (Table A5).

Structure is the most commonly used method for population structure analysis but it can be affected by unequal sample sizes between populations and the presence of related individuals in a dataset (Anderson and Dunham, 2008; Kalinowski, 2011; Wang, 2017). Tess and Geneland are also affected by isolation by distance but can better incorporate spatial information (Safner et al., 2011). Clumpp v1.1.2 (Jakobsson and Rosenberg, 2007) was then used to confirm individual membership assignments for each population cluster. Individuals with a membership coefficient of $q \geq 0.7$ were assigned to a single cluster, and individuals with membership coefficients of $0.25 \leq q \leq 0.7$ were considered to have shared membership between clusters.

3. Results

A total of 148 scats were collected over 15 months of sampling, and scat contents included hair, bone fragments, body parts (claws, quills), soil, and vegetation. Transect length varied from ~2.5–10 km and the number of scats encountered at each site varied due to differences in survey effort and terrain, with lowland sites yielding far more samples than submontane regions. More scats were observed on open trails and logging roads compared to forest animal trails, due to the presence of heavy leaf litter and decomposition on the forest floor. Most scats were dried or partially decomposed on collection and varied in age (judged subjectively) from > 7 days old to > 1 month old. Preliminary analysis did not reveal any significant correlation between PCR success and scat location (e.g. animal trail, road, etc.) or scat contents (e.g. bones, hairs, etc.), though fresher samples (< 1 month old) stored in ethanol generally performed well (data not shown). We had variable success with

the detection dog, mostly due to the high temperatures and humidity, and the logistical challenges of transporting the dog with our field teams. This combined with the ongoing cost of the dog's husbandry meant that it was far more effective to rely on field teams searching visually alone.

DNA concentration per extract ranged from $\sim 6\text{--}192\text{ ng }\mu\text{l}^{-1}$, but this did not correlate well with PCR success. Thirty-seven samples were positive for tiger DNA and variable results were obtained with the sex and microsatellite primers. Ten samples with very low PCR success rates across all loci ($< 10\%$) were discarded immediately from the dataset, and we were able to determine putative sex for 15 of the remaining 27 samples (8 males and 7 females). The mean number of positive PCRs estimated with Gimlet was 0.54 (range 0.25–0.88) across loci and 0.54 (range 0.36–0.77) across samples, with the proportion of missing data per locus ranging from 12 to 72%. The 24 microsatellite loci gave a PI_{SIB} value of 1.57×10^{-8} , locus Fca 161 was monomorphic, and the most informative locus was Fca 94. Average allelic dropout and false allele rates were 0.39 and 0.10 as estimated by Pedant with 15,000 search steps. Microchecker identified two loci with possible stuttering (Fca 201, Fca 220), and analysis with MicroDrop highlighted two other samples with allelic dropout rates > 0.50 . Dropout rates above 0.50 have been shown to bias estimates of genetic diversity and population structure (Smith and Wang, 2014). These two individuals plus the three problematic loci (Fca 161, 201, and 220) were therefore also removed from the dataset before subsequent analysis. Shaza suggested that all the remaining samples represented unique individuals apart from a possible match between two pairs of samples. Analysis with Colony suggested that these two pairs were most likely to be full-sibs so they were retained. The final dataset thus contained 25 individuals genotyped at 21 loci.

Overall, mean observed heterozygosity was 0.52 ± 0.03 s.e. and unbiased expected heterozygosity (UHe) was 0.66 ± 0.03 s.e. (Table 1; Table A4). The population sample did not appear to be in Hardy-Weinberg equilibrium ($F_{IS} = 0.201$), which may be due to non-random mating or population subdivision. NeEstimator v2 gave an estimate of effective population size ($N_e = 22.2$ (95% CI 14.9–37.5), comparable to that from the sibship assignment method in Colony ($N_e = 18$ with 95% CI 9–40). We also found a significant pattern of isolation by distance, which gave a neighborhood size estimate of 29 individuals (95% CI 16–115).

Overall differentiation between regions was low ($\theta_w = 0.08$), with a θ_w value of ≤ 0.15 (95% CI 0.05–0.18) between the southern group and the rest of the island. This agreed with the AMOVA analysis, which suggested that most of the genetic variance could be explained by grouping the regions into North-West-East and South (Table 2; Table A6). Results from BayesAss suggested that there was little migration into our out of the south region (mean migration rates ≤ 0.06). Most gene flow occurred from the west to the north, and from the west to the east (mean migration rates $\cong 0.20$).

We found evidence of spatial autocorrelation with a significant relationship between genetic and geographic distance up to 850 km (Fig. A1). This is roughly equivalent to half the length of Sumatra. Analysis with Structure, Tess and Geneland suggested two to four genetic

Table 1

Estimates of genetic diversity in subspecies of *Panthera tigris*. Microsatellite loci were not identical between studies, but showed some degree of overlap between the loci used.

Tiger subspecies	No. of individuals	No. of loci	Observed heterozygosity	Expected heterozygosity	Reference
<i>P.t. sumatrae</i>	24	21	0.52 ± 0.03 s.e.	0.64 ± 0.03 s.e.	This study
<i>P.t. sumatrae</i>	16	30	0.47 ± 0.02	0.49 ± 0.04	Luo et al. (2004)
<i>P.t. altaica</i>	34	30	0.47 ± 0.02	0.46 ± 0.04	Luo et al. (2004)
<i>P.t. altaica</i>	95	8	0.26 ± 0.11	–	Henry et al. (2009)
<i>P.t. corbetti</i>	33	30	0.64 ± 0.02	0.67 ± 0.03	Luo et al. (2004)
<i>P.t. jacksoni</i>	22	30	0.56 ± 0.02	0.57 ± 0.03	Luo et al. (2004)
<i>P.t. tigris</i>	6	30	0.52 ± 0.04	0.57 ± 0.04	Luo et al. (2004)
<i>P.t. tigris</i>	73	5	0.70 ± 0.16 s.d.	–	Mondol et al. (2009)

Table 2

Pairwise differentiation (θ_w) for regional groups in the Sumatran tiger population. Estimates were computed in Genepop and significant values ($p < 0.05$) are indicated with an asterisk.

	North ^a	East	West	South
North	–			
East	0.07*	–		
West	0.06	0.03	–	
South	0.15*	0.15*	0.13*	–

^a North - Ulu Masen-Gunung Leuser ecosystem; East - Tesso Nilo NP, Kerumutan Wildlife Reserve, Berbak NP; West - Kerinci Seblat NP, Batang Hari protection forest; South - Way Kambas NP.

clusters with inconsistent assignment of individuals to the clusters (Fig. 2; Table A7). Structure analysis inferred two main clusters – (i) Riau samples north of Tesso Nilo NP and (ii) the rest of Sumatra, including Ulu Masen, Kerinci Seblat NP/Batang Hari, and Way Kambas NP. Structure results may have been biased by the unequal sample sizes between regions, as it has been shown to assign all the individuals from the largest sample to the same cluster (in this case the Riau samples). The output from TESS also suggested two main clusters: one large group encompassing the majority of the island, and a southern subgroup containing the Way Kambas samples. In contrast, Geneland suggested 4 clusters: (i) an admixed northern group encompassing Ulu Masen, (ii) a separate eastern group in Riau, (iii) an admixed east-west grouping including Kerinci Seblat NP/Batang Hari and Jambi province, and (iv) a southern Way Kambas group. These Geneland results infer some influence of underlying clinal variation within the Sumatran population. Thus, due to unequal sampling and the presence of isolation by distance, it was not possible to combine results from these three clustering methods to infer one pattern of population structure.

4. Discussion

This study represents the first genetic survey of the wild tiger population on Sumatra to include all the Tiger Conservation Landscapes and protected areas with global or long-term priority. Overall, estimates of heterozygosity were higher than expected for an island subspecies, with some evidence of southern Sumatran tigers becoming genetically differentiated from the rest of the island. This is most likely due to reduced migration into and out of this region as a consequence of an expanding human population and agricultural footprint. With ongoing deforestation and land conversion also occurring in Riau province, it is likely that tigers in eastern Sumatra will eventually suffer a similar fate.

Sample collection over a period of 15 months generated 148 scats, which yielded useable DNA from 25 different tiger individuals. The limited number of samples is in part due to the vast sampling area considered ($> 140,000\text{ km}^2$ of occupied forest) and the low average population density of tigers on Sumatra ($\sim 1\text{--}2$ individuals/ 100 km^2) (Wibisono and Pusparini, 2010). It also serves to highlight that while non-invasive samples such as feces and hair are valuable sources of DNA for threatened mammal species in humid, tropical environments,

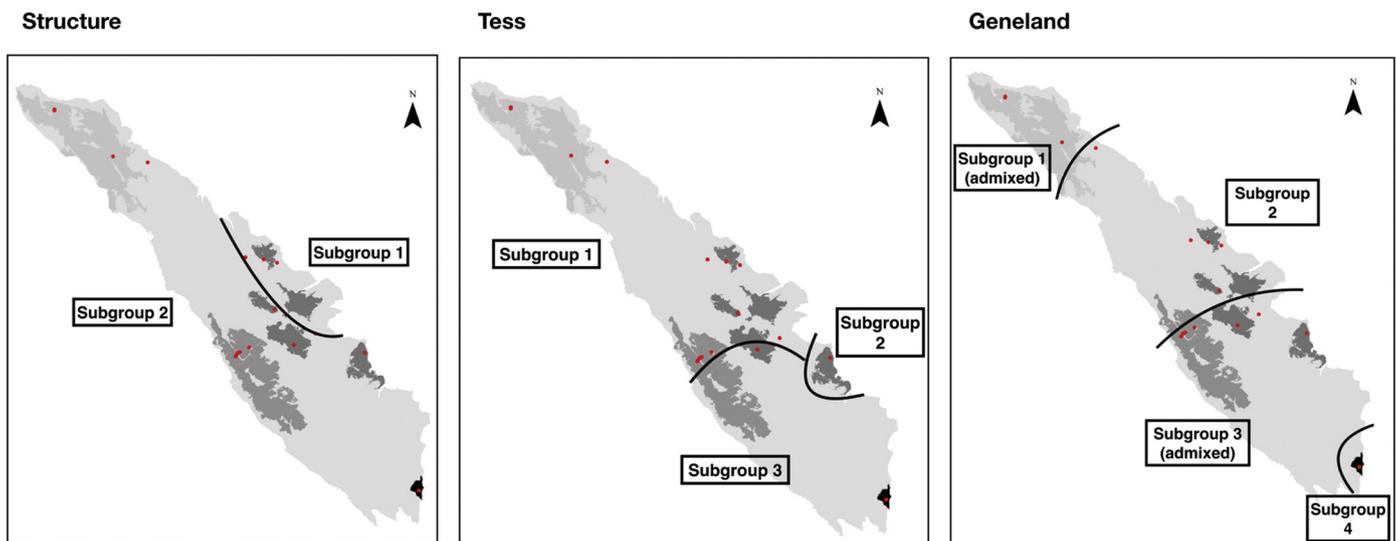


Fig. 2. Maps showing the genetic subgrouping of positive tiger samples using 3 different algorithms in Structure, Tess, and Geneland. Structure preferentially separated northern Riau samples from the rest of Sumatra. Tess placed southern Way Kambas samples into a separate group. Geneland suggested 4 subgroups, which could reflect underlying isolation by distance.

the proportion of samples that can ultimately be used for genetic analysis ranges from ~25–75%, necessitating prolonged and repeated surveys for sample collection (e.g. Bhagavatula and Singh, 2006; Ernest et al., 2000; Janečka et al., 2008; Lucchini et al., 2002).

We also attempted to use a detection dog to increase sample detection during our surveys. Despite cultural sensitivities to handling dogs, the field teams adjusted well to working alongside the detection dog once introductory training had been completed. However, the high heat and humidity, hilly terrain, and changing locations challenged both the dog's stamina and concentration, resulting in short periods of work before his motivation and focus tailed off. Therefore, for this study, we found that the field teams were more effective with consistent survey effort rather than the alternating rest and work periods required for the dog surveys.

Although DNA quality has been shown to deteriorate with increasing sample age (Piggott et al., 2004; Santini et al., 2007; Panasci et al., 2011), we collected all scats during our surveys due to the expected low encounter rate for intact scats in this tropical environment. Fecal DNA is particularly prone to genotyping errors such as allelic dropout and false alleles, but our results are similar to other non-invasive studies in carnivores (Broquet and Petit, 2004). Many different methods such as pre-amplification and sample dilution have been proposed to improve PCR success for non-invasive samples, but they had little effect in this study (data not shown). An ongoing pilot study in our research group suggests that combining an appropriate method of sample preservation (e.g. DNA/RNA Shield; Zymo Research), a DNA extraction method including homogenization (e.g. using FastPrep-24; MP Biomedicals), and amplification with inhibitor-resistant polymerases (e.g. KAPA2G Robust; KAPA Biosystems) can greatly improve data quality (data not shown). As it is difficult to obtain good quality scats in humid, tropical environments, others have explored the use of alternative sources of DNA, such as swabs taken from urine scent marks, which have much higher detection rates than scats in some sites (Caragiulo et al., 2015). For example, scent mark to scat detection ratios in Tambling Wildlife Nature Conservation, southern Sumatra, are typically between 3:1 and 4:1 (unpublished results). Tigers preferentially spray scent on overhanging trees or leaves along territory boundaries with up to 3.7 and 1.0 marks per km for males and females, respectively (Smith et al., 1989; Protas et al., 2010). Lipids contained within the urine sprays enable them to persist on the surfaces of vegetation (Andersen and Vulpius, 1999; Burger et al., 2008), and their characteristic scent is easily detected by people for up to 3 weeks after deposition.

In this study, we used a subset of the microsatellite loci used by Luo et al. (2004) to show that wild Sumatran tigers retain levels of genetic variation comparable to mainland subspecies. Low heterozygosity has been shown to correlate with a high risk of extinction for many species, and threatened or island species are thought to have ~60–65% of the microsatellite heterozygosity of similar or related non-endangered species (Frankham and Ralls, 1998; Brook et al., 2002). Therefore, the level of genetic variation found bodes well for Sumatran tigers as it suggests that overall the population has not experienced significant genetic drift. Given that heterozygosity is expected to be lost at a rate of $1/2N_e$ per generation due to genetic drift alone (Hedrick, 2005; Hamilton, 2009), we would expect Sumatran tigers to lose 1–3% of their genetic variation every generation (~ every 5–7 years). This is in the absence of other threats and assumes that current estimates of effective population size ($N_e \cong 18$ –29) and generation time remain unchanged in the future. This rate could be higher for the smallest subpopulations of tigers ($N < 30$ individuals), which would result in a faster rate of decline and increased differentiation from other subpopulations. While genetic drift and loss of genetic variation at the subpopulation level could be counterbalanced to some extent by migration or gene flow (e.g. Vilà et al., 2003), those at the subspecies level cannot be ameliorated by migration. Hence, while maintaining or increasing connectivity is an important part of the management of low density, wide-ranging species, the fundamental management strategy should be to increase the overall population size by expanding tiger habitat and/or improving habitat quality which then may also lead to increasing connectivity.

Our results may also represent a type of genetic extinction debt, in which population changes resulting from increased forest loss and poaching are subject to a time delay (Habel et al., 2015). The delay between the environmental change and a genetic effect is likely to be greater for long-lived species with low rates of population turnover (Kuussaari et al., 2009). Ultimately, if the pace of forest loss and human activity continues at its current rate it is likely that we will start to see signs of reduced heterozygosity and greater population isolation on Sumatra (Helm et al., 2009). Increased homozygosity (and the resulting inbreeding depression) have been associated with increased extinction risk due to factors such as reduced reproductive success, a decrease in population fitness, and increased susceptibility to disease (Amos and Balmford, 2001; Spielman et al., 2004). Although these changes have been noted in some carnivore populations (e.g. Johnson et al., 2010; Fredrickson et al., 2007), for tigers there is little empirical evidence to determine at what level these changes would occur.

The presence of isolation by distance suggests that Sumatran tigers are partly structured by a neighborhood mating system in which individuals are more likely to mate within a given area governed by dispersal distance. The estimated values of effective population size in this study ($N_e = 18\text{--}29$) give an $N_e:N_c$ ratio in the range of $\sim 0.04\text{--}0.06$, where N_c represents the total estimated population of 500 tigers. This is in line with previous studies in Bengal and Amur tigers ($N_e = 27\text{--}35$) using genetic data and variance in reproduction (Henry et al., 2009; Smith and McDougal, 1991), and is close to the average ratio of 0.1–0.11 for wildlife populations (Frankham, 1995). However, it is lower than other cat species such as the leopard, cheetah, and puma in which ratios of 0.25–0.64 have been recorded (Nowell and Jackson, 1996; Spong et al., 2000; Kelly, 2001). Analysis with MRatio (Garza and Williamson, 2001) did not provide evidence for a recent population bottleneck (Smith, 2012) and thus other factors such as a polygynous mating system, in which dominant males mate with most available females, or sampling scale, may account for the low effective population size (Kaeuffer et al., 2004; Neel et al., 2013). However, restricting our analysis to the neighborhood size suggested by spatial autocorrelation (< 850 km) did not result in a significant difference in the estimates of N_e (data not shown).

In the absence of gene flow, populations lose alleles under the influence of genetic drift and become increasingly differentiated (Falconer and MacKay, 1996). It was expected that geographic features such as Lake Toba and the Bukit Barisan mountain range might influence tiger population structure as they interrupt the distribution of other large mammals on Sumatra such as the tapir, orangutan, and rhino (Wich et al., 2016; Pusparini et al., 2015; Linkie et al., 2013). However, telemetry data shows that some tigers are capable of using ridgelines to cross the Bukit Barisan mountain range (Priatna et al., 2012), and our study did not find any obvious genetic discontinuity caused by these features. This is likely due to the tiger's dispersal ability, which can reach up to 65 km for males and 33 km for females (Smith, 1993; Goodrich et al., 2010), and highlights the importance of understanding differences in species' abilities to disperse across natural and anthropogenic barriers.

The high concentration of roads, settlements and plantations across parts of central Sumatra were also expected to act as dispersal barriers (Smith et al., 1998; Kerley et al., 2002; Linkie et al., 2006), but again our results suggest that either tigers have been able to maintain a fairly continuous distribution using patches of 'stepping stone' habitat, or more likely that insufficient time has passed for measurable genetic drift to have occurred in this region. Given the tiger's long generation time of 7 years (Seal et al., 1994), it could take up to 105 years (15 generations) for a landscape barrier to produce a detectable genetic signature (Holzhauer et al., 2006; Landguth et al., 2010). Therefore, it appears that the current Sumatran tiger population still exhibits evidence of the continuous distribution and genetic variation present within the ancestral Sunda population (Bay et al., 2014). However, given the current rates of land conversion to commercial crops such as oil palm and agroforestry, it is probable that much of the primary forest at lower elevations outside of conservation areas will be lost in the next 30–50 years (Holmes, 2002). Repeating a genetic study such as this in the future is therefore likely to show a more extensive pattern of population isolation and a more profound loss of genetic variation (Wearn et al., 2012; With, 2004).

In contrast to central Sumatra, there appears to be very little gene flow into or out of Way Kambas NP in the southern tip of Sumatra - this national park showed the highest pairwise F_{ST} and the lowest migration rates. These high F_{ST} values represent a separation from the sampled TCL populations in western and eastern Sumatra. While we acknowledge that there may be some exchange of individuals with the nearest protected areas in Berbak/Sembilang NP and Bukit Balai Ranjang NP, Way Kambas covers a relatively small area of isolated habitat (~ 1300 km²) and has a small population of ~ 30 tigers with low occupancy rates (Wibisono et al., 2011; Sanderson et al., 2006).

Maintaining gene flow or connectivity and the quality of the surrounding matrix is thought to be crucial to the survival of tigers within smaller protected areas (Ranganathan et al., 2008). However, the prospects for increasing connectivity in this region are bleak.

Primary lowland forest has been replaced by a mosaic of agricultural crops and plantations, (Miettinen et al., 2008; Miettinen and Liew, 2010), and a zone of urbanization surrounds the park such that there are no significant buffer zones suitable for wildlife (Nyhus and Tilson, 2004; Imbernon, 1999). Although the early stages of forest conversion may be beneficial to tigers due to the creation of secondary forest and edge habitats that support many prey species (Berry et al., 2010; Maddox et al., 2007; Barlow et al., 2007; Sunquist, 1981; Santiapillai and Ramono, 1987), many degraded or previously logged areas are quickly converted to oil palm or other agricultural plantations which are not as beneficial to tigers (Barber and Schweithelm, 2000). Frontier activities by local communities at the borders of national parks/wildlife reserves and agricultural concessions also commonly progress to more wide-scale operations or permanent rural settlements (Smith, 2009). And some habitat degradation, encroachment and hunting also occurs within park borders such that these are not the inviolate refugia their names suggest (e.g. Forrest et al., 2011; Gaveau et al., 2009b).

Lowland peat swamp forest in eastern Sumatra is suffering a similar fate with land being cleared at a rate of up to 2.3% per year (Uryu et al., 2008; Hansen et al., 2009; Broich et al., 2011; Koh et al., 2011; Miettinen et al., 2012). Riau lost $> 50\%$ of its primary lowland forest between 1990 and 2010, and focus has now shifted to primary peat swamp forest (Margono et al., 2012, 2014). This rate of deforestation is likely to continue as Indonesia plans to increase its land allocation to oil palm, paper and pulp to just under 15 million hectares by 2030 (Wilcove et al., 2013). These land use types support much lower species richness compared to primary forest ($\sim 38\%$), and tigers are commonly extirpated from these areas (Maddox et al., 2007; Smith, 2009; Danielsen et al., 2009; Fitzherbert et al., 2008). This combination of poaching pressure and impoverished habitat is therefore likely to result in a population decline and increased genetic differentiation between protected areas as the options for tiger movement across the agricultural matrix are reduced (Kenney et al., 1995; Linkie et al., 2006; Chapron et al., 2008).

Tiger populations are more likely to suffer extinction debt in areas where there is overlap with agricultural or rural development, and these could serve as priority hot spots where intervention is most likely to be effective (Helm et al., 2009; Wearn et al., 2012). Despite a national government moratorium on conversion of peatland and primary forest since 2011 (Austin et al., 2014), the Ministry of Forestry has also pledged a commitment to expanding the oil palm and timber industries to support national and international demand (Kementerian Kehutanan, 2011; Brockhaus et al., 2012; Harahap et al., 2017). Uncertainties around land classification and implementation of the moratorium have resulted in continued loss of primary forest on Sumatra, particularly in Riau province and around Teso Nilo NP (Harris et al., 2017). Therefore, there needs to be greater coordination across different government policies (biofuels, climate, forestry and agriculture) to ensure adequate protection of the primary and secondary forested lands which are key to supporting the remaining tiger populations.

5. Conclusions

We present the first assessment of the effects of landscape change on the tigers on Sumatra. Our results show that the Sumatran tiger has retained levels of genetic diversity comparable to mainland subspecies and that there is evidence to suggest reduced gene flow for tigers in the extreme south of Sumatra. While we acknowledge the limited sample size, the distribution of sampling sites represents a good proportion of the remaining tiger habitat on Sumatra. Precise estimates of genetic variation can be made with as few as 10 individuals (Smith and Wang,

2014), and therefore, our results provide a good overview of the genetic status of the wild Sumatran tiger population. This study also demonstrates that the genetic data obtained from non-invasive samples is critical to understanding the genetic diversity and population structure of large-bodied, low-density mammals such as the tiger; individuals are not easily captured for biological sampling, baited hair traps are not reliable, and dens or latrines are rarely seen. Similar methods are being used to study the Sumatran elephant and Sumatran rhino, which will provide more information on the effects of land conversion on other threatened large mammal species.

Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2018.03.014>.

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