Title

Do rivers influence fine-scale population genetic structure of tigers in the Sundarbans?

Author names and affiliations

M. Abdul Aziz[1,*], Olutolani Smith[2], Adam Barlow[3], Simon Tollington[1,5], Md. Anwarul Islam[4], Jim J. Groombridge[1]

[1] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury CT2 7NZ, UK

[2] Department of Genetics, Evolution & Environment, University College London, London, UK

[3] WildTeam, Surfside, St Merryn, Padstow PL28 8NU, Cornwall, UK

[4] Department of Zoology, University of Dhaka, Dhaka 1000, Bangladesh

[5] The North of England Zoological Society, Chester Zoo, Caughall Road, Chester, CH2 1LH, UK

* Present address & corresponding author: Department of Zoology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh; Email: maaziz78@gmail.com; Cellphone: +88 0176256193

Abstract

Global tiger *Panthera tigris* populations mostly survive within the geographically fragmented forest patches, thereby limited genetic exchange between isolated populations. Assessing the genetic status of these populations can reveal the effects of dispersal barriers and provide critical insights to guide future conservation actions. Using non-invasively collected biological samples, we investigated fine-scale genetic structure of tigers in the Sundarbans mangrove forests intersected by the complex river systems, and which holds one of the largest global tiger populations. We genotyped 52 tiger samples at 10 polymorphic microsatellite loci, and sequenced 33 of them for a total of 1,263 base-pairs at four mitochondrial gene fragments. Microsatellite analyses exhibit a signature of fine-scale genetic structure, which might have been the consequence of limited tiger dispersal due to wide rivers across the Sundarbans. Similarly, mitochondrial data show a historic pattern of population isolation that might be due to wider rivers across the entire Sundarbans shared by Bangladesh and India. Given the intrinsic nature of the mangrove habitat embedded with numerous rivers, increased commercial traffic and human activities may further impede tiger dispersal across wide rivers, escalating further genetic isolation of the Sundarbans tigers.

Introduction

Human-induced historic land change across Asia has transformed the once widespread forested areas into a series of isolated patches, threatening the survival of many forest-dependent species (Walker et al. 2000; Loxterman 2011). Large carnivores like the tigers can be acutely affected by such habitat fragmentation, which greatly restricts their genetic viability through reducing the effective population size in any one forest patch (Smith, 1993). Moreover, habitat fragmentation resulting in a limited tiger dispersal (Ewers and Didham 2005; Joshi et al. 2013) promotes the loss of genetic diversity, and induces genetic 'isolation by distance' between populations (Wright 1943; O'Brien et al. 1985; Vila et al. 2003; Liberg et al. 2005). Such isolated populations are highly susceptible to environmental stochasticity, increasing the likelihood of population extinction (Woodroffe 2000). Approximately 4,000 individual tigers left in the world (Seidensticker, 2010) now live within isolated forest patches (Walston et al. 2010) across just 7% of their historic range (Sanderson et al. 2006), and have already lost 93% mitochondrial genetic variants (Mondol et al. 2013).

The Sundarbans mangrove forests shared between Bangladesh and India is one of the six regional Tiger Conservation Landscapes (TCLs) of global priority (Sanderson et al. 2006), and supports one of largest populations of Bengal tigers (Barlow 2009; Dey et al. 2015; Aziz et al. 2017a). However, tigers in the Sundarbans have long been isolated from the nearest TCL (e.g., Similipal Tiger Reserve in West Bengal, India) by approximately 200 km of agricultural lands and human settlements (Sanderson et al. 2006), thereby limiting any opportunity of gene flow between populations. The Sundarbans itself is a series of muddy, densely vegetated islands separated by waterways of varying widths, some of which may be barriers to tiger dispersal within this landscape (Naha et al. 2016). For instance, radio-collaring studies have revealed that tigers rarely cross the rivers wider than 400 m in India Sundarbans (Naha et al. 2016) as well as 600 m in Bangladesh Sundarbans (Barlow 2009), suggesting that tiger movement might have been compromised by wide rivers in this mangrove forests. Several studies have highlighted the morphological adaptation (Barlow et al. 2010) and genetic distinctiveness (Singh et al. 2015) of the Sundarbans tigers, however none have examined the effect of barriers on the fine-scale genetic structure of the population. Understanding how landscape barriers facilitate or deter gene flow is a high priority management need for an endangered population surviving in a changing forest habitat (Sork and Waits 2010).

The objectives of this study were therefore to investigate the fine-scale genetic structure and to assess how rivers influencing the genetic architecture of tigers living in the Sundarbans. This study is the first of its kind for the Sundarbans tigers that provides useful information to guide long-term genetic management of this uniquely adapted tiger population.

Methods

Study area

Of the 10,263 km^2 of the world largest mangrove forest shared between Bangladesh and India (Giri et al. 2007), the Bangladesh part covers 6,017 km^2, of which 4,267 km^2 is forest and the remaining area is water (Iftekhar and Islam 2004). The north and east sides of the forest are bounded by dense human settlements and agriculture land, and to the south by the Bay of Bengal (Fig. 1). The Bangladesh

Sundarbans (hereafter Sundarbans) is managed as Reserve Forest, except three forest areas that have been designated as wildlife sanctuaries: Sundarbans West (715 km^2), Sundarbans South (370 km^2) and Sundarbans East (383 km^2).

The Sundarbans supports a relatively high species diversity compared to other mangrove forests of the world. For instance, previous studies have reported 330 species of plants, over 400 species of fishes, 35 species of reptiles, more than 300 species of birds and 42 species of mammals from this habitat (IUCN–Bangladesh 2001; Islam and Wahab 2005). Although leopards (*Panthera pardus*) coexist with tigers in many of the Asian forest landscapes (Karanth and Sunquist 2000), the leopard however became extinct from the Sundarbans about a century ago (Seidensticker and Hai 1983). Currently, several species of small cats including fishing cat (*Prionailurus viverrinus*), jungle cat (*Felis chaus*) and leopard cat (*Prionailurus bengalensis*) are found in the Sundarbans.

Topography of the Sundarbans is less than 1 m above the sea level (Canonizado and Hossain 1998), and consists of tangled region of estuaries, rivers and watercourses, enclosing a vast number of low-lying swampy forest islands of various shapes and sizes (Prain 1979). All major rivers flow from north to south, which are interconnected by the east-west narrow channels of rivers and creeks (Islam and Wahab 2005). Three mighty rivers, namely the Arpangassia (width: 1.2-2.8 km), the Sibsa (width: 1.3-3.4 km) and the Passur (width: 1.4-3.1 km) divide the forest into three larger isolated regions (Fig. 1). In addition, the rivers Raimangal (width: 1.5-2.8 km) and Harinbhanga (width: 2.9-4.4 km) mark the international boundary and bisect the entire forest into Bangladesh and Indian Sundarbans. Most of these major rivers have been used as cargo channels as well as other human activities for centuries.

Sample collection

Non-invasive biological samples (scat and hair of tigers) were collected from different areas of the Sundarbans, with purposive sampling from isolated forest patches separated by the rivers Arpangassia, Sibsa and Passur. Firstly, we intensively sampled four geographically isolated areas of the Sundarbans using 2×2 km sample grids (detail protocol and sampling effort were described in Aziz et al. 2017a). Briefly, six survey teams comprising four members in each walked on transect line in each sample grid whilst actively searched for suspected scats and hairs. Once a sample block was surveyed with one to three repeated transects, teams moved to the next sample block. Secondly, we sampled the remaining forest areas opportunistically, with specific focus to cover outside of these four sample blocks. We collected deposited scats, and hairs left in scratch marks on trees, and recorded the location of each sample using a handheld Global Positioning System (GPS) *Garmin GPSMAP 64*. During the survey, a total of 512 biological samples were collected (Table 1). Winter months were chosen for sampling to avoid extreme weather conditions (e.g., rains and tidal surges in summer), with sampling from 20 November 2014 to 26 February 2015.

All biological samples were transported from Bangladesh to the United Kingdom under the Convention on International Trade in Endangered Species (CITES) (Permit No. BD 9118404), and authorisation of the Department for Environment, Food and Rural Affairs, United Kingdom (AHVLA authorization: TARP/2015/111).

DNA extraction and sample screening

DNA extraction and analyses were performed at the Conservation Genetics Laboratory of the Durrell Institute of Conservation and Ecology, University of Kent, UK. Two isolated laboratory spaces were used for analyses of all biological samples to prevent potential contamination. All scat samples were prepared for DNA extraction under pre-sterilized fume hood conditions in batches of 10 samples. The workstation was sterilized before and after each use by irradiation using UV light and cleaned using 10% bleach. All PCR reactions were carried out in a separate laboratory under a fume hood pre-irradiated using UV light.

Genomic DNA from scat and hair samples was extracted using QIAamp DNA Stool mini kits and QIAamp DNA Blood and Tissue kits (QIAGEN Inc.), respectively, following the manufacturer's instructions. Approximately 200 mg of scat material was scraped from the outer surface of each scat sample with a sterilized razor blade and then incubated overnight with 1.5 ml ASL buffer on a mechanical rotator at 56 ℃. The DNA supernatant from the sample was lysed with 300 µl AL buffer plus 25 µl proteinase K and incubated at 70 ℃ for 15 min. Four microliters of carrier RNA (ThermoFisher Scientific, UK) was added to AL buffer to increase DNA yield from scat samples. To extract DNA from blood, tissue, and hair samples, we used DNeasy™ Blood and Tissue Kits (QIAGEN Inc.); approximately 10 hairs of each sample was added to 300 µl AL buffer incorporating 20 µl of proteinase K and 20 µl of DTT (Dithiothreitol, Biotech) and then incubated at 56 ℃ overnight or until the sample was completely digested. The DNA was eluted in 75 µl of buffer solution. We maintained strict protocols to reduce the chances of contamination by using aerosol barrier pipette tips, separate pre and post PCR rooms and UV PCR hoods for sample preparation. A negative control (with no biological material) was included with each batch of extractions to monitor for possible contamination during the DNA extraction procedure. To confirm that scats had been deposited by tigers rather than non-target wild cat species, extracted DNA was screened using tiger-specific primers to amplify a 245 base-pair fragment (fwd TTACTAGGACTCCTCCTAGCC; rev GAATAGGGTTGTGATGGCCCC) of the tiger cytochrome *b* gene (Mondol et al., 2009a; Mukherjee et al., 2007). In this screening process, PCR cycling conditions consisted of an initial hot start of 95 ℃ for 1 min followed by 45 cycles of 95 ℃ for 15 s, 55 ℃ for 15 s and 72 ℃ for 15 s, and a final incubation period of 10 mins at 72 ℃ using a G-Storm Thermal Cycler (Labtech France). PCR reaction volumes (total 27 µl) contained 3 µl of template DNA, 12.5 µl MyTaq Redmix (containing dNTPs and $MgCl_2$; Bioline, UK), 5 µM of each forward and reverse primer, 4 µM BSA (Bovine Serum Albumin, New England Biolabs Inc.) and 8.5 µl dH_2O. PCR products were purified and sequenced using a 3730XL analyser (Macrogen, Amsterdam, Netherlands). Sequences were edited with Jalview v2 (Waterhouse et al. 2009) and then cross-checked and aligned with sequences from the GenBank database (National Center for Biotechnology Information, NCBI) to confirm species identity for each sample prior to downstream genotyping analysis.

Microsatellite genotyping and individual identification

Microsatellite markers (repeated sequence of nucleotides) are commonly used to investigate genetic structure (Mondol et al. 2009b; Reddy et al. 2012), spatial genetics (Sharma et al. 2012), and genetic connectivity between tiger populations (Joshi et al. 2013). In this study, we used a panel of 10 microsatellites from a set of 14 optimised polymorphic loci (Aziz et al. 2017a), to amplify tiger-

authenticated DNA samples (Table S1). Four multiplexes were designed to include the full set of loci. All forward primers were fluorescently labelled for gene-scanning (Table S1). Each microsatellite PCR reaction volume (10 µl) contained 3 µl of DNA template, 5 µl Qiagen multiplex PCR buffer mix (Qiagen Inc.), 0.2 µM forward primer, 0.2 µM reverse primer (Eurofins Genomics), and 2 µM BSA. For all multiplex reactions, the PCR temperature regime included an initial denaturation step for 15 min at 95 °C with 45 cycles of denaturation (94 °C for 30 s), annealing (*Ta* ranges from 52 °C to 57 °C for 90 s for four multiplexes; details in Table 2), extension (72 °C for 90 s), and a final extension of 10 mins at 72 °C, using a G-Storm Thermal Cycler. All PCR products were genotyped using an Applied Biosystems 3730 DNA Analyser and ROX 500 ROX™ as the size-standard. Alleles were identified and scored using GENEMAPPER v3.7 (Applied Biosystems, MA, USA). We employed the comparative genotyping approach (Frantz et al. 2003; Hansen et al. 2008) by ensuring that equivalent heterozygote genotype profiles were scored at least twice and corresponding homozygote genotypes at least three times (up to a maximum of five) for each sample. This approach ensured a level of rigour in resolving the true genotype and was less laborious and more cost-effective than the multiple tube approach (Taberlet et al. 1997). A consensus genotype was achieved if genotypes matched 100% at all loci in at least three repeats. Any samples that could not be scored consistently across amplifiable loci in the repeated genotype profiles were removed from the analysis (Jackson et al. 2016).

We used CERVUS v3.0 (Marshall et al. 1998) to identify unique or recaptured genotypes from pooled samples. A detailed procedure of individual identification can be found in Aziz et al. (2017a). In short, matching genotypes based on five or more loci were considered to be sourced from the same individual (Mondol et al. 2009a). Incomplete or partial genotype profiles amplified at a minimum of five of the 10 loci set were also used to identify unique and recaptured individuals following the approaches of previous studies in tigers (Bhagavatula and Singh 2006; Mondol et al. 2009a), and badgers (Frantz et al. 2003).

We used MICROCHECKER v2.2.3 (van Oosterhout et al. 2004) to check genotyping errors due to stuttering (e.g., incorrect repeats, typographic errors). Allele frequencies, observed (*Ho*) and expected (*He*) heterozygosity, allelic dropout and false alleles were estimated using GIMLET v1.3.3 (Valiere 2002). Linkage disequilibrium and departure from Hardy-Weinberg equilibrium (HWE) were tested in GENEPOP v4.2 (Raymond and Rousset 1995).

Mitochondrial DNA sequencing

Variable mitochondrial gene fragments are commonly used in investigating phylogenetic relationship (Luo et al. 2004) as well as genetic status of tigers (Mondol et al. 2009b). To generate mitochondrial DNA dataset from tiger-authenticated DNA samples, a total of nine primer sets (optimised from Mondol et al. 2009b; Table S2) were used to amplify four gene fragments: control region (CR), cytochrome *b* (cyt *b*), NADH dehydrogenase subunits 2 (ND2), and NADH dehydrogenase subunits 5 (ND5). These four fragments were chosen primarily because these genes showed sufficient variability across tiger populations (Luo et al. 2004; Mondol et al. 2009b), which also allowed us to compare our sequence data with these studies. To amplify DNA fragments, PCR reactions were conducted in 27 µl reaction volumes which contained 3 µl template DNA, 12.5 µl MyTaq Redmix (Bioline), 5 µM of each primer, and 10.5 µl of dH$_2$O. PCR amplification was performed using a G-Storm Thermal Cycler

(Labtech France). The PCR profile comprised of initial denaturation (95 °C for 1 m); 45 cycles of denaturation (95 °C for 30 s), annealing (T_a for 15 s), extension (72 °C for 30 s) and a final extension phase (72 °C for 10 m). All amplicons were examined by agarose gel electrophoresis to check for a clean single band and to check for any signs of contamination. PCR products were purified and amplified using a 3730xl analyser (Macrogen, Amsterdam, Netherlands).

Fine-scale genetic structure analysis

We used Bayesian clustering approach to assess the fine-scale genetic structure in the program STRUCTURE v2.3.4 (Pritchard et al. 2000). The STRUCTURE determines the most likely number of genetic clusters (K) by assigning each individual tiger to the most likely clusters using multiple Markov Chain Monte Carlo (MCMC) algorithms for multilocus genotypes. The STRUCTURE was run with admixture and correlated allele frequency models (Falush et al. 2003), using an initial burn-in length of 20,000 followed by a total run length of 500,000 iterations (Pritchard et al. 2000). The admixture model assumes that individuals can be of mixed ancestry, and is thus more suitable to studying populations that are harder to split into arbitrary predefined populations. Ten independent runs were performed, each time inferring the number of genetically distinct clusters ($K = 1$-10), in order to verify that the estimates are consistent across the runs (Pritchard et al. 2000). Posterior likelihood values for the most likely K and ΔK were evaluated following the Evanno method (Earl and VonHoldt 2012) in STRUCTURE HARVESTER (Evanno et al. 2005; Earl and VonHoldt 2012), and averages for the proportions of individual assignment for the 10 independent runs were estimated using CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007). The proportion of individuals assigned to each cluster was then graphically displayed using POPHELPER (Francis 2017) and geographically plotted with ArcGIS v10.3 to denote clustered individuals across the Sundarbans landscape.

The GENELAND v1.0.7 using geo-referenced multilocus genotypes of sampled individuals was applied to infer and locate genetic discontinuities between tiger populations within the domain of the Sundarbans (Guillot et al. 2005b) which further improve the STRUCTURE inferences. In GENELAND, we processed all parameters including K simultaneously by the MCMC algorithm following recommendations of Guillot et al. (2005a). We followed two steps to run the algorithm: a first run to infer K, and a second run with K fixed at the previously inferred value to estimate other parameters (individual assignment to the inferred populations). The first step was replicated 10 times to check for convergence, allowing K to vary between 1and 10 clusters. The parameters set for the run were 5×10^5 MCMC iterations, maximum rate of Poisson process fixed to 500, maximum number of nuclei in the Poisson-Voronoi tessellation fixed to 200, and with true spatial coordinates. The Dirichlet model was used for its better performance than the alternative model (Guillot et al. 2005a). We then inferred the number of population clusters from the modal K of these 10 runs, and ran the MCMC 100 times with K fixed to this number, and other parameters similar to the runs with variable K. We calculated the mean logarithm of posterior probability for each of the 100 runs, and selected the 10 runs with the highest values. The posterior probability of population membership for each pixel of the spatial domain was computed for each of these 10 runs (with a burn-in of 5×10^4 iterations). The number of pixels was set to 900 pixels along the X axis and 750 along the Y axis, to avoid having two individuals in the same

7

pixel. We then computed the posterior probability of population membership for each individual within the spatial domain. We finally checked the consistency of results across these 10 runs.

Spatial genetic structure analysis

We investigated spatial genetic structure, i.e., isolation by distance by autocorrelation analyses that reveals the level of variations of genetic distance as a function of geographic distance (Peakall et al. 2003; Guillot et al. 2005a; Peakall and Smouse 2012). This multivariate spatial analysis aids in understanding the fine-scale genetic structure generated by multiple loci on spatial scale (Peakall et al. 2003). First, a linear pairwise geographic distance was calculated as the Euclidean geographic distance between geographic locations of all sampled tigers. Then a pairwise squared genetic distance was calculated using the microsatellite genotype data. These two distance matrices were then used to estimate a spatial autocorrelation coefficient (r), bounded by -1, +1, which is a measure of the genetic similarity between pairs of individuals whose geographic separation falls within the specified distance classes (Smouse and Peakall 1999). These distance classes were determined in the program GenAlEx v6.5 using default setting (Smouse and Peakall 1999; Peakall and Smouse 2012), which created 11 geographic distance classes with 8 km interval between the minimum and maximum sample distances. A test for statistical significance was conducted by 9,999 random permutations to create a 95% confidence interval around a null hypothesis of 'no spatial genetic structure' ($r = 0$), and 9,999 bootstrap resampling to create a 95% confidence interval around the mean estimate of r.

To complement the spatial autocorrelation analysis, a Mantel test was performed using the pairwise geographic and genetic distances (Mantel 1967; Smouse and Peakall 1999). The Mantel test was carried out with 9,999 permutations to test for statistical significance of the correlation coefficient (r) between the geographic and genetic distances, with a significant correlation being indicative of spatial genetic structure exists in the sampled population (Mantel 1967; Smouse et al. 1986). The spatial genetic autocorrelation, and Mantel test analyses were performed using the software package GenAlEx v6.5 (Peakall and Smouse 2012).

Phylogenetic structure analysis

The sequenced datasets of four mitochondrial gene fragments were edited and aligned in Jalview v2 (Waterhouse et al. 2009), and concatenated into a complete dataset in SEQUENCEMATRIX (Vaidya et al. 2011). To assess phylogenetic relationships between tiger populations of Bangladesh and Indian Sundarbans, previously reported haplotype sequence data (TIG23 and TIG 29; Mondol et al., 2009b) were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/; accession number for TIG23: EU661642 and TIG29: EU661648), and added to our concatenated sequence dataset. A median joining haplotype network was constructed in the program PopART v1.7 (http://popart.otago.ac.nz) to demonstrate evolutionary relationship of tiger population in the Sundarbans.

Results

Genetic diversity

We utilized 53 individual tiger samples from a total of 125 samples that yielded genotype profiles for a minimum of five to 10 microsatellite loci. The mean proportion of loci typed was 87% across the dataset, with a mean polymorphic information content (PIC) of 0.64. All loci were polymorphic with a mean number of alleles of $5.50 \pm SD\ 1.65$ per locus. The overall expected heterozygosity (He) and observed heterozygosity (Ho) were $0.68 \pm SD\ 0.04$ and $0.37 \pm SD\ 0.02$, respectively. This result indicates that the tiger population in the Sundarbans might have lost some levels of genetic diversity. Several loci exhibit significant deviations from Hardy-Weinberg equilibrium (HWE) but no linkage disequilibrium was found between loci pairs (Table 2). Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3f1647m.

Fine-scale genetic structure

The model-based clustering technique implemented in STRUCTURE suggested three ancestral gene clusters within the tiger population of the Bangladesh Sundarbans ($K = 3$, where K is the number of genetic population cluster) (Fig. 2, 3). The STRUCTURE also detected another peak at $K = 7$, indicating a possibility of further fine-scale genetic structure within the population (Fig. 2, 3). At $K = 3$, the cluster 1 (red) included the highest (40%) number of individuals predominantly distributed to the western part of the river Arpangassia. The estimated probability (q) of individual membership was higher for this cluster, ranging from 0.495 to 0.969, with $q > 0.817$ for 71% individuals. The population cluster 2 (blue) and cluster 3 (cyan) containing 32% and 28% of individuals respectively were distributed to the regions between Arpangassia and Sibsa, and Sibsa and Baleshwar rivers respectively. The estimated probability of individual membership to the cluster 2 ($q = 0.429$-0.712) and cluster 3 ($q = 0.372$-0.716) showed relatively higher amount of shared alleles among the populations across eastern regions of the Arpangassia (Fig. 4). With regard to the clusters at $K = 7$, the estimated probability of individual membership was unevenly distributed, specific to several clines in the remote southeast islands, lending additional support for subtle genetic structure within the population.

The spatial model-based clustering technique implemented in the GENELAND resulted in an interesting genetic structure at $K = 7$, corresponding to the fine-scale genetic patterns detected at STRUCTURE analysis. The distribution of membership coefficients across the sampled population has revealed six genetics clusters containing majority of sampled populations, alongside the seventh 'ghost cluster'. Specifically, the cluster a (Dobeki; denoted by location name; Fig. 1, 5) contained individuals with high membership coefficients distributed principally between Kalagachhia and Jamuna rivers on the east of the Arpangassia river. Similarly, the genetic cluster b (Supati) was located in the further east of the Passur river; the cluster c (Notabeki) between Mahmuda and Raimongal rivers; the cluster d (Hangsha Raj) between Passur and Sibsa rivers; the cluster e (Harbaria) between Sibsa and Passur rivers, and the cluster f (Haldibunia) between Harinbhanga and Raimongal rivers (Fig. 1, 5). The distribution of membership coefficients was higher for the clusters Supati, Hangsha Raj, Notabeki and Haldibunia than the Passur and Dobeki. The seventh cluster did not contain any individuals, therefore considered as a ghost cluster.

9

Spatial genetic structure

Genetic isolation by distance analysis exhibited a pattern of genetic differentiation with increase of geographic distances. Analysis showed that distance between genotypes as a function of genetic correlation was significantly positive from zero to 24 km ($r = 0.038$, $p = 0.002$) whilst significantly negative ($r = -0.044$, $p = 0.002$) from 72 km onwards (Fig. 6). Similarly, the Mantel test with pairwise geographic and pairwise genetic distance revealed a significant positive relationship ($r = 0.161$, $p = 0.01$), indicating significant genetic differentiation in relation to geographic distance (Fig. 7). Both of these tests showed a reasonable decline of genetic similarity among individuals living further apart, however this pattern is unusual for the highly dispersal animals such as tigers.

Phylogenetic structure

We detected two haplotypes (denoted as SBT1 and SBT2) from concatenated sequences of four mitochondrial genes comprising 1263 base-pairs from 33 tiger samples (Fig. 8). Sixty four percent samples contained haplotype SBT2 while 36% the SBT1. Remarkably 90% sampled individuals in the western region of the Arpangassia carried the haplotype SBT1, suggesting a clear distinct pattern of historical isolation of these individuals from the remaining populations. Conversely, 80%, 67%, and 94% individuals in the eastern regions of the Arpangassia, Sibsa and Passur respectively shared the haplotype SBT2. The overall haplotype distribution clearly showed a gradient that distinguishes tigers in the western region of the Arpangassia from the rest of the Sundarbans (Fig. 8a). These two haplotypes have been submitted to Genbank with accession numbers MH427526-MH427533.

The haplotypes reported from the Indian Sundarbans (Mondol et al. 2009b) and this study revealed that there are three haplotypes within the entire tiger population of the Sundarbans. Interestingly, the haplotype SBT1 was identical to the haplotype TIG29 reported by Mondol et al. (2009b) from the Indian Sundarbans. Therefore the haplotype SBT2 is unique to the Bangladesh Sundarbans while the haplotype TIG23 to the Indian Sundarbans (Fig. 8b).

Discussion

Genetic diversity

Overall a low level of genetic diversity was found in the Sundarbans tiger population compared to the populations in the Indian landscapes. For example, the mean number of alleles observed in this study (5.50) is reasonably lower than reported for Northeast India (6.61; n=15; Borthakur et al. 2013). Similarly, the observed heterozygosity in the tiger population of the Bangladesh Sundarbans ($Ho = 0.37$) is also lower than the tiger population in the Indian Sundarbans ($Ho = 0.49$; n=13; Singh et al., 2015) and Northeast India ($Ho = 0.47$; n=15; Borthakur et al. 2013). The overall lower levels of allele diversity and heterozygosity in the Sundarbans population could be attributed to the fact that this population has long been isolated from the nearest tiger populations of Similipal tiger reserve in West Bengal of India, thereby completely wiped out any chance of gene flow between the populations. Moreover, it has been shown that habitat fragmentation limits population connectivity (Frankham 1996) that reduces genetic diversity and increases population differentiation (Johansson et al. 2007; Dixo et al. 2009). Furthermore, the lower level of genetic diversity might be linked to limited connectivity between

locally isolated populations created by large river systems within the Sundarbans. Of note, it is not unlikely that the observed differences could also be due to the differences in sample area, nuclear markers and size of sample used in these studies (Borthakur et al. 2013; Singh et al. 2015).

Fine-scale genetic structure

The genetically most distinct population cluster exists in the western regions of the Arpangassia river, suggesting that a fine-scale genetic structure has been developed within tiger population of the Sundarbans. With regard to the three genetic clusters ($K = 3$) detected by the STRUCTURE, a stronger population genetic differentiation was revealed in the western part of the Arpangassia rivers from the remaining tiger populations. Although the analysis of the posterior likelihood values in Evano procedure suggesting $K = 3$ as the most reliable genetic clusters for the tiger population (Pritchard et al. 2000), a subtle signal for substructure also appeared at $K = 7$. Overall individual membership probabilities to the population cluster 2 and cluster 3 showed a moderate level of shared alleles between the rivers Sibsa and Passur, whilst the population in the western region of Arpangassia river retained the lower level of shared alleles with the rest of the populations. Although STRUCTURE has failed to detect genetic evidences for stronger population isolation between Sibsa and Passur rivers, some level of gene flow might have been maintained between these populations. However, the weaker genetic structure in these regions could be linked to limited number of samples (n=3) obtained from the area between Sibsa and Passur rivers, and relatively low level of polymorphic markers used (5.5 alleles/locus) compared to other studies (Oyler-McCance et al. 2012; Yumnam et al. 2014). In regard to the substructure at $K = 7$, it is not entirely unlikely that the cryptic genetic substructures might already have developed within the population because of isolated forest islands due to hundreds of rivers. This finding was further corroborated by the results of GENELAND which detected cryptic genetic signature for the Sundarbans populations. Therefore, all six genetic clusters detected in the GENELAND might not be unexpected as these clusters have been widely distributed as well as isolated by rivers having high (1.2-4.4 km) and moderate (>1 km) widths (Fig. 1, 5). Specifically, the cluster Supati, Hangsha Raj, Notabeki and Haldibunia showed a profound genetic structure than the Passur and Dobeki. This pattern of genetic clustering could be explained by the river systems as such that these four distinct genetic clusters (Supati, Hangsha Raj, Notabeki and Haldibunia) are separated by rivers laid at north-south direction. Conversely the latter two clusters (Dobeki and Passur) are within the same spatial domain between wide rivers (Fig. 1). This further suggests that wider rivers might be precluding tiger dispersal from east to west and vice-versa whilst the north-south movement might not have been restrained to that extent. It is understood that in GENELAND, the ghost cluster, with no individuals assigned, is not uncommon but is a poorly understood phenomenon (Guillot et al. 2005b) which however could be linked to heterogeneous sample distribution (Fontaine et al. 2007).

A recent study using GPS-Satellite and VHS radio-collars fitted to three male and three female tigers in the Indian Sundarbans found that tigers rarely cross the rivers greater than 400 m (Naha et al. 2016) as well as 600 m wide (Barlow 2009). Therefore, we reasonably expect that the tiger population of the Sundarbans would have developed a fine-scale genetic structure.

The genetic connectivity between tiger populations across Indian landscapes has been hampered by human-induced landscape features such as human density, intensity of roads and human habitations

(Joshi et al. 2013; Yumnam et al. 2014), fortunately no such landscape barriers yet exist in the Sundarbans mangrove forests. Therefore, in the absence of other landscape barriers in the Sundarbans, the wider rivers might have been the effective barrier to genetic exchange between locally colonised tiger populations within the forest islands. Several studies have detected profound influence of rivers on genetic structure of a range of species in the Amazon where rivers are particularly wide enough to be an efficient barrier (Lougheed et al. 1999; Moritz et al. 2000; Hayes and Sewlal 2004). In particular, rivers influenced fine-scale genetic structure in the mammalian carnivore of Stone marten, *Martes foina* (Basto et al. 2016), strictly limited gene flow in primates (Pastorini et al. 2003; Goodman and Ganzhorn 2004; Gehring et al. 2012), Raccoon, *Procyon lotor* (Cullingham et al. 2009), and White-tailed deer, *Odocoileus virginianus* (Blanchong et al. 2008). However, the higher resolution of distinct genetic clusters in the entire Sundarbans population could be obtained using reasonable number of samples from each region separated by major rivers (overall sample size >200) and use of highly polymorphic loci (10 alleles/locus) (Oyler-McCance et al. 2012).

Spatial genetic structure

The autocorrelation analysis detected a pattern of fine-scale genetic structure within the population with positive correlations at smaller geographic distances, however with significance negative correlation beyond 72 km. This result indicates that the patterns of gene flow are not homogeneous due to natural (e.g., rivers) or human-induced barriers which partitions the Sundarbans habitat into discrete forest patches (Mona et al. 2014). This result further endorses the findings of fine-scale population genetic structure and phylogenetic clines detected within the population. It is generally expected that genetic distance increases linearly with the geographic distance in a continuously distributed population (Rousset 2000), however which may not be the case if gene flow is restricted within a population (Johansson et al. 2007; Dixo et al. 2009). The significantly negative genetic relatedness beyond 72 km is inconsistent whilst tiger is a long dispersal carnivore, and there are evidences that tiger are able to maintain reasonable genetic connectivity between populations (Sharma et al. 2012; Joshi et al. 2013). Therefore spatial genetic structure could have been developed within this population due to restrictive gene flow owing to limited dispersal between landscapes separated by wider rivers (Sokal and Wartenberg 1983; Epperson 1990; Naha et al. 2016). The influence of rivers and watersheds in developing spatial genetic structure were documented in a range of species including cetaceans (Fontaine et al. 2007), Sitka black-tailed deer, *Odocoileus hemionus sitkensis* (Colson et al. 2013), and Red foxes, *Vulpes vulpes* (Kirschning et al. 2007).

Phylogenetic structure

Spatial distribution of haplotypes in the Bangladesh Sundarbans shows a distinct pattern from west to east with a remarkably higher frequency of haplotype SBT1 on western side of the Arpangassia than the eastern regions. This trend is also aligned to the genetic structure demonstrated by the microsatellite data. The distribution of detected haplotypes suggests that the Arpangassia might have been a historical dispersal barrier in developing genetic clines within tiger populations. Similarly, the spatial distribution of three haplotypes within the entire tiger population displayed an interesting gradient, where the common haplotype shared between Bangladesh and Indian populations was surprisingly detected in the

western region of the Arpangassia; while the haplotype in the Bangladesh Sundarbans was predominantly distributed between Sibsa and Passur. This result clearly suggests that tiger population in the further eastern regions between Sibsa and Passur are phylogenetically divergent from the Indian Sundarbans, which might be due to the Raimangal and Harinbhanga rivers being significant dispersal barrier across the international border.

Conservation implications

Habitat connectivity is fundamental to sustaining regional tiger populations that allow normal dispersal thereby gene flow (Smith 1993). The Sundarbans tigers have been completely isolated from nearby populations by settlement and agriculture landscapes, removing any chance of future genetic exchange that is vital to long-term persistence of the population. Consequently, the immediate conservation effort should focus on increasing the tiger population size up to the carrying capacity to ensure a genetically viable population in the Sundarbans. This would require to curb direct poaching of tigers and their major prey animals, in addition to improving the quality of habitat that can support sufficient prey base for tigers (Aziz et al. 2017b).

Microsatellite and mitochondrial data shows that the rivers might have been the major contributing factor to the genetic architecture of tigers in the Sundarbans, so ensuring population connectivity across wider rivers is vital to prevent further genetic differentiation. Although human activities were not incorporated into this analysis, similar studies in the Indian landscapes revealed that human density could significantly limit tiger dispersal between populations (Joshi et al. 2013; Yumnam et al. 2014). Therefore the future tiger management effort in the Sundarbans should aim to reduce the intensity of human activities (e.g., regulation of cargo movement at night and control of loud whistle from the vessels) across wider rivers to facilitate normal dispersal of tigers. Given the historical genetic clines detected within the populations between Bangladesh and Indian Sundarbans, tigers in the entire Sundarbans should be managed as a single population disregarding the political boundary.

References

Aziz MA, Tollington S, Barlow A, et al (2017a) Using non-invasively collected genetic data to estimate density and population size of tigers in the Bangladesh Sundarbans. Glob Ecol Conserv 12:272–282

Aziz MA, Tollington S, Barlow A, et al (2017b) Investigating patterns of tiger and prey poaching in the Bangladesh Sundarbans: Implications for improved management. Glob Ecol Conserv 9:70–81

Barlow ACD (2009) The Sundarbans tiger: evolution, population status and conflict managment. University of Minnesota

Barlow ACD, Mazak J, Ahmad IU, Smith JLD (2010) A preliminary investigation of Sundarbans tiger morphology. Mammalia 74:329–331

Basto MP, Santos-Reis M, Simoes L, et al (2016) Assessing genetic structure in common but ecologically distinct carnivores: The stone marten and red fox. PLoS One 11:1–26

Bhagavatula J, Singh L (2006) Genotyping faecal samples of Bengal tiger Panthera tigris tigris for population estimation: a pilot study. BMC Genet 7:48

Blanchong JA, Samuel MD, Scribner KT, et al (2008) Landscape genetics and the spatial distribution of chronic wasting disease. Biol Lett 4:130–133

Borthakur U, Das C, Das AK, et al (2013) Noninvasive genetic assessment of population status of tigers (Panthera tigris tigris) in Buxa Tiger Reserve , West Bengal, India. Int J Biodivers Conserv 5:27–32

Budowle B (2004) SNP typing strategies. Forensic Sci Int 146:

Canonizado JA, Hossain MA (1998) Integrated forest management plan for the Sundarbans reserved forest. Dhaka

Colson KE, Brinkman TJ, Person DK, Hundertmark KJ (2013) Fine-scale social and spatial genetic structure in Sitka black-tailed deer. Conserv Genet 14:439–449

Cullingham C, Kyle C, Pond B, et al (2009) Differential permeability of rivers to raccoon gene flow corresponds to rabies incidence in Ontario, Canada. Mol Ecol 18:43–53

Dey TK, Kabir MJ, Ahsan MM, et al (2015) First Phase Tiger Status Report of Bangladesh Sundarbans. Bangladesh Forest Department, Ministry of Environment and Forests, Government of Bangladesh

Dixo M, Metzger JP, Morgante JS, Zamudio KR (2009) Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. Biol Conserv 142:1560–1569

Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Epperson BK (1990) Spatial autocorrelation of genotypes under directional selection. Genetics 124:757–71

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611–2620

Ewers RM, Didham RK (2005) Confounding factors in the detection of species responses to habitat fragmentation. Biol Rev 81:117

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–87

Fontaine MC, Baird SJ, Piry S, et al (2007) Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. BMC Biol 5:30

Francis RM (2017) POPHELPER : an R package and web app to analyse and visualize population structure. Mol Ecol Resour 17:27–32

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500–1508

Frantz AC, Pope LC, Carpenter PJ, et al (2003) Reliable microsatellite genotyping of the Eurasian badger (Meles meles) using faecal DNA. Mol Ecol 12:1649–1661

Gehring PS, Pabijan M, Randrianirina JE, et al (2012) The influence of riverine barriers on phylogeographic patterns of Malagasy reed frogs (Heterixalus). Mol Phylogenet Evol 64:618–632

Giri C, Pengra B, Zhu Z, et al (2007) Monitoring mangrove forest dynamics of the Sundarbans in Bangladesh and India using multi-temporal satellite data from 1973 to 2000. Estuar Coast Shelf Sci 73:91–100

Goodman SM, Ganzhorn JU (2004) Biogeography of lemurs in the humid forests of Madagascar: the role of elevational distribution and rivers. J Biogeogr 31:47–55

Guillot G, Estoup A, Mortier F, Cosson JF (2005a) A spatial statistical model for landscape genetics. Genetics 170:1261–1280

Guillot G, Mortier F, Estoup. A (2005b) Geneland: A computer package for landscape genetics. Mol Ecol Notes 5:708–711

Hansen H, Ben-David M, McDonald DB (2008) Effects of genotyping protocols on success and errors in identifying individual river otters (Lontra canadensis) from their faeces. Mol Ecol Notes 8:282–289

Hayes FE, Sewlal J-AN (2004) The Amazon River as a dispersal barrier to passerine birds: effects of river width, habitat and taxonomy. J Biogeogr 31:1809–1818

Iftekhar MS, Islam MR (2004) Managing mangroves in Bangladesh: A strategy analysis. J Coast Conserv 10:139–146

Islam M, Wahab M (2005) A review on the present status and management of mangrove wetland habitat resources in Bangladesh with emphasis on mangrove fisheries and aquaculture. Hydrobiologia 542:165–190

IUCN–Bangladesh (2001) The Bangladesh Sundarbans: a photoreal sojourn. IUCN - The Intenational Conservation Union Bangladesh country office, Dhaka, Bangladesh

Jackson HA, Bunbury N, Przelomska N, Groombridge JJ (2016) Evolutionary distinctiveness and historical decline in genetic diversity in the Seychelles Black Parrot Coracopsis nigra barklyi. Ibis (Lond 1859) 158:380–394

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–6

Johansson M, Primmer CR, Merila J (2007) Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (Rana temporaria). Mol Ecol 16:2693–2700

Joshi A, Vaidyanathan S, Mondol S, et al (2013) Connectivity of tiger (Panthera tigris) populations in the human-influenced forest mosaic of Central India. PLoS One 8:e77980

Karanth KU, Sunquist ME (2000) Behavioural correlates of predation by tiger (Panthera tigris), leopard (Panthera pardus) and dhole (Cuon alpinus) in Nagarahole, India. J Zool 250:255–265

Kirschning J, Zachos FE, Cirovic D, et al (2007) Population genetic analysis of serbian red foxes (Vulpes vulpes) by means of mitochondrial control region sequences. Biochem Genet 45:409–420

Liberg O, Andren H, Pedersen HC, et al (2005) Severe inbreeding depression in a wild wolf (Canis lupus) population. Biol Lett 1:17–20

Lougheed SC, Gascon C, Jones DA, et al (1999) Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (Epipedobates femoralis). Proceedings Biol Sci 266:1829–35

Loxterman JL (2011) Fine scale population genetic structure of pumas in the Intermountain West. Conserv Genet 12:1049–1059

Luo SJ, Kim JH, Johnson WE, et al (2004) Phylogeography and genetic ancestry of tigers (Panthera tigris). PLoS Biol 2:e442

Mantel N (1967) The Detection of Disease Clustering and a Generalized Regression Approach. Cancer Res 27:14–16

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

Mona S, Ray N, Arenas M, Excoffier L (2014) Genetic consequences of habitat fragmentation during a range expansion. Heredity (Edinb) 112:291–9

Mondol S, Bruford MW, Ramakrishnan U (2013) Demographic loss , genetic structure and the conservation implications for Indian tigers. Proc R Soc Biol Sci 280:20130496

Mondol S, Karanth KU, Kumar NS, et al (2009a) Evaluation of non-invasive genetic sampling methods for estimating tiger population size. Biol Conserv 142:2350–2360

Mondol S, Karanth KU, Ramakrishnan U (2009b) Why the Indian subcontinent holds the key to global tiger recovery. PLoS Genet 5:e1000585

Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of Rainforest Faunas: An Integrated Molecular Approach. Annu Rev Ecol Syst 31:533–563

Mukherjee N, Mondol S, Andheria A, Ramakrishnan U (2007) Rapid multiplex PCR based species identification of wild tigers using non-invasive samples. Conserv Genet 8:1465–1470

Naha D, Jhala Y V., Qureshi Q, et al (2016) Ranging, activity and habitat use by tigers in the mangrove forests of the Sundarban. PLoS One 11:1–16

O'Brien SJ, Roelke ME, Marker L (1985) Genetic basis for species vulnerability in the cheetah. Science (80-) 227:1428–1434

Oyler-McCance SJ, Fedy BC, Landguth EL (2012) Sample design effects in landscape genetics. Conserv Genet 14:275–285

Pastorini J, Thalmann U, Martin RD (2003) A molecular approach to comparative phylogeography of extant Malagasy lemurs. Proc Natl Acad Sci U S A 100:5879–84

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, Rattus fuscipes. Evolution 57:1182–1195

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28:2537–9

Prain D (1979) Flora of Sundarbans. The Superindent of Government Printing, India, Calcutta

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. Genetics 155:945–959

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. J Hered 86:248–249

Reddy PA, Gour DS, Bhavanishankar M, et al (2012) Genetic evidence of tiger population structure and migration within an isolated and fragmented landscape in Northwest India. PLoS One 7:e29827

Rousset (2000) Genetic differentiation between individuals. J Evol Biol 13:58–62

Sanderson E, Forrest J, Loucks C, et al (2006) Setting Priorities for the Conservation and Recovery of Wild Tigers: 2005-2015. New York - Washington D.C.

Seidensticker J (2010) Saving wild tigers: A case study in biodiversity loss and challenges to be met for recovery beyond 2010. Integr Zool 5:285–299

Seidensticker J, Hai MA (1983) The Sundarbans Wildlife Management Plan: Conservation in the Bangladesh Coastal Zone. Bangladesh Forest Department. Dhaka

Sharma S, Dutta T, Maldonado JE, et al (2012) Spatial genetic analysis reveals high connectivity of tiger (Panthera tigris) populations in the Satpura-Maikal landscape of Central India. Ecol Evol 3:48–60

Singh SK, Mishra S, Aspi J, et al (2015) Tigers of Sundarbans in India: Is the population a separate conservation unit? PLoS One 10:1–25

Smith JLD (1993a) The Role of Dispersal in Structuring the Chitwan Tiger Population. Behaviour 124:165–195

Smith JLD (1993b) The Role of Dispersal in Structuring the Chitwan Tiger Population. Behaviour 124:165–195

Smouse P, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity (Edinb) 82:561–573

Smouse PE, Long JC., Sokal RR. (1986) Multiple Regression and Correlation Extensions of the Mantel Test of Matrix Correspondence. Syst Zool 35:627–632

Sokal RR, Wartenberg DE (1983) A Test of Spatial Autocorrelation Analysis Using an Isolation-by-Distance Model. Genetics 105:219–37

Sork VL, Waits L (2010) Contributions of landscape genetics - approaches, insights, and future potential. Mol Ecol 19:3489–3495

Taberlet P, Griffin S, Uhres E, et al (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Mol Ecol 6:869–876

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171–180

Valiere N (2002) GIMLET: A computer program for analysing genetic individual identification data. Mol. Ecol. Notes 2:377–379

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vila C, Sundqvist AK, Flagstad O, et al (2003) Rescue of a severely bottlenecked wolf (Canis lupus) population by a single immigrant. Proc R Soc London - B Biol Sci 270:91–97

Walker CW, Harveson LA, Pittman MT, et al (2000) Microsatellite Variation in Two Populations of Mountain Lions (Puma concolor) in Texas. Southwest Nat 45:196

Walston J, Robinson JG, Bennett EL, et al (2010) Bringing the tiger back from the brink-the six percent solution. PLoS Biol 8:6–9

Waterhouse AM, Procter JB, Martin DMA, et al (2009) Jalview V.2: A multiple sequence alignment editor and analysis workbench. Bioinformatics 25:1189–1191

Woodroffe R (2000) Predators and people: using human densities to interpret declines of large carnivores. Anim Conserv 3:165–173

Wright S (1943) Isolation by distance. Genetics 28:114–138

Yumnam B, Jhala Y V., Qureshi Q, et al (2014) Prioritizing tiger conservation through landscape genetics and habitat linkages. PLoS One 9:

Figure captions

Fig. 1 The Bangladesh Sundarbans showing sampling locations and regions separated by the major rivers.

Fig. 2 Plot of (A) mean likelihood L(K); (B) rate of change of the likelihood distribution of mean; (C) absolute value of second order rate of change of the likelihood distribution of mean, with variance per K value from the STRUCTURE on a dataset containing 53 individuals genotyped at 10 microsatellite loci. Evanno method (D) detected ΔK values indicating the higher probability of number of K, that best fit the data. Here, three clusters are clearly detected, with the next higher ΔK at K=7.

Fig. 3 Genetic structure of tiger population showing in the bar plot from STRUCTURE at $K = 2$, $K = 3$, $K = 4$ and K=7 for 53 individuals typed at 10 microsatellite loci. Each bar represents one individual, where colouration corresponds to the percentage of genotype shared with the respective cluster. For instance, at $K = 3$, red colour corresponds to the tiger population cluster *1* on the western side of the river Arpangasia whilst the cyan denotes the population cluster *2* between the rivers Arpangasia and Sibsa and the blue denotes the population cluster *3* between the rivers Sibsa and Baleshwar.

Fig. 4 Geographical representation of the assignment probabilities for 53 tiger samples typed at 10 microsatellite loci to each of the clusters, proportional to the colour of each pie chart: (a) $K = 2$, (b) $K = 3$, and (c) $K = 7$. The placement of each pie chart indicates the sample location. Note that a distinct pattern of spatial genetic structure of tigers appeared in all clustered K across the western regions of the Sundarbans.

Fig. 5 Maps of GENELAND individual assignments to $K = 7$ clusters for 53 individuals typed at 10 microsatellite loci. The six plots represent the assignment of pixels to each cluster: cluster *a* (Dobeki) between Kalagachhia and Jamuna rivers on the east of the Arpangassia river; cluster *b* (Supati) located in the further east of the Passur river; cluster *c* (Notabeki) between Jamuna-Mahmuda and Raimongal rivers; cluster *d* (Hangsha Raj) between Passur and Sibsa rivers; cluster *e* (Harbaria) between Sibsa and Passur rivers, and cluster *f* (Haldibunia) between Harinbhanga and Raimongal rivers. The assignments of pixels to the seventh cluster were not shown, as no individuals were assigned to it ("ghost cluster", see text for details). The plot is based on the highest-probability run at that value of K. The highest membership values are in light yellow and the level curves illustrate the spatial changes in assignment values.

Fig. 6 The spatial autocorrelation at various distance classes for 53 tiger samples of the Sundarbans genotyped at 10 microsatellite loci. The correlograms showing genetic correlation, *r* as a function of distance, with 95% CI about the null hypothesis of a random distribution of genotypes, and 95% confidence error bars around *r* as determined by bootstrap resampling.

Fig. 7 The diagram showing the results of Mantel test between pairwise geographic and genetic distance matrices, with test of significance by permutation. The dots represent the permuted tiger samples, with regression line indicating the level of genetic differentiation over geographic distances.

Fig. 8 Distribution of haplotypes identified in 33 tiger samples for 1,263 base pairs sequences across the (a) Bangladesh Sundarbans, and (b) the entire Sundarbans, using data from Mondol et al. (2009b) for Indian Sundarbans. Two haplotypes were shown in inset with sample sizes of SBT1 (n = 12) and SBT2 (n = 21). Note that sample locations for Mondol et al. (2009b) are approximate.
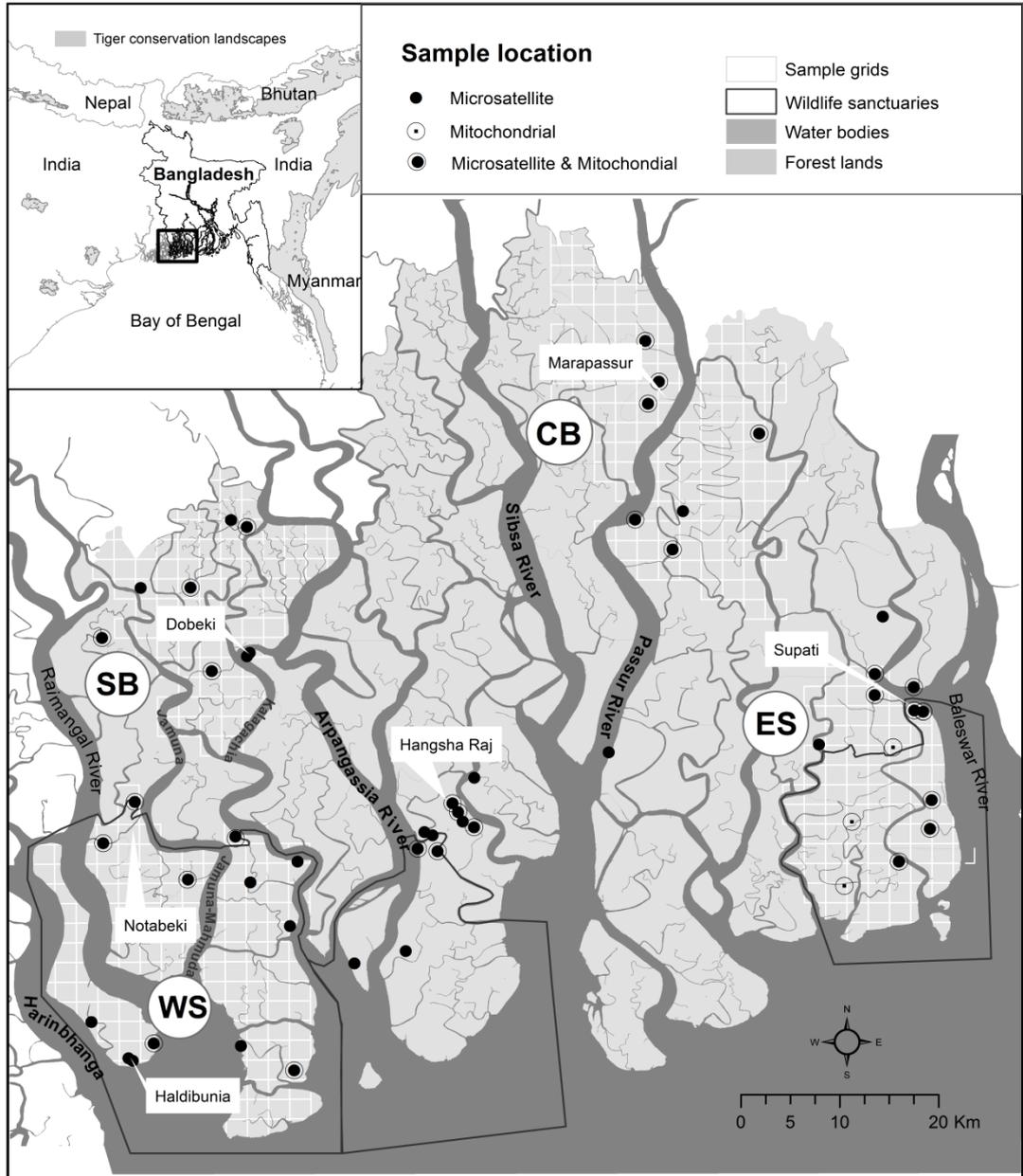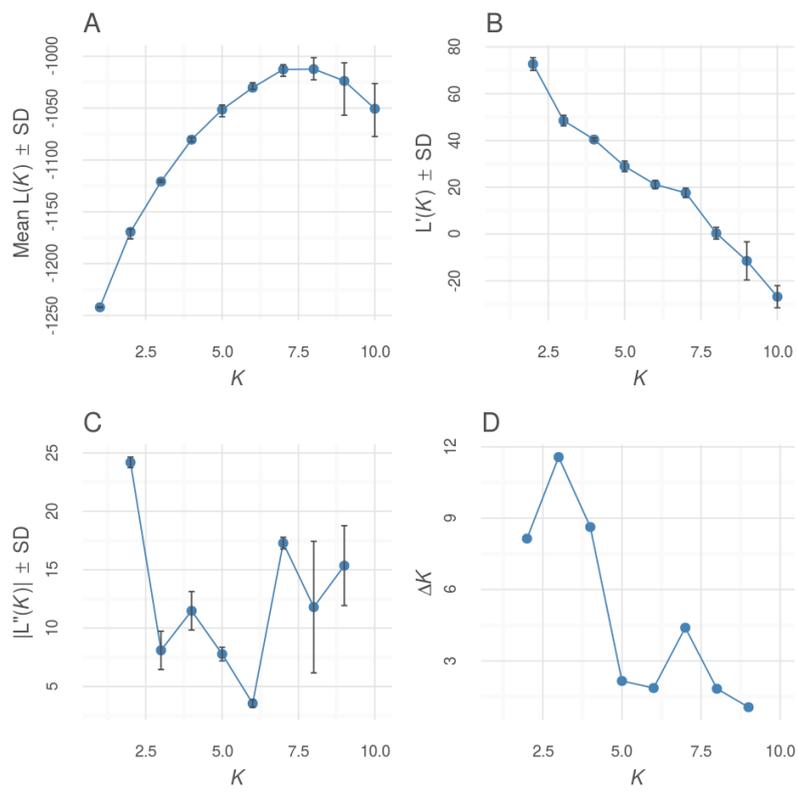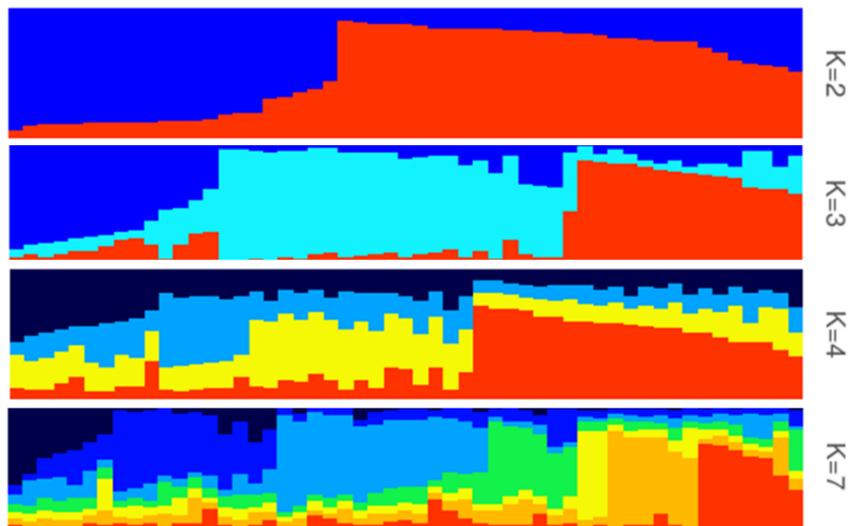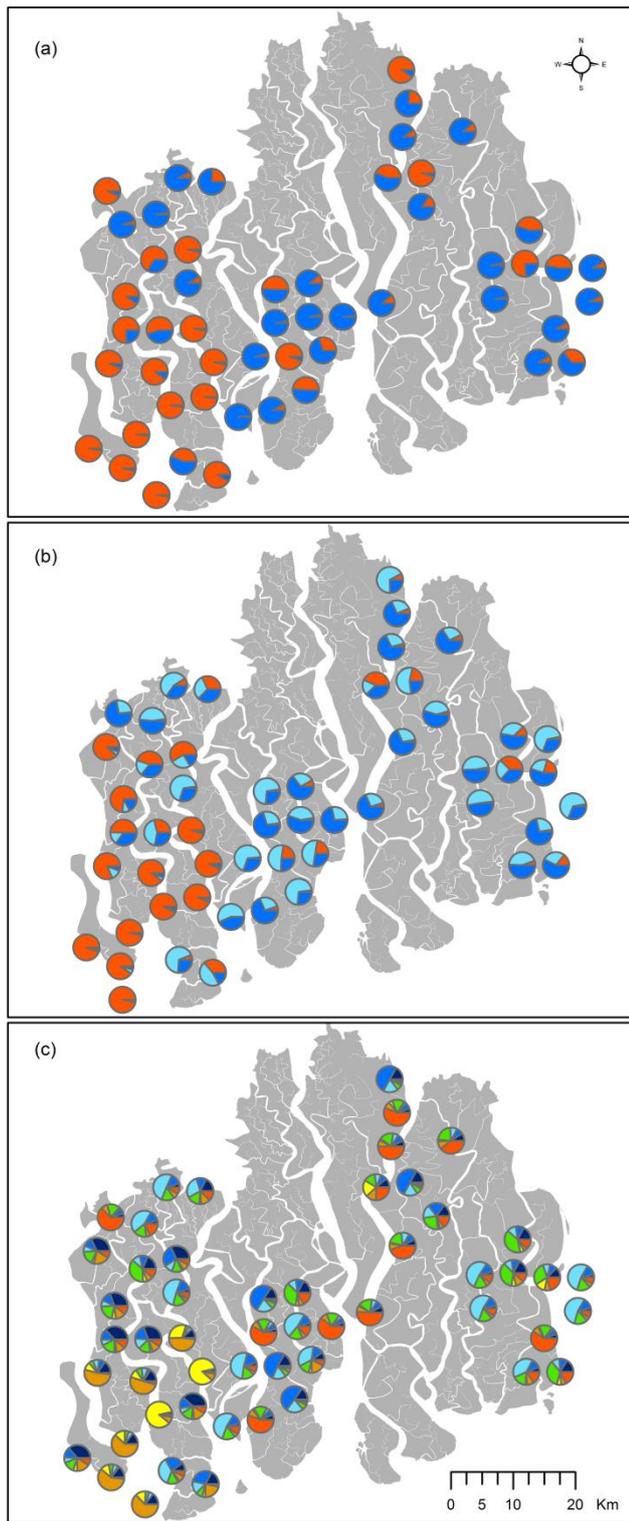
Fig. 1

Fig. 2

Fig. 3

Fig. 4
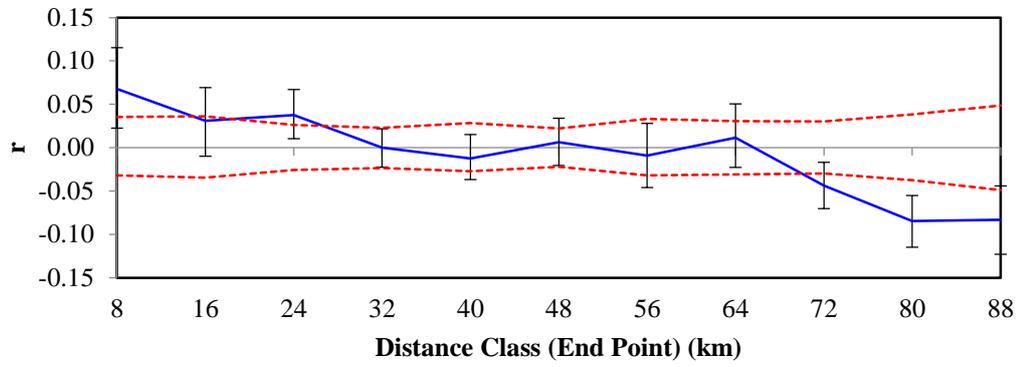
Fig. 5

22

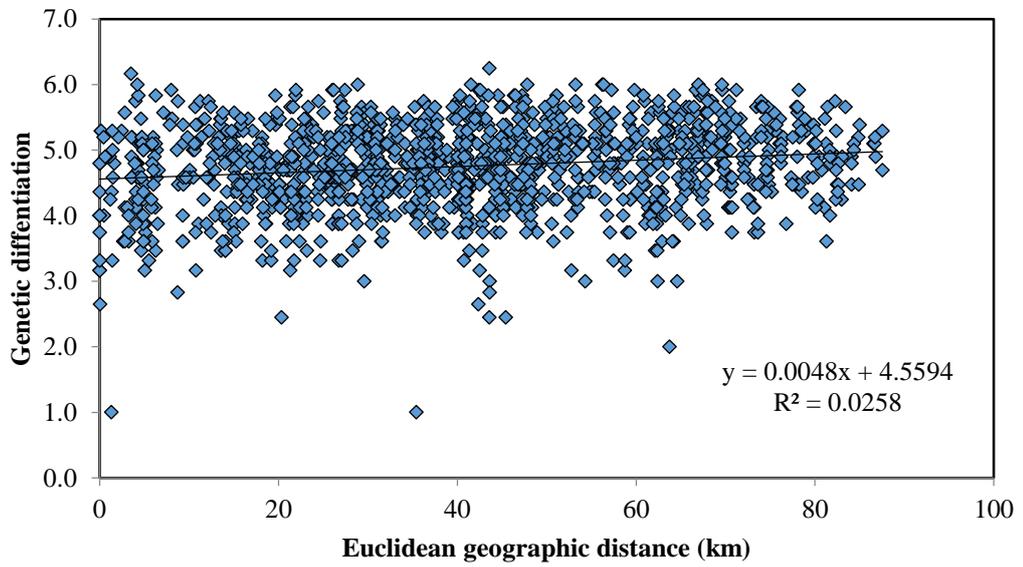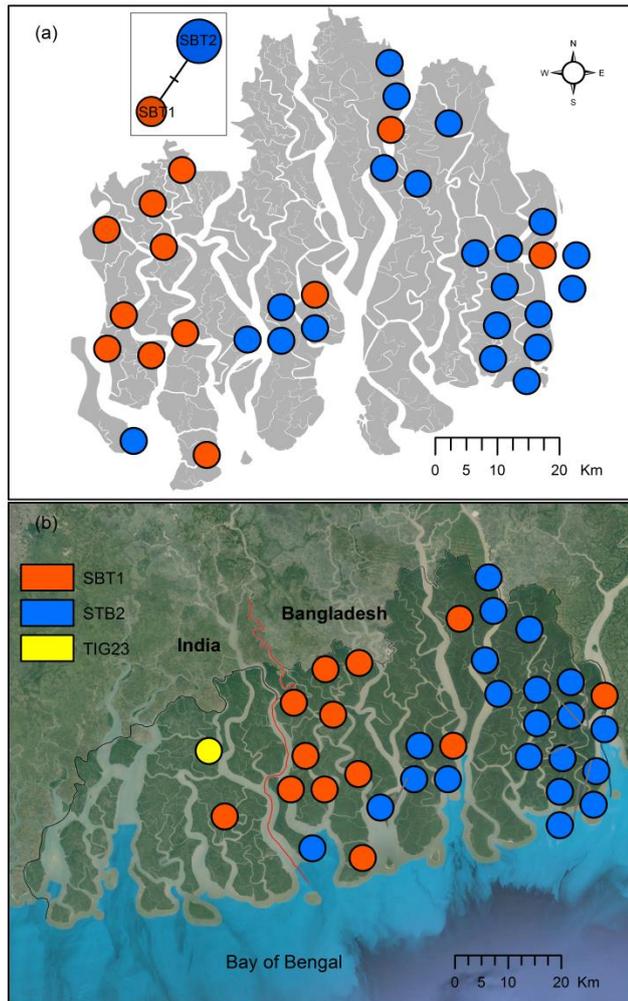**Fig. 6**

Fig. 7

23

Fig. 8

Table 1 Sample area and number of samples used in this study

Sample area	Area (Km^2)	Protection status	Samples collected	Samples screened	Samples genotyped
Satkhira block	342	Reserve forest	77	30	15
West wildlife sanctuary	715	Protected area	152	82	33
Chandpai block	544	Reserve forest	127	74	33
East wildlife sanctuary	383	Protected area	84	44	24
Opportunistic sample	--	Both types	72	35	20

Table 2 Characteristics of microsatellite loci used: locus name, number of allele (A), observed heterozygosity (Ho), expected heterozygosity (He), polymorphic information content (PIC), null allele (NA), allelic dropout (AD), False allele (FA), Hardy-Weinberg Equilibrium (HWE), and the probability of identity for siblings (PID_{sibs}) at 10 microsatellites for 53 individuals

Locus name	A	Ho	He	PIC	NA	AD	FA	HWE	PID(sibs)
Fca304	4	0.35	0.71	0.65	0.33	0.00	0.11	No	9.77E-03
Fca126	5	0.18	0.69	0.62	0.48	0.00	0.02	No	1.91E-03
F41	5	0.61	0.65	0.58	0.02	0.00	0.40	Yes	9.10E-04
Fca230	9	0.13	0.70	0.66	0.37	0.00	0.14	No	4.26E-03
E7	4	0.30	0.45	0.38	0.19	0.00	0.10	Yes	3.00E-04
Fca279	7	0.62	0.81	0.77	0.13	0.16	0.00	Yes	3.67E-01
D15	4	0.49	0.74	0.68	0.20	0.11	0.00	Yes	2.25E-02
Fca43	5	0.44	0.58	0.50	0.11	0.00	0.13	Yes	4.80E-04
Fca90	5	0.34	0.79	0.75	0.39	0.00	0.12	No	1.40E-01
Fca672	7	0.28	0.78	0.74	0.47	0.34	0.00	No	5.43E-02