CONSERVATION GENETICS OF THE BENGAL TIGER
(PANTHERA TIGRIS TIGRIS) IN INDIA

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CONSERVATION GENETICS OF
THE BENGAL TIGER (PANTHERA
TIGRIS TIGRIS) IN INDIA

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**Abstract**

Tigers are endangered in the wild and face increasing threats from habitat loss and fragmentation. The majority of their range occurs in the Indian subcontinent, which is therefore a critical area for tiger conservation. Bengal tigers are distributed across many small protected areas in India. Two important Bengal tiger landscapes – Terai Arc Landscape (TAL) and Sundarbans in India were lacking in basic genetic information and needed to address the impact of anthropogenic pressure and climate change on their genetic makeup in order to identify conservation units. Therefore, I employed nuclear and mitochondrial genetic markers on TAL and Sundarbans tiger individuals to respond these demands for the first time. Thirty-nine heterologous microsatellite loci were screened on Bengal tigers and thirteen of these loci were selected to genotype Bengal tiger samples from western TAL (WTAL) and Sundarbans. After I had genotyped seventy-one Bengal tiger individuals from WTAL, I found cryptic population genetic structure, moderate gene flow and asymmetric migration among the subpopulation. Genetic diversity was moderate and there were no signs of population bottlenecks. In order to maintain the connectivity of subpopulations and avoid human–wildlife conflict, relocation of villages is necessary. Preventive measures against habitat encroachment and a ban on sand and boulder mining in the corridor area should also be implemented. Noninvasively-collected tiger samples from Sundarbans were analyzed for mitochondrial and microsatellite markers and compared with mainland (northern and peninsular) Bengal tiger populations in India. Sundarbans tigers were found to be genetically distinct and had lower genetic variation in comparison to other mainland tiger populations. Demographic analysis indicated recent historical isolation (600–2000 years ago) of the Sundarbans tiger from the mainland. Both historical and genetic evidence supported that the Sundarbans tiger was genetically connected to other mainland tigers until recently. Conclusively, genetic isolation from the mainland tiger population and adaptation to the mangrove ecosystem might have jointly shaped the genetic architecture of the Sundarbans tiger. Hence, the Sundarbans tiger needs special conservation attention for the preservation of its unique ability to adapt and for its genetic individuality. It should be managed as an evolutionary significant unit (ESU) under the adaptive evolutionary conservation (AEC) criteria. I also addressed a problem in the previously suggested sex-specific gene flow estimation method and recommended an alternative approach for a more precise estimation.

**Keywords:** anthropogenic pressure, conservation unit, population genetic structure, Sundarbans, Terai Arc Landscape
Tiivistelmä

Dedicated to my parents and family
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Oulu, April 2017

Sujeet Kumar Singh
### Abbreviations

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<th>Definition</th>
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<tr>
<td>$A$</td>
<td>Number of alleles</td>
</tr>
<tr>
<td>$A_R$</td>
<td>Allelic richness</td>
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<tr>
<td>$bp$</td>
<td>Base pair</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>Inbreeding coefficient</td>
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<tr>
<td>$F_{NULL}$</td>
<td>Null allele frequency</td>
</tr>
<tr>
<td>$F_{ST}$</td>
<td>Index of population differentiation</td>
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<tr>
<td>$h$</td>
<td>Haplotype diversity</td>
</tr>
<tr>
<td>$H_E$</td>
<td>Expected heterozygosity</td>
</tr>
<tr>
<td>$H_O$</td>
<td>Observed heterozygosity</td>
</tr>
<tr>
<td>$HWE$</td>
<td>Hardy–Weinberg Equilibrium</td>
</tr>
<tr>
<td>$k$</td>
<td>Number of clusters</td>
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<tr>
<td>$LD$</td>
<td>Linkage Disequilibrium</td>
</tr>
<tr>
<td>$m$</td>
<td>Migration rate</td>
</tr>
<tr>
<td>$mtDNA$</td>
<td>Mitochondrial DNA</td>
</tr>
<tr>
<td>$N$ or $n$</td>
<td>Sample size</td>
</tr>
<tr>
<td>$N_E$</td>
<td>Effective population size</td>
</tr>
<tr>
<td>$p$</td>
<td>Statistical test value</td>
</tr>
<tr>
<td>$S$</td>
<td>Number of polymorphic sites</td>
</tr>
<tr>
<td>$\pi$</td>
<td>Nucleotide diversity</td>
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Contributions

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<td>SKS, SPG</td>
<td>SKS, SPG, PN</td>
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<td>SKS, SPG, JA, PP</td>
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1 Introduction

The past five to six centuries have marked the beginning of the epoch ‘Anthropocene’ (Crutzen 2002), which has witnessed the high human-mediated decline and loss of species. Although it has contributed to the extinction of species of all kingdoms variably, large-bodied mammals have been particularly vulnerable (Crutzen & Stoermer 2010, Ripple et al. 2014, Dirzo et al. 2014). Most carnivores are declining and have lost much of their historical range (Ripple et al. 2014). Tigers (Panthera tigris), the largest extant felids, are a case in point; of the nine indigenous subspecies, three (Java, Bali, and Caspian) are now extinct and the remaining six have experienced drastic declines in numbers and range (Luo et al. 2004). Presently, tigers are estimated to occupy a mere 7% of their historical range (Dinerstein et al. 2006). India harbors almost 70% of global tiger populations (Jhala et al. 2015), which makes it a stronghold for tiger conservation and survival. Securing the future of Bengal tigers (Panthera tigris tigris) is hence crucial for species endurance. Until a few centuries ago, tigers were widespread in the region. Today, tigers are scattered in small, often isolated populations, none of which are viable on their own (Karanth et al. 2010). In recent studies (Mondol et al. 2009, 2013), genetic data suggested a 90% population decline about 200 years ago and a concomitant loss of 93% mitochondrial genetic variants in modern Bengal tigers (Panthera tigris tigris) in comparison to the historical population. This decline and loss of genetic variation was attributed to anthropogenic impact.

The Bengal tiger is distributed in six global priority ‘tiger landscape complexes’ in India: Viz (i) Western Ghats, (ii) Eastern Ghats, (iii) Central India, (iv) Sunderbans, (v) Shivalik-Gangetic Plain or Terai Arc Landscape (TAL) and (vi) North-eastern India and the Brahmaputra flood plain (Jhala et al. 2008). Wide-ranging ecological data on population size, density (Jhala et al. 2008, 2011, 2015) and the level of population connectivity (Qureshi et al. 2014) are available from all the six global priority landscapes. Population genetic structure and connectivity of the Bengal tiger has been extensively studied, especially in Central India (Sharma et al. 2012, 2013, Yumnam et al. 2014), Eastern Ghat (Reddy et al. 2012b), Western Ghat (Mondol et al. 2009) and Western India (Reddy et al. 2012a). There has also been some information from north-eastern India and the Brahmaputra flood plain (Borthakur et al. 2011). However, information from TAL and Sunderbans tiger landscapes has been lacking. Hence, there is an urgent need to investigate genetic variation and population genetic structure in western TAL and to examine the
genetic status of the completely isolated Sundarbans tiger population, which inhabits a unique swampy location.

Several ecological studies in western TAL have highlighted the seriousness of the anthropogenic impact on tigers (Johnsingh & Negi 2003, Johnsingh et al. 2004, Harihar et al. 2009a, Harihar & Pandav 2012, Singh et al. 2015). Corridors that link the source population (Corbett Tiger Reserve) to adjoining protected areas have been decimated by resorts, village encroachments, expansion of agricultural land, industries, and sand and boulder mining. These anthropogenic pressures hamper the movement of tigers from Corbett to the neighboring protected areas.

The Sundarbans tiger landscape is an entirely isolated swampy habitat that is shared between India and Bangladesh (Barlow et al. 2010, Jhala et al. 2011). Habitat destruction, prey depletion, a direct loss of individuals due to human-tiger conflict and the consequences from the rise in sea level due to climate change in Sundarbans have been extensively studied in both countries (Khan 2004, Bera & Sahay 2010, Barlow et al. 2010, Loucks et al. 2010). Barlow et al. (2010) compared skull size and body weight of Sundarbans tigers with nine tiger subspecies and found that the skulls of Sundarbans tigers were significantly smaller, and their body weight was just half of that of mainland tigers. Due to this morphological distinctiveness, Barlow et al. (2010) recommended that the Sundarbans tigers should be managed separately, and genetic evaluation was also recommended to determine the conservation status of the population.

Therefore, this study was designed to characterize the genetics of the Bengal tiger in WTAL and Sundarbans tiger landscapes for the first time ever, and to determine the level of genetic and ecological exchangeability between Sundarbans and mainland Bengal tiger populations.

1.1 Habitat fragmentation

Habitat fragmentation is the process in which a continuous habitat divides into smaller or unfavorable patches, or is sometimes destroyed completely due to habitat loss. Several human activities, such as mining, building of road networks, the spread of urbanization and indirect human-induced changes in the environment are some of the causes behind the habitat loss and fragmentation. Nevertheless, the expansion of agricultural land is the major cause, as more than 30% of the terrestrial area is used for agricultural production (Foley et al. 2011). Large-scale commercial agriculture in particular has threatened biodiversity and caused habitat loss and fragmentation (Belfrage 2006, Rosset 1999). Apart from agriculture, an increase in
human population and profound economic growth from 1999–2006 has led to urbanization and habitat encroachment for extensive infrastructure development, which have also asserted unprecedented pressure on remaining habitats and biodiversity (McNeely 1997, Sodhi et al. 2004, Shahabuddin 2010) which eventually led to habitat fragmentation.

Habitat fragmentation due to habitat loss is a grave peril to species endurance (Laurance et al. 2002, Sekercioglu et al. 2002). Fundamentally, the consequence of habitat fragmentation is the isolation of patches from a continuous habitat, which affects the local populations by restricting immigration and emigration, which eventually leads to a decrease in population size. Small populations are vulnerable to several stochastic processes, which may lead to local extinctions. Stochastic processes include changes in demographic parameters, loss of genetic diversity due to inbreeding and genetic drift, and the adverse impact of environmental fluctuations and catastrophic events. All these stochastic processes pose fewer threats to a large population, but they might eliminate a smaller one (Laurance 2008).

Habitat fragmentation has the potential to perturb genetic population structures among wildlife populations, especially in large carnivores because of their elusive, solitary and long-ranging nature. For species like large carnivores which need to traverse wide distances and are able to endure a diverse range of environmental conditions (Sweanor et al. 2000, Sunquist & Sunquist 2002), habitat fragmentation is a serious threat. They are at a high risk of extinction when their numbers decline and their populations lose connectivity in degraded habitat fragments. The shrinkage in geographic range and habitat fragmentation due to anthropogenic disturbances may also lead to human-carnivore conflicts (Singh et al. 2015), dispersal and ranging behaviour constraints and thus further reduce population densities (Woodroffe et al. 2005, Loxterman 2011). Ultimately, this leads to isolation of the population and evidently, long-term isolation results in the reduction of genetic variation that increases risks of extinction due to inbreeding and reduced fitness.

1.2 Genetic diversity and inbreeding

Genetic diversity is crucial for populations to adapt to environmental changes. It has been considered as the raw material for the adaptive evolutionary change, which is required for populations to evolve and survive in the changing environment (Franklin 1980, Soulé 1986, Allendorf et al. 2013). Neutral and
adaptive diversity can be considered as two major classes of genetic diversity. Adaptive diversity is more associated with natural selection while many other evolutionary processes can be inferred with the assistance of neutral genetics variations (Avise 2000). Several factors are involved in regulating the quantity and pattern of genetic diversity. They are natural selection, mutation, gene flow, genetic drift, demographic history, breeding system, other life-history traits and interactions between all these factors (Hartl & Clark 2007, Frankham et al. 2009). Gene flow, mutation and genetic drift are the key processes for the regulation of neutral genetic variation within a population. Variation generated due to mutations counter the effect of genetic drift; however, mutations are very rare natural events and are unable to counterbalance the effect of the rapid genetic drift (Frankham et al. 2009).

The rate of loss of rare alleles in a single generation is higher in small populations as compared to large ones (Beebee & Rowe 2008). Loss of genetic variation due to genetic drift is a problem especially for small populations (Frankham et al. 2009). The level and pattern of genetic diversity of a population can give clues on evolutionary history and demography of the population. For example, low level of genetic variation can indicate recent decline in population size and the level of genetic divergence within the population might reflect the level of migration between populations (Allendorf et al. 2013).

The loss of genetic diversity is directly associated with inbreeding (mating between related individuals of the population) and inbreeding depression in small populations. Inbreeding depression can be seen as reduced fitness in survival and reproduction of offspring (Slate et al. 2000, Allendorf et al. 2013). Inbreeding may occur in populations of all sizes, but small populations are more prone to it because in small populations the probability to mate with related individuals is higher compared to in large populations (Lynch 1996). Patterns of inbreeding in a variety of species have revealed that inbreeding affects reproduction, survival, and resistance to stress and diseases in natural populations. Thus, inbreeding increases the risk of extinction (Allendorf et al. 2013, Balkenhole et al. 2016).

1.3 Effective population size and population bottleneck

Sewall Wright (1931) initiated the concept of the effective population size ($N_e$) which was further utilized to address different issues in population genetics, evolutionary biology and applied broadly to conservation genetics (Schwartz et al. 2007, Dudgeon et al. 2012). It is one of the valuable parameters in evolutionary
biology, which affects essential evolutionary processes like the loss of genetic diversity due to drift, accumulation of deleterious mutation and response of the populations to artificial or human-mediated changes (Frankham et al. 2009, Allendorf et al. 2013).

Fundamentally, the concept of effective population size is centered on the change in allele frequency due to drift and the rate of inbreeding. With reference to the Wright-Fisher ideal population (Fisher 1930, Wright 1931), $N_E$ can be defined as the ideal population size, which will experience a similar number of consequences of the rate of change in variance of allele frequency (genetic drift) and inbreeding as the actual or census population size ($N_c$). Several genetic assessment methods which have been used are based on the different concepts of the effective population size ($N_E$) i.e. inbreeding effective size ($N_E I$), variance effective size ($N_E V$), eigenvalue effective size ($N_E E$) and coalescent effective size ($N_E C$) (Ewens 1982, Caballero 1994, Charlesworth et al. 2003). All these methods vary in the time frames i.e. contemporary, past and ancient and are influenced by various processes, such as gene flow, genetic substructure, changes in population size and life history parameters (Wang 2005, Waples 2005). Thus, the comparison of $N_E$ estimates is complicated, since they have been estimated from several dissimilar measures of the genetic changes.

Among different approaches to estimate the $N_E$ of the current or past few generations, the temporal approach which has been most commonly used, is also known as the variance $N_E$ ($N_E V$) (Nei & Tajima 1981, Schwartz et al. 1999, Luikart et al. 2010). The temporal method is principally based on the assumption that change in the allele frequency over time due to drift is inversely related to the effective population size ($N_E$). The temporal method which assumes discrete non-overlapping populations might lead to bias in the results if the assumption does not hold (Luikart et al. 2010). Inbreeding $N_E$ ($N_E I$) is another commonly used method which is based on the rate of increase in inbreeding (loss of heterozygosity over time) (Ewens 1982, Crow & Denniston 1988), as dissimilar to the rate of allele frequency change due to drift i.e. $N_E V$. Theoretically, if the population size is constant, both ($N_E V$ & $N_E I$) will give similar estimates (Crow & Denniston 1988). Recent advances in the temporal method overcome the bias resulting from age structure (Waples & Yokota 2007, Jorde 2012), but sampling difficulties persist in various studies. The temporal method needs to obtain either multiple samples over intervals of one or more generations or samples from associates accompanied by precise demographic information, which is hard to get in wild populations. These sampling limitations provided substantial scope to single sample based $N_E$
estimation. Genetic process in parental generations (heterozygosity, gametic equilibrium and relatedness) forms the foundation for individual sample estimators; hence they can be thought of as measures of inbreeding. A single sample based \( N_E \) estimator includes the linkage disequilibrium (LD) (Laurie-Ahlberg & Weir 1979, Hill 1981, Waples 2006), approximate Bayesian computation (Tallmon et al. 2008, Beaumont et al. 2009), estimator by parentage assignment (Wang et al. 2010) and sibship assignment (Wang 2009) methods.

The LD based approach is one of the most widely-used single sample \( N_E \) estimators. It is a significant tool for assessing the effective population size of species lacking in historical samples and information. It also has the potential to detect population fragmentation and subdivision (Young & Clark 2000, Luikart et al. 2010). A LD based \( N_E \) estimator rests on the idea that as \( N_E \) reduces, genetic drift generates non-random associations among alleles at different loci, i.e. gametic disequilibrium or linkage disequilibrium (LD). Thus, LD can be used in estimating \( N_E \), provided that the source of LD is from a small \( N_E \). LD-based \( N_E \) estimates can be biased due to substructure, immigration or admixture, extensive inbreeding and overlapping generations (Luikart et al. 2010, Wang et al. 2016).

\( N_E \) influences the rate of inbreeding and reduction in genetic variations (Waples 2005). In association with other causes such as mutation, natural selection, and migration, it also determines the amount and distribution of genetic diversity present in the populations (Wang 2005). \( N_E \) is inversely related to the loss of genetic diversity due to inbreeding and genetic drift in finite, randomly mating populations (Frankham 2005, Charlesworth and Wills 2009) and it is considered to be an indicator of augmented extinction risk (Newman & Pilson 1997). Consequently, understanding the effective population size and the different factors affecting it is important in wildlife management and conservation (Luikart et al. 2010).

Effective population size in wild animals is crucial information for sustained management and may allow conservationists to recognize the status of a population and assist in predicting extinction threats. \( N_E \) is typically smaller than the census size (Frankham 1995) and it generally depends on the demography and life history of the species. It is extremely useful in determining the level of variability in a population, and effectiveness of the selection and genetic drift (Charlesworth & Wills 2009). \( N_E \) also predicts the fixation probabilities of alleles and can be used to forecast the survival and fitness of populations (Wang 2005). In order to manage the natural population and avoid inbreeding, a threshold value of \( N_E \geq 100 \) as the effective population size is often used while \( N_E \geq 1000 \) is recommended to maintain the evolutionary potential of the population (Frankham et al. 2014).
A recent decline in the $N_e$ of the population results in a relatively faster decline in the number of alleles compared to the heterozygosity at the polymorphic loci (Lande 1988, England et al. 1996). Most populations fluctuate in size and range over time and if the fluctuations are pronounced enough, populations may become very small, experiencing a so-called bottleneck (Garza & Williamson 2001, Allendorf et al. 2013). Comparatively, a faster reduction in allelic diversity than that in heterozygosity (Nei et al. 1975, Denniston 1978) in a bottleneck situation, can be detected as a consequential transient deficiency in the number of alleles in a population (Maruyama & Fuerst 1985). If a species recovers from a bottleneck, some genetic diversity, especially rare alleles, might be permanently lost and consequently, its evolutionary potential may be decreased. Bottlenecks reduce heterozygosity and affect allelic diversity, but a short-lasting bottleneck has to be quite severe to have a significant impact on heterozygosity. In a single generation bottleneck, a proportion of $1/(2N)$ of the original heterozygosity is lost (Wright 1931, Frankham et al. 2009).

1.4 Gene flow and population structure

Migrations are essential in conservation biology as they are responsible for gene flow between populations, through movements of individuals among populations, thereby enabling the transfer and establishment of new alleles in populations. Gene flow among populations acts as the cohesive force holding geographically separated populations together as a single conservation unit (Allendorf et al. 2013). The rate of gene flow can be characterized as the difference in allele frequencies among populations. High rates of gene flow tend to homogenize populations, thus resulting in similar allele frequencies, while low rates of gene flow tend to result in population isolation and genetic differentiation (Balkenhol et al. 2016). In the absence of gene flow, the populations would diverge over time as a result of genetic drift and selection (Beebee & Rowe 2008).

The rate of gene flow in wild animals is directly correlated with their dispersal, hence meticulous information regarding the rate of gene flow can help researchers to understand the consequences of changing environments and habitat fragmentation on the population structure and demography. It provides important information which can be used to aid conservation practices such as demographic rescue (Tallmon et al. 2004, Allendorf et al. 2013). Reduction in gene flow can result in inbreeding, whereas increased gene flow can bound local adaptation and result in outbreeding depression by removing locally-adapted traits or breaking
gene complexes. On the other hand, gene flow counteracts genetic drift and can spread potentially adaptive genes (Segelbacher et al. 2010). It also reduces the negative genetic effects of population fragmentation and inbreeding. This is why gene flow is needed to maintain the long-term viability of populations. However, because gene flow is a homogenizing force, it can lead to loss of local genotypes and rare alleles and there by aloss of local adaptations and/or manifestations of maladaptive traits. Ultimately, the genetic effect of gene flow depends on the difference in allele frequencies between the receiving population and the immigrants, and on the proportion of alleles contributed by the migrants (Frankham et al. 2009).

Several studies have reported a significant disparity in gene flow between sexes (males and females) (Hedrick 2007, Hoffman et al. 2006). Sex-biased gene flow is connected to behavioral and social features and is well recognized in many species including birds and mammals. Higher male dispersal and female philopatry is common in mammals while higher female dispersal and male philopatry is generally found in birds (Greenwood 1980). This behavioral pattern might have evolved via improvement of reproductive output through enhanced access to mates, kin competition or by avoiding inbreeding. The directionality of sex-biased dispersal is significant in mate choice (Greenwood 1980, Lawson Handley & Perrin 2007). Behavioral sex-biased dispersal patterns can influence the population structure as reported in several mammal species like Steller’s sea lions (Eumetopias jubatus; Hoffman et al. 2006), California sea lions (Zalophus californianus; Gonzales-Suarez et al. 2009), bison (Halbert et al. 2012), Saimaa seals (Valtonen et al. 2014), and Bengal tigers (Gaur et al. 2013). Variable levels of genetic differentiation between males and females among sub-populations are one of the consequences of sex-biased dispersal and it ultimately leads to increased heterozygosity in the progeny. Therefore, measuring sex-biased gene flow is important in understanding the sex-specific evolutionary drivers (Prugnolle & Meeus 2009, Hedrick 2007). As male gene flow can not be easily and directly estimated in many organisms, Hedrick et al. (2013) have suggested an approach to estimate male gene flow using estimates of diploid nuclear and female differentiation.

Assessment of population genetic structure is one of the key components in conservation, population and landscape genetics. Profound knowledge of population structure and association among populations is fundamental in assuring proper management of endangered species (Pacioni et al. 2010). In panmictic populations, i.e. populations where completely random mating takes place, there
are no apparent differences in allele frequencies and estimates of gene flow become infinite. Sometimes the physical distance between populations (isolation by distance; IBD; Wright 1943, Epperson et al. 2010) and variables related to the landscape (topographic barriers) (Forbes & Boyd 1997, Carmichael et al. 2001, Geffen 2004) and environment (Stenseth et al. 2004) may limit the gene flow. If the gene flow is restricted for multiple generations, it will create a particular pattern of population subdivision and genetic structure. Behavioral processes, such as mating systems, dispersal and social relationships among individuals may also influence the genetic structure of the populations (Way & Koepfli 1996).

Apart from the above mentioned factors, anthropogenic factors and the demographic history of populations also strongly influence the structure of populations (Frankham et al. 2009, Hewitt 2000). Anthropogenic factors, such as habitat destruction and encroachment, railway and road networks and agricultural land-use, negatively influence the genetic structure of populations and have an adverse effect on the species (Stornen et al. 2012). Demographic history, i.e. historical and recent fluctuations in the population size of species, always leave its mark on the current genetic pattern of the species, and it can be revealed by modern genetic methods (Flagstad et al. 2003). Therefore, understanding the genetic structure of populations and sub-structuring within populations is crucial for conservation plans and identifying units in need of conservation (Frankham et al. 2009, Allendorf et al. 2013).

At the species level, many different population structures exist. It has been thought that large-bodied habitat generalists, such as large carnivores, exhibit lower levels of genetic structuring and higher migration rates than habitat specialists, with more restricted ranges (Coltman 2008). Large-bodied habitat generalists are often top predators, and by virtue of their high resource requirements, they occur at low densities, have large home ranges and require vast areas to harbor viable populations (Purvis et al. 2000, Gittleman et al. 2001). Therefore, they exhibit lower levels of genetic structuring and higher migration rates. Thus, on fragmented landscapes such habitat generalists face threats of restricted gene flow that may change the genetic structures and ultimately affect the evolutionary processes of the species.

Large carnivores are more susceptible to habitat fragmentation (range narrowing) due to their elusive behavior, long-ranging lifestyle and need for a lot of energy (Woodroffe & Ginsberg 1998, Seidensticker 2010). Fragmentation restricts the movements of individuals between the populations reducing the number of individuals and density of the populations such that populations become
isolated and small. Reduction in the population size and density of the populations might have an adverse effect on gene flow and thus increase the loss of genetic variation due to genetic drift. These factors need to be taken into account when planning for suitable management strategies for conservation. The correct approaches are required to protect enough habitats and resources that fulfill the energy requirements of large carnivores (Karanth & Gopal 2005).

1.5 Conservation unit

A conservation unit is the smallest element of organism or species considered in the conservation planning of species. It may be a group of individuals, a set of populations or a species living in a given environment (Bunnell et al. 2003). For the purpose of management, the delineation of conservation units is crucial (Fraser & Bernatchez 2001), as it helps to design and manage conservation reserves, to set precedence for allocating resources, and to implement protection measures (Ryder 1986, Allendorf et al. 2013). Identification of conservation units also helps to maximize evolutionary potential and minimize extinction risks. Therefore, it has been emphasized that recognition and protection of conservation units in genetically diverse local populations, should be given conservation priority (Luck et al. 2003, Hilborn et al. 2004).

Since more than two decades ago, the delineation of conservation units has been a controversial issue and the problem of how the two commonly used conservation units called ESU (Evolutionary Significant Unit) and MU (Management Unit) should be defined, has still not been resolved. Ryder (1986) introduced the term ESU and suggested that it should describe a population that represents significant adaptive variation or a high level of distinctiveness from other populations. Moritz (1994) criticized the term ESU due to overly high distinctiveness requirements and proposed a less stringent term, MU, which accommodates subdivided populations, in which divergence time has not been sufficient enough to accumulate evolutionary diagnostic characters (morphological differences and reproductive isolation). Several other definitions and suggestions have been proposed (Waples 1991, Dizon et al. 1992, Crandall 2000) and the debate on the definition and applicability of these units continues. Many of the concepts of ESUs and MUs emphasize historic and contemporary population structure but overlook the maintenance of adaptive differences among populations. These rigid criteria of ESUs and MUs create a conflictual situation in planning management strategies. Populations with a history of genetic isolation coupled with current
threats like habitat fragmentation should be managed separately to begin with (Fraser & Bernatchez 2001). Holycross & Douglas (2008) discussed about the dichotomization (panmixia, paraphyly, reciprocal monophyly) of ESUs and suggested that it is very difficult to assess the rigid criteria of ESUs i.e. reproductive isolation and adaptive divergence in rare species and thus an ESU should not be an absolute criterion for conservation. Subsequently, they recommended that in formulating management policies, a population’s adaptive variation should be considered once the population has been recognized as an isolated one to be on a separate evolutionary trajectory.

1.6 The Bengal tiger (*Panthera tigris tigris*)

Historically, the tiger was widely distributed, extending from roughly 10° latitude south of the equator to more than 60° north and through a longitude of more than 100° (Mazak 1996, Nowell & Jackson 1996). It was distributed from the equatorial rain forests of Sumatra to the temperate, boreal forests of Siberia and the dry tropical forests in Western India (Seidensticker *et al.* 1999). Subspecies of tigers are usually demarcated by body size, skull characters, pelage coloration, and striping patterns (Mazak 1981, Herrington 1987). It is usually assumed that the biggest tigers live in the Russian Far East, and the smallest are found on the Sunda Islands. The occiput shape in the skull is typically narrow in Javan and Bali tigers and considerably wider in Caspian tigers (Mazak 1996). Herrington (1987), Kitchener (1999) and Kitchener & Yamaguchi (2010) revealed a wide range of morphological variations within the subspecies that are to some extent overlapping among the subspecies. This corroborates the validity of the traditional subspecies. Substantial morphometric dissimilarities can only be validated between the mainland Asian tigers and Sunda Island tigers, but dissimilarities are typically clinical in the mainland subspecies (Mazak & Groves 2006, Mazak 2010). A recent study based on morphological, ecological and genetic parameters suggested only two broader groups of tigers (Wilting *et al.* 2015). Debate is ongoing.

The nine accepted tiger subspecies are in accordance with their geographic distribution (Luo *et al.* 2004). Extant populations of tigers are grouped into six remaining subspecies, viz. Bengal tiger (*P. t. Tigris*), Sumatran tiger (*P. t. sumatrae*), Siberian tiger (*P. t. altaica*), South China tiger (*P. t. amoyensis*), Indo-Chinese tiger (*P. t. Corbetti*) and Malayan tiger (*P. t. jacksoni*) whereas Caspian tiger (*P. t. Virgata*), Javan tiger (*P. t. Sondaica*) and Bali tiger (*P. t. Balica*) became extinct in the 20th century (Luo *et al.* 2004). Of the subspecies, the Bengal tiger (*Panthera*
*tigris tigris*) inhabits the Indian subcontinent (Bangladesh, Bhutan, India, western China, western Myanmar, and Nepal) and it is considered a flagship species that indicates the health of the forests and ecosystems (Seidensticker *et al.* 1999).

The Bengal tiger is the national animal of India and a conservation-dependent species. It has been placed under the endangered category by IUCN in 2006 and is listed under Schedule-I of the Wildlife (Protection) Act in 1972 in India and Appendix-I of CITES. According to the latest tiger census report, the current Bengal tiger population is estimated to be 2,226 individuals with a range of 1,945 to 2,491 and it consists of over 70% of the global tiger population (Jhala *et al.* 2015) but only 11% of its original habitat still exists (Sanderson *et al.* 2006, Seidensticker 2010). On the basis of tiger occupancy and potential for connectivity, the entire Indian landmass is divided into six landscape complexes (Fig. 1). Until the recent past, all the six tiger landscape complexes in India were contiguous. Hence, it is possible that they shared a common gene pool (Jhala *et al.* 2011).
Fig. 1. Distribution map of the Bengal tiger (*Panthera tigris tigris*) showing the six tiger landscape complexes in India. Adopted from Singh and Sen (2015).

Tigers are mostly solitary, except during the short mating season and the time when the young are dependent on their mothers (Schaller 1967, Hemmer 1987). Mating may take place at any time of the year. However, it is most common from at the end of November to the mid of April (Corbett 1961, Sankhala 1967, 1978). Normally the number of cubs is two or three although it varies from one to four (Mazak 1981, Sunquist & Sunquist 2002). In the wild tiger, female produce new litters only after their families have disintegrated, usually after two or sometimes even three or four years after the birth of preceding young (Mazak 1981). The mother tigress teaches her young to stalk, attack and finally kill their prey (Locke 1954, Schaller 1967, Sankhala 1967, 1978). Tigers have a sex-biased dispersal behavior, in which females are philopatric in nature and males disperse longer distances than females (Smith 1993, Gour *et al.* 2013). The cubs usually separate from their mother (mostly males) at about two years of age while some cubs remain with their mother (mostly females) for three years (Schaller 1967). In the wild,
sexual maturity is reached at the age of three to four years in females, and four to five years in males (Mazak 1981). The size of the territory and home range varies with habitat type, prey density, and sex and age of the animal (Mazak 1981). The Bengal tiger territories usually vary from about 200 to 1,000 km², but areas as small as 64 km² and as large as 9,252 km² have also been documented (Anderson 1957, Schaller 1967).
2 Objectives of the study

In order to strengthen the effective management of tigers in fragmented and human-dominated Western Terai Arc Landscape (WTAL) and Sunderbans Tiger Landscape in India, the present study aims to:

1. Estimate genetic diversity and structure of tiger populations using microsatellite markers in WTAL (I & II);
2. Determine the level of gene flow and migration patterns between different populations of tigers using microsatellite markers in WTAL (II);
3. Examine genetic drift, founder events and genetic bottlenecks in tiger populations of WTAL using microsatellite markers (II);
4. Determine the conservation status of the Sunderbans tiger population (III).
5. Evaluate possible problems in the methodology used to estimate sex-specific gene flow and suggest alternative approaches (IV).
3 Material and methods

3.1 Study area

In the present study, we chose two populations of Bengal tigers without any previous information on their conservation genetics i.e. Western Terai Arc Landscape (WTAL) and Sundarbans tiger landscape out of the six tiger landscape complexes. In this section, I will describe these two tiger landscapes (II & IV).

3.2 Western Terai Arc Landscape

Terai Arc Landscape (TAL) has an area of 42 700 km² and has been divided into 3 areas, namely the western, central and eastern landscapes. There is a lack of connectivity between them. The study area of Western Terai Arc Landscape (WTAL) includes the Corbett Tiger Reserve (CTR) and its surrounding forests divisions of Lansdown, Terai west, central and east, and Ramnagar with an area of 1500 km². Rajaji Tiger Reserve (RTR) is another smaller tiger habitat (Fig. 2) occupying an area of 390 km² (Walston et al. 2010, Jhala et al. 2011). WTAL is a unique habitat at the foothills of the Himalayas and is known for the richness of its species of prey (Johnsingh & Negi 2003, Seidensticker 2010). CTR is the only source population of tigers in this area and is responsible for maintaining genetic connectivity among the entire tiger population of WTAL (Jhala et al. 2008).

Vegetation types in WTAL include both moist as well as dry deciduous forests. The northern slopes are dominated by Shorea robusta (Sal) while the southern slopes are covered with mixed forests consisting of tree species such as Aegle marmelos, Anogeissus latifolia, Holoptelia integrifolia, Terminalia alata, Lagerstroemia parviflora and Ehretia laevis (Champion & Seth 1968). Some of the large mammal species encountered in WTAL are tiger (Panthera tigris tigris), leopard (Panthera pardus fusca), elephant (Elaphus maximus), sambar (Rusa unicolor), chital (Axis axis) and Asian black bear (Ursus thibetanus). Over 585 species of avifauna, both local and migratory, have been recorded. Indian marsh crocodiles (Crocodylus palustris), King cobras (Naja naja) and Indian pythons (Python molurus) are some of the common reptiles found there (Johnsingh & Negi 2003).
3.3 Sundarbans tiger landscape

Sundarbans is the world’s largest estuarine wetland and mangrove forest habitat, located at the confluence of the deltas of the rivers Ganga, Brahmaputra, and Meghna. Sundarbans shares boundaries with two countries—India (38%) and Bangladesh (62%) (Chakraborty 1992, Mitra 2000). The Indian part of Sundarbans extends across an area of 9,630 km². Out of the total area of Indian Sundarbans, 4,266 km² has been classified as reserve forests in 24 pargnas districts, located within 21° 40’ 04” N and 22° 09’ 21”N latitude, and 80° 01’ 56”E and 80° 06’ 01”E longitude (Jhala et al. 2015). It has also been classified as a coastal biogeographic zone (Rodger & Panwar 1988). The mangrove forest in Sundarbans is rich in biodiversity and home to many endangered and highly threatened species, such as tigers (Fig. 3). Therefore, UNESCO (United Nation Educational, Scientific and Cultural Organization) has declared Sundarbans as a World Heritage site (Qureshi et al. 2006).

From 1973–1974, the Indian government declared 2,585 km² of Sundarbans as a tiger reserve, which includes 1,330 km² of a core area of Sundarbans National
Park. Subsequently in March 1989, the entire Indian side of Sundarbans was defined as a biosphere reserve, which includes a core zone (Sundarbans National Park), a buffer zone, a protected mangrove area and wildlife sanctuaries of Sundarbans tiger reserve. The human population density of the area is amongst the highest in the county with 1,437 persons/km² (Qureshi et al. 2006).

Sundarbans is an important tidal forest, consisting of almost 35 mangrove species and 117 other halophytic mangrove associates (Qureshi et al. 2006). On the overall almost 350 vascular plant species belonging to 254 genera are present in Sundarbans (Chakraborty 1992). Five vegetation types i.e. mangrove scrub, mangrove forest, salt water mixed forest (Heritiera), brackish water mixed forest and palm swamp have been documented in Sundarbans (Champion & Seth 1968).
The dominant trees in the northern part of the area belong to genus *Heritiera* (Malvaceae), and have the local name ‘sundari’, giving the name “Sundarbans” to this landscape.

Sundarbans consists of around 163 species of birds (Sen & Naskar 2003), 165 species of fish, 23 species of molluscs and 56 species of reptiles (Dinda 2010). Nevertheless, the Bengal tiger is the flagship species of Sundarbans which forms the only mangrove tiger population in the world. As a result, this area is of tiger conservation unit (TCU) level–1 importance (Dinerstein *et al.* 2006). Here, tigers live an amphibious life which makes this population unique when compared to other Bengal tiger populations and tiger subspecies. Small-sized prey i.e. chital (*Axis axis*), wild pig (*Sus scrofa*), rhesus macaque (*Macaca mulatta*) and lesser adjutant (*Leptoptilos javanicus*) (Khan & Monirul 2008) are common. Native mega herbivores, including Javan Rhinoceros (*Rhinoceros sondaicus*), swamp deer (*Cervus duvaucelii*), gaur (*Bos taurus*), and hog deer (*Axis porcinus*) are now extinct in Sundarbans (Chakraborty 1992).

### 3.4 Sample collection

In the present study, I used biological samples of tigers, such as blood, tissue, scat, and hair, as a source of DNA (I, II & III). All the biological samples were collected by the Forest Department of Uttarakhand and Sundarbans Tiger Reserve, west Bengal. Tissue and blood samples were collected from tigers which have died a natural death, are road kill or radio-collared, and those killed due to human-tiger conflicts (man-eaters). Scat and hair samples were collected during the day-to-day trail surveys and monitoring by the forest department. Collected biological samples were given to the national repository at Wildlife Forensic and Conservation Genetics Cell (WFCGC), Wildlife Institute of India (II & III). All the tissue, blood and hair samples were stored at −80°C. Scat samples were dried at 55°C and stored at room temperature. Information and place of collection and/or geographical coordinates are available for all the biological samples and are well-catalogued in the national repository.

Altogether 71 individual tiger samples were collected from different sampling locations in western TAL. The tissue (*n = 57*) and blood (*n = 2*) samples were obtained from animals which had died naturally or were killed due to human-tiger conflicts (man-eaters). The samples were collected between years 2005 and 2014 across western TAL (RTR, *n = 3*; CTR, *n = 27* and Terai west, central and east FDs, *n = 15* and Ramnagar FDs, *n = 14*). Scat samples (*n = 12*) were collected from
different sampling locations from RTR. A total of 56 samples were obtained from CTR and adjoining FDs with 15 samples from RTR (II) (Fig. 2).

In contrast to WTAL, not many samples were obtained from Sundarbans, because collection of non-invasive samples like scat and hair or tissue of dead animals is very difficult in the inaccessible swampy habitat. Therefore, only 16 samples were collected by the forest department (blood $n = 5$, tissue $n = 1$, hair $n = 1$ and scat $n = 9$) (Fig. 3). For the comparison with other mainland tiger populations, I also obtained tiger samples from the tiger reserves located in peninsular India ($n = 73$; scat) and northern India ($n = 62$; tissue, blood, and scat) (III).

3.5 DNA extraction, multilocus genotyping and sequencing

Various DNA extraction methods were used for the different types of biological samples. DNeasy Tissue and Blood Kit (QIAGEN, Germany) were used for tissue, blood and hair samples with some modifications and the addition of DTT for hair sample digestions. DNA from blood spots on the FTA Classic Cards (Whatman™) was extracted following Smith & Burgoyne (2004) protocol. Scat DNA isolation was performed with the QIAamp Stool DNA isolation Kit (QIAGEN, Germany). All extraction processes were performed as suggested by manufacturer’s protocols with some modifications in the time for sample digestion and amount of final elution (II & IV).

Thirty-nine heterologous microsatellite loci were screened and nine to thirteen markers were selected to genotype the Bengal tiger samples from western TAL and Sundarbans. Details about the method of screening are in the original article I. Bengal tiger DNA samples from western TAL were amplified and genotyped with the set of thirteen microsatellite markers (II), and Sundarbans tiger samples were amplified and genotyped with the set of nine microsatellite markers (III). Details about PCR amplification and genotyping can be found in the original articles (I, II, III). Amplified PCR products were subjected to Applied Biosystems 3130 Genetic Analyzer for fragment analysis. Alleles were scored by using the program, GENEMAPPER 4.0 (Applied Biosystems). All the genotype data were rigorously checked for possible null alleles, stutter bands and large allele dropouts using the program MICROCHECKER (Van Oosterhout et al. 2004). Deviations from Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) were inspected using the web version of GENPOP (Raymond & Rousset 1995, II, III).
To understand the phylogenetic status of the Sundarbans tiger, 3kb of mitochondrial DNA (mtDNA) fragment comprising of the gene \textit{NADH dehydrogenase subunits 2, 5 and 6} (\textit{ND2}: 960 bp, \textit{ND5}: 1139 bp, \textit{ND6}: 443 bp) and \textit{cytochrome b} (\textit{cytb}) (555 bp) were amplified and sequenced from six samples of the Sundarbans tigers using the forward and reverse primers developed by Luo \textit{et al.} (2004). The description of PCR amplification and sequencing are described in the original article (III).

All the sequenced data were manually aligned and compared with previously published Bengal tiger sequences by Luo \textit{et al.} (2004), Mondol \textit{et al.} (2009) and Sharma \textit{et al.} (2009, 2011) and are available at the GenBank. Details can be found in the original paper III. As the length of the mtDNA sequences analyzed in the previous studies (Luo \textit{et al.} 2004, Mondol \textit{et al.} 2009) were not identical to ours, I formed two independent data sets and analyzed them separately. In total, I analyzed 2600 bp of mtDNA sequence with sequences by Luo \textit{et al.} (2004), concentrating on the phylogenetic status of the Sundarbans tigers. Altogether 1063 bp of mtDNA sequences were aligned with sequences by Mondol \textit{et al.} (2009), focusing on genetic variation, genetic differentiation and phylogeny among different tiger populations.

### 3.6 Genetic analyses

All the objectives of the present study were examined with microsatellite genotyping data. The mtDNA data were only used for objective 4 which is to understand the phylogenetic status and demographic history of the Sundarbans tiger.

#### 3.6.1 Genetic diversity and inbreeding

Heterozygosity is the most commonly used estimate to elucidate genetic diversity from allelic data. Observed heterozygosity ($H_O$) can be estimated by the proportion of heterozygous individuals at a particular locus and it is the realized measure, while expected heterozygosity ($H_E$) is estimated based on the observed frequency of alleles. Allelic richness ($A_R$) and the number of alleles ($N_A$) are also commonly used diversity estimates. Genetic diversity estimates such as $H_O$ and $N_A$ are biased due to differences in sample sizes because rare alleles tend to be missing if sample sizes are small (e.g. Nei 1987, Petit \textit{et al.} 1998). Therefore, these estimates are not comparable if samples sizes differ. Allelic richness avoids this problem by using a rarefaction method to estimate the allelic richness at a locus for a fixed sample size.
If more populations are sampled, it uses the smallest sample size (Petit et al. 1998). In the present study, all the diversity estimates ($H_o$, $H_e$, $N_i$, & $A_e$) and inbreeding coefficient ($F_{IS}$) in the Bengal tiger population of WTAL and Sundarbans were calculated using the program FSTAT (Goudet 1995, II, III).

In order to estimate the mtDNA diversity of the Sundarbans tiger and to compare it with the other Bengal tiger populations in India, 1036 bp sequences (including ND2, ND5, and cytb), were aligned with sequences by Mondal et al. (2009). Genetic diversity was estimated by calculating haplotype diversities ($h$), nucleotide diversities ($\pi$) and the number of segregating sites ($S$) with the program, DnaSP 5.0 (Librado & Rozas 2009, III).

3.6.2 Effective population size and population bottleneck

Genetic and demographic methods are common ways to estimate the effective population size but the genetic method is more often used due to unavailability of appropriate demographic data. Estimates of effective population size can be obtained based on the loss of heterozygosity over generations, changes in allele frequencies over time due to genetic drift and the rate of decay in linkage disequilibrium among loci (see section 1.3 for details). In the present study, the program LDNe v.1.3.1 (Waples & Do 2008, II, III; based on linkage disequilibrium) was used to estimate the effective population sizes in Bengal tiger populations. The analysis was repeated using different critical p-values i.e. 0.01, 0.02 and 0.05 and finally, $P_{crit} = 0.02$ was used because it has a balance between precision and bias from rare alleles (England et al. 2010, II, III).

Populations that have decreased in size or are experiencing bottlenecks, are vulnerable to many unfavorable chance events; therefore detection of genetic bottleneck signatures is critical for conservation. Several population genetic methods, such as departure from allele frequency distribution expected in an equilibrium population, excess in heterozygosity relative to that predicted on the basis of the number of alleles (Cornuet & Luikart 1996) and the mean ratio of the number of alleles to the range in allele size test (Garza & Williamsons 2001) are aimed at detecting signatures of genetic bottlenecks. Program BOTTLENECK 1.2.02 (Piry et al. 1999, II) uses three different quantitative tests (sign test, standardized difference test and Wilcoxon test) to detect departure from the mutation–drift equilibrium and one qualitative test (the mode shift test) to detect bias in the allele frequency distribution. In addition, the distortion in allele frequency distribution can be quantified by the Garza Williamson index (G-W) also
known as the M-ratio test. The G-W index was estimated by Arlequin 3.1 (Excoffier & Lischer 2010). Details of the analysis can be found in the original article II.

### 3.6.3 Population genetic structure

Different approaches (Bayesian and non-Bayesian) were used to examine the population genetic structure of the Bengal tiger. Bayesian clustering method implemented in the program, STRUCTURE (Pritchard et al. 2003, II, III), TESS (Chen et al. 2007, II, III) and GENELAND v.4.0.3 (Guillot et al. 2005, II) were used. All these programs are based on individual Bayesian clustering (IBC) and each individual was assigned to a population based on the allele frequencies. Among the IBC based programs, STRUCTURE works exclusively with multilocus genotypes, while TESS and GENELAND can use both multilocus genotypes data and geo-referenced information. The admixture model was used for all three programs. Additionally, in STRUCTURE, I used both options i.e. with and without prior information of the sampled population using the admixture model (II, III).

Bayesian clustering approaches are sensitive to deviation in Hardy–Weinberg equilibrium and linkage disequilibrium, which might lead to a bias in the output of the population genetic structure. Therefore, a non-Bayesian clustering approach i.e. multivariate analysis (non-model-based program) was also used to validate the IBC results. Multivariate ordination analyses, such as the discriminant analysis of principal components (DPAC) and spatial principle component analysis (sPCA) are commonly used in non-Bayesian clustering approaches (II). DAPC and sPCA both are implemented in the adegenet R package (Jombart et al. 2008, II) and provide an efficient description of genetic clusters using a few synthetic variables, called discriminant functions. These analyses seek linear combinations of the original variables (alleles) that show differences between groups, as clearly as possible, while minimizing variation within clusters. DAPC recognizes genetic groups through sequential clustering and model selection. Initially, it changes the genotype data into principal components and then uses k-means clustering to account for groups of individuals, with the best-suited number of clusters recognized by the Bayesian Information Criteria (BIC) (Jombart et al. 2008, 2010). Spatial principle component analysis (sPCA) takes spatial information into account along with genetic information. It explores spatial genetic patterns by simultaneously taking into account spatial auto-correlation and genetic variance. It summarizes the data in positive and negative scores which infer global (positive) and local (negative) genetic structure patterns. Permutations were performed for reliability of the results.
and scores were used to understand the spatial genetic pattern (Jombart et al. 2008, II).

To investigate the population genetic structure of Sundarbans (III), mtDNA haplotype networks were drawn by combining mitochondrial haplotype data of Sundarbans with the published sequences of Bengal tigers from different tiger landscapes in India (1036 bp) and other subspecies of tigers across the global tiger distribution range (2600 bp). The program NETWORK 4.6.1.1 (Bandelt et al. 1999, III) was used to draw the haplotype network with default settings (weight = 10 and e = 0). Details can be found in the original article III.

3.6.4 Gene flow and migration rate

In order to measure the long-term gene flow between the different sampling (subpopulations) sites of Bengal tiger populations, F-statistics were calculated using microsatellite genotype data according to the method of Weir and Cockerham (1984) and their significance was tested with 10,000 permutations using Arlequin 3.15 (Excoffier & Lischer 2010, II, III). In addition, mitochondrial data (mtDNA) were used to calculate the pairwise $F_{ST}$ based on the haplotypes frequency (II). Arlequin 3.15 (Excoffier & Lischer 2010) was also used to perform the hierarchical molecular variance (AMOVA) analysis (II & III).

Migration rates between the populations were estimated using three different genetic methods, i.e. likelihood based estimator (II & III), posterior probability distribution (II & III) and sibship analysis (II). The recent migration (over the last 5–6 generations) was calculated using a Bayesian MCMC method implemented in BAYESASS 1.3 (Wilson & Rannala 2003). To identify first generation migrants, admixed and resident individuals from the populations, a likelihood-based estimator was used. The Bayesian criterion of Rannala & Mountain (1997) and the re-sampling method of Paetkau et al. (2004) as implemented in the program GENECLASS 2.0 (Piry et al. 2004) were applied to identify possible first generation migrants (II & III). Apart from the likelihood and posterior probability based estimators, a sibship analysis method was also used to detect migrants, admixed and resident individuals in a population. The COLONY software package (Wang 2004) was used to carry out the sibship analysis with the full likelihood approach (II).
3.6.5 Sex-biased gene flow

The indirect approach for gene flow estimation can be performed to acquire estimates of the sex-specific gene flow by using gender-specific genetic markers. Maternally inherited mtDNA can be used for female gene flow, while the paternally inherited Y-chromosome can be used for male gene flow. Other approaches have also been used, especially when lacking Y-chromosomal data. I estimated the sex-specific gene flow between Bengal tiger populations using the approach by Hedrick et al. (2013). Male differentiation was first estimated using the equation 7 (a) in Hedrick et al. (2013)

\[
F_{ST(m)} = \frac{2F_{ST(f)}F_{ST(f)}}{F_{ST(f)} - 2F_{ST} + 3F_{ST}F_{ST(f)}}
\]

and then the ratio of male to female gene flow was assessed using equation 7 (b) in Hedrick et al. (2013) (IV).

\[
\frac{m_m}{m_f} = \frac{F_{ST(f)}(1 - F_{ST(m)})}{F_{ST(m)}(1 - F_{ST(f)})}
\]

3.6.6 Demographic history

In order to understand the demographic history of the populations, the coalescent theory framework is the most commonly used (Kingman 1982a, Kingman 1982b) and most reliable method. The coalescent process is random genetic drift viewed in reverse, starting with a sample of size N at the present and following the ancestral lines back in time until they reach their most recent common ancestor (MRCA) (Avise 2000). Demographic analysis of the Bengal tiger was performed using both mitochondrial and microsatellite markers. Bayesian skyline plots (Drummond et al. 2005) of mitochondrial effective population sizes through time were produced from the sequences of ND2, ND5 and cytb genes of Sundarbans tigers with sequences of northern and peninsular populations using the program BEAST (Drummond & Rambaut 2007). In order to estimate divergence times and population history, we also used the coalescent-based Approximate Bayesian Computation (ABC) algorithm in the program DIY-ABC (Cornuet et al. 2014) with microsatellite data of Sundarbans, peninsular, and northern tiger populations (III).
3.6.7 Ecological exchangeability

Identification of a distinct conservation unit requires profound understanding (i.e. historical and recent) of the intensity of genetic as well as ecological exchangeability between the distinct populations (Crandall et al. 2000, Allendorf et al. 2013). Concordance between genetic and adaptive differences between geographically distinct populations supports their definition as population units, significant for conservation (Allendorf et al. 2013). Therefore, in order to investigate the ecological characteristics which distinguish Sundarbans tigers from other mainland Bengal tiger populations, a large amount of available, published literature was reviewed to search for the evidence of adaptive differences or differences in various traits, such as morphology (Barlow et al. 2010), habitat type (Chakraborty 1992, Karanth et al. 2004), body size of prey (Khan & Monirul 2008, Karanth 2003), competition with other predators (Jhala et al. 2011) and density (Jhala et al. 2011, III).
4 Results and discussion

4.1 Conservation genetics of the Bengal tiger in western Terai Arc Landscape (WTAL)

Among thirteen microsatellite markers amplified with WTAL samples, three were showing higher rate of null allele and HWE deviation across the sampling site in WTAL (II). Hence, these three loci were excluded from subsequent analysis. Therefore, I used 13 loci to calculate genetic diversity indices and 10 loci were used for gene flow, migration rate, population structure and sibship analysis.

4.1.1 Genetic diversity and inbreeding

The microsatellite loci used in the present study were polymorphic with a mean number of alleles (MNA) of 6.6 (range 4–10). Genetic diversity estimates show a moderate level of genetic variation ($H_O = 0.50$, $H_E = 0.64$; $A_R = 6.5$) in the western TAL tiger population (Table 2). The level of genetic variations in WTAL was lower than reported in other Bengal tiger populations ($H_O = 0.65–0.71$, $H_E = 0.74–0.81$, $A_R = 7.76$) in India (Mondol et al. 2009, Yumnam et al. 2014). Nevertheless, the diversity estimates are not directly comparable with each other because different studies used different sets of microsatellite markers. The lower level of genetic variation in WTAL might be due to its geographic position at the northern limit of the tiger distribution range. Populations at the edge of the distribution range of a species often have lower genetic diversity compared to more central populations as suggested by the “rear-edge” (Petit et al. 2003) or “abundant-center” hypotheses (Brussard 1984). The tiger habitat in WTAL is structured in patches that occur as “stepping-stones”, due to the recent anthropogenic activities (development of highways, resorts and increased agriculture and human pressure). This may restrict gene flow between the subpopulations and be responsible for the observed genetic erosion and positive inbreeding coefficient ($F_{IS} = 0.23$). Philopatric felids, which are threatened by poaching, are known to have high $F_{IS}$ values as reported by studies in for example other tiger populations (Sharma et al. 2012), African leopards (Panthera pardus pardus, McManus et al. 2015), African lions (Panthera leo, Tende et al. 2014), mountain lions (Puma concolor, Holbrook et al. 2012) and ocelots (Leopardus pardinis, Wultsch et al. 2016).
Table 2. Genetic characterization of the 13 microsatellite loci applied to the Bengal tigers from WTAL, India (Article II).

<table>
<thead>
<tr>
<th>Locus</th>
<th>CTR</th>
<th>RTR</th>
<th>Overall (CTR &amp; RTR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>A</td>
<td>H₀</td>
</tr>
<tr>
<td>PttA2</td>
<td>55</td>
<td>6.0</td>
<td>6.3</td>
</tr>
<tr>
<td>PttE5</td>
<td>54</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>PttF4</td>
<td>51</td>
<td>4.0</td>
<td>3.7</td>
</tr>
<tr>
<td>PttD5</td>
<td>56</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td>FCA304</td>
<td>55</td>
<td>5.0</td>
<td>6.7</td>
</tr>
<tr>
<td>FCA272</td>
<td>53</td>
<td>5.0</td>
<td>7.5</td>
</tr>
<tr>
<td>F41</td>
<td>52</td>
<td>6.0</td>
<td>4.9</td>
</tr>
<tr>
<td>PUN327</td>
<td>56</td>
<td>7.0</td>
<td>5.8</td>
</tr>
<tr>
<td>PUN100</td>
<td>56</td>
<td>5.0</td>
<td>6.5</td>
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<tr>
<td>FC126</td>
<td>47</td>
<td>7.0</td>
<td>5.6</td>
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<td>FGC572</td>
<td>54</td>
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<td>FCA232</td>
<td>50</td>
<td>5.0</td>
<td>0.1</td>
</tr>
<tr>
<td>FCA090</td>
<td>50</td>
<td>6.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean</td>
<td>56.9</td>
<td>4.28</td>
<td>0.51</td>
</tr>
</tbody>
</table>

N= sample size, A = number of alleles, AR = allelic richness, H₀ = observed heterozygosity, Hₑ = expected heterozygosity, Fₛ = inbreeding coefficient, F₀ = null allele frequency, * = P < 0.05

1 includes CTR (Corbett Tiger Reserve) and Forest Divisions of Lansdown, Ramnagar, Terai West and Terai central, RTR = Rajaji Tiger Reserve
4.1.2 Population genetic structure and gene flow

STRUCTURE identified the most probable number of genetic clusters in WTAL to be two (k = 2) (II), based on both the Δk and the mean likelihood value, both with and without prior information. The clustering pattern was more well-defined with prior information than without.

![Fig. 4. Bayesian clustering analysis results from the program, STRUCTURE based on the 10 microsatellite markers and admixture model. a) Summary bar plot of STRUCTURE at k = 2 showing assignment of each individual to either of the two clusters in WTAL, including Rajaji Tiger reserve (RTR), Corbett Tiger Reserve (CTR) and adjoining Forest Divisions (FDs); b) Map showing individuals by dots, colored to reflect the cluster they were assigned to with a probability higher than > 70% (Article II). When no prior information on sample collection location was used, 50% of the individuals were assigned (q > 70%) to their respective clusters and 50% showed](image)
mixed ancestry (q < 70%) (Fig. 4a, b). On the other hand, with prior information, all the individuals were assigned to their sampling locations even though some individuals with mixed ancestry were found (II). Ambiguity in the clustering pattern in STRUCTURE (with and without locprior information) might have been due to the presence of a weak population structure in WTAL (II). Hubisz et al. (2009) suggested that this type of ambiguity in the individual assignment with STRUCTURE can arise because in the case of a weak population structure without a prior information model, it is sometimes not possible to detect the population structure. Separate STRUCTURE analysis with samples from CTR and the adjoining FDs also suggested two clusters, but the clustering pattern was not distinct and individuals showed mixed ancestries (II).

TESS output converged with STRUCTURE’s finding of two genetic clusters and was more similar to the results with prior than without prior information clustering pattern. In TESS, RTR individuals were grouped in one cluster while CTR and adjoining FDs individuals were clumped in a separate cluster with few individuals migrating between these clusters (II). GENELAND identified four genetic clusters in western TAL, but most samples were assigned to only three clusters with high spatial uniformity and noticeably clear cluster boundaries. Only four individuals were assigned to the fourth cluster (II). These identified genetic clusters supported the genetic clustering of CTR and adjoining FDs as identified by STRUCTURE. In STRUCTURE, the clustering pattern was not as distinct but one cluster was formed in GENELAND, CTR and Terai west FD while in Ramnagar FD, a separate group was formed. Individuals from Terai central and east showed mixed ancestry in both clusters.

Non-Bayesian clustering approaches i.e. DAPC and sPCA also supported the clustering pattern of the Bayesian methods and suggested at least two genetic clusters in WTAL. The multivariate DAPC analysis identified 3–4 genetic clusters, of which only RTR clustered separately, while CTR and adjoining FDs were overlapping despite separate centroids (Fig. 5a, b). The shade gradients in sPCA quantify the degree of genetic differentiation and reveals that the tigers from WTAL form two genetic clusters (II).

To conclude, both Bayesian and non-Bayesian clustering methods concurred with a minimum of two genetic clusters and identified the presence of cryptic genetic structuring in WTAL populations.
As the tigers have good dispersal abilities (Joshi et al. 2013) and connecting corridors assist their movements between subpopulations, maintenance of this connectivity is crucial. Ecological and anthropogenic factors are known to change the genetic structure of the population even in contiguous habitats (Pilot et al. 2006, Frankham 2006). WTAL is known to be a good habitat for tigers, because of its abundance of prey and moderate forest cover (Harihar & Pandav 2012, Johnsingh & Negi 2003, Jhala et al. 2011, 2015). As summarised in article II, there is no difference in the habitat quality and prey distribution in the different protected areas in western TAL. Therefore, the present study supports the assumption that recent (in the last century) population fragmentation and increased urbanization have had an impact on the genetic structure of the Bengal tiger in WTAL. In support of the structuring described above, AMOVA and pairwise $F_{ST}$-analysis also revealed
significant genetic differentiation ($F_{ST} = 0.061, p < 0.01$) in WTAL. AMOVA analyses showed that most of the molecular variance (93.14%) originates from within the populations while variance between populations and among the group was lower (1.56% & 4.54%) (II). In WTAL, tigers are distributed across protected areas (Tiger Reserves) and outside protected areas (Forest Divisions). Hence there are different administration control and forest management practices. Therefore, different sampling sites (i.e. Tiger Reserves and forest divisions) in WTAL were treated as separate subpopulations. I grouped all the tiger reserves and forest divisions of WTAL into four different subpopulations i.e. Corbett Tiger Reserve (CTR), Rajaji Tiger Reserve (RTR), Ramanagar Forest Division and Terai Forest Divisions (including Terai east, central, and west). The pairwise $F_{ST}$-value between RTR and CTR suggested significant genetic differentiation and moderate historical gene flow ($F_{ST} = 0.060, p = 0.01$), while the level of gene flow between CTR and adjoining forest divisions was higher ($F_{ST}$ values ranged from 0.01 to 0.04, Table 3). There was rather low but significant differentiation and the high gene flow between CTR and Ramanagar FD ($F_{ST} = 0.04, p = 0.001$) reflects the functionality of the corridor between these populations. Likewise, the lack of genetic differentiation and high gene flow ($F_{ST} = 0.01$) between CTR and Terai FDs indicate sufficient migration and gene flow (Table 3).

These results (levels of divergence between RTR and CTR, and CTR and adjoining FDs) provide evidence that within western TAL, the eastern part of CTR can be considered as a contiguous tiger habitat that has maintained a higher rate of gene flow compared to the west of CTR. The western part of CTR is relatively flat and has fewer networks of “drainages” or “rivulets” than eastern CTR. It has also always been more prone to human encroachment than the more mountainous terrains. Such “rivulets” in mountainous terrains may provide secluded places for tigers to move from one area to another (II). This could have been one of the reasons for the higher gene flow in the east than the west of CTR.

The current scenario of gene flow also supports our hypothesis that recent anthropogenic activities (habitat fragmentation and urbanization) in TAL have influenced the genetic structure and gene flow of the Bengal tiger in this region. Ecological studies (Semwal 2005, Wikramanayake et al. 2010) also suggested that TAL is the most threatened and fragmented landscape in Asia for many species, especially for large mammals. This region had already been inhabited by ancient tribes. However, it was not until after the successful malaria eradication in the 1960s that people started to migrate to Terai from different parts of India and
cleared forests for agricultural purposes, which resulted in only 2% of the forest remaining connected on this landscape (Semwal 2005, Wikramanayake et al. 2010).

Development activities in the form of industrial setups in WTAL has resulted in the conversion of natural mixed forest to monoculture plantations in Haridwar, Bijnore, Terai west, Terai central and Terai east FDs (Fig. 2, Johnsingh & Negi 2003, Semwal 2005). Johnsingh & Negi (2003) also accentuated the severity of anthropogenic pressure on some parts of this landscape, especially along the RTR–CTR corridor. Subsequently, Joshi et al. (2010) reported that in the last two decades, movements of wild elephants and tigers have become rare due to heavy vehicle traffic on the Kotdwar–Lansdowne and Kotdwar–Kalagarh highways (Fig. 2). The townships and agricultural lands both in Kotdwar severely threatened the corridor connecting Rajaji to Corbett and Bimore especially (Qureshi et al. 2014). The seriousness of the townships and agricultural development in Kotdwar can also be observed by the night-light pollution in this area (Fig. 2). Qureshi et al. (2014) also reported a similar kind of night-light pollution in Kotdwar, which is situated along the RTR-CTR corridor. Recent studies (Joshi et al. 2010, Harihar & Pandav 2012, Qureshi et al. 2014) also confirmed that the loss of functionality of the regional corridors has resulted in a decrease in tiger abundance in eastern RTR and suggested maintaining the RTR–CTR corridor by eliminating disturbances and facilitating movement between the populations.

| Table 3. Pairwise $F_{ST}$-estimates between the tiger subpopulations in WTAL, India (the subpopulations are defined based on the sampling sites) (Article II). |
|-----------------|--------|-----------------|-----------------|--------|
| Populations     | CTR    | Terai West, Central and East FDs | Ramnagar FDs | RTR    |
| CTR             | 0      | 0.016           | 0.041*        | 0.060* |
| Terai West, Central and East FDs | 0.016  | 0               | 0.033*        | 0.011* |
| Ramnagar FDs    | 0.041* | 0.033*          | 0              | 0.093* |
| RTR             | 0.060* | 0.011*          | 0.093*        | 0      |

*P < 0.05
FD: Forest Divisions, CTR: Corbett Tiger Reserve, RTR: Rajaji Tiger Reserve

### 4.1.3 Migration rate in western Terai Arc Landscape

Three different approaches were utilized to estimate migration rates between sub-populations in WTAL and they all pointed to similar conclusions of low current migration between the sub-populations. GENECLASS detected three first generation migrants individual (II), BAYESASS reflected low and asymmetric
contemporary migration (CTR to RTR; \( m = 9\% \), II) and the sibship analysis by COLONY found some first and second order sibship relationships between and within the subpopulations in WTAL (II). On the whole, the number of half sibships was greater than that of full sibships and there was a greater number of full and half sibship assigned within populations than between populations (II). Findings of first generation migrants and asymmetric migration in the last 3–5 generations from CTR to RTR indicate that there is a low level of migration between the subpopulations despite the high level of disturbance along the connecting RTR–CTR corridor (II). This asymmetric migration might be due to source–sink population dynamics, with individuals moving from the more stable, higher density population CTR into a neighboring low-density population (RTR). Emigrant tigers are probably exploring areas of low disturbance in order to settle there. Harihar et al. (2009b) reported a high turnover rate of tigers in eastern RTR, determined using camera traps. This high turnover is probably a result of tigers coming from CTR, as this is the only source population for tigers in this landscape.

Several ecological studies (Jhala et al. 2008, Johnsingh et al. 2004, Harihar et al. 2009a, Harihar & Pandav 2012) have emphasized on the current anthropogenic pressure along the connecting corridors in WTAL and recommended conservation of the RTR–CTR corridor for a better future for tigers and elephants in RTR. Harihar et al. (2009a) reported a photo capturing a lactating female with her cub in the eastern part of the region and suggested that this is evidence of the recovery of the tigers. They credited this recovery to the possible, successful movement of tigers from the source population (CTR) through the RTR–CTR corridor after the relocation of the Gujjars (pastoralist community) from eastern RTR. Relocation of the Gujjars reduced the disturbance caused by livestock grazing, and lumbering while providing a habitat for prey species (chital, swamp deer). Ultimately, it facilitated the settlement of breeding tigers in a low disturbance area.

The sibship relationships of individuals between RTR and CTR also indicate movements between the two populations. Though there is no direct genetic confirmation of migration of particular individuals from CTR to RTR, the detection of first-generation migrants, considerably asymmetric migration, and sibship relationships provide more evidence of the functionality of the corridor. This also provides good reason to curtail human disturbance from the west of CTR to establish another source population. At present, the prey abundance in eastern RTR may sustain around 11–15 tigers (Harihar & Pandav 2012).
4.1.4 Effective population size and population bottlenecks

LD-based estimators for effective population sizes in WTAL were higher for CTR and adjoining FDs populations ($N_E = 81.2; 95\% \text{ CI}: 47.7–195.9$) than for RTR ($N_E = 46.8; 95\% \text{ CI}: 13.6 \text{ to infinite, Table 4}$). All the bottleneck tests based on an excess of heterozygosity or on allele frequency distributions showed that there were no consistent strong signals for a bottleneck. This was also supported by the Garza-Williamson index. Garza & Williamson (2001) suggested that values of the G-W index that are lower than 0.68 are evidence of a bottleneck, whereas values greater than 0.68 would denote no bottleneck history. In the present data set, the G-W values were between 0.73 and 0.77 (Table 4).

On the overall, all the different genetic tests indicate that WTAL has not experienced a population bottleneck in the recent past (Table 4, II). However, genetic methods do not always detect recent population declines (Peery et al. 2012). Sometimes the demographic decline may not result in a bottleneck, as numerous factors, such as the time-frame of the population decline, pre-bottleneck diversity, and gene flow between populations can affect the probability of detecting a bottleneck (Cornuet & Luikart 1996, Garza & Williamson 2001, Girod et al. 2011). Several other genetic studies on the Bengal tiger population in central (Sharma et al. 2012, Yumnam et al. 2014) and southeastern (Reddy et al. 2012b) tiger landscapes also did not detect the population bottleneck. Therefore, the absence of a recent bottleneck in WTAL population advocates that the larger source population, i.e. CTR in TAL, have had a more stable population history than smaller populations. This has also been observed on central Indian landscape by Sharma et al. 2013, Yumnam et al. 2014. Though, in light of the level of genetic variation (moderate; $H_O = 0.50$) in WTAL, should the future population size remain low or even decrease due to current anthropogenic pressures, the WTAL population will probably lose even more of its genetic diversity due to drift and inbreeding.
Table 4. Summary of bottleneck analyses and effective population sizes ($N_e$) in Western Terai Arc Landscape. Pop = population, TPM = two phase mutation model, SMM = stepwise mutation model, SD-test = standardized difference test (Article II).

<table>
<thead>
<tr>
<th>Pop</th>
<th>Mutation model</th>
<th>Sign Test</th>
<th>SD test</th>
<th>Wilcoxon test</th>
<th>Mode shift test</th>
<th>G-W index</th>
<th>$N_e$ (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR1</td>
<td>TPM</td>
<td>$p=0.065$</td>
<td>$p=0.385$</td>
<td>$p=0.99$</td>
<td>L-shaped</td>
<td>0.76±0.21</td>
<td>81.2</td>
</tr>
<tr>
<td></td>
<td>SMM</td>
<td>$p=0.013^*$</td>
<td>$p=0.000^*$</td>
<td>$p=0.99$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTR</td>
<td>TPM</td>
<td>$p=0.580$</td>
<td>$p=0.393$</td>
<td>$p=0.46$</td>
<td>L-shaped</td>
<td>0.72±0.22</td>
<td>46.8</td>
</tr>
<tr>
<td></td>
<td>SMM</td>
<td>$p=0.407$</td>
<td>$p=0.116$</td>
<td>$p=0.78$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$includes Corbett Tiger Reserve (CTR) and Forest Divisions of Terai East, Central, West and Ramnagar

$^*_{significant}$ p-value ($p < 0.05$)

RTR= Rajaji Tiger Reserve

4.2 Conservation status of the Sundarbans tiger

4.2.1 Genetic diversity and effective population size

All the nine microsatellite markers used were polymorphic with three to four alleles in the Sundarbans tiger population. Estimated genetic diversity indices of Sundarbans and mainland tiger populations are summarized in the Table 5. Allelic richness ($A_R$) and expected heterozygosity ($H_E$), were lower in Sundarbans tigers ($A_R = 3.24, H_E = 0.58$) as compared to other mainland populations i.e. peninsular ($A_R = 4.80, H_E = 0.70$) and northern India ($A_R = 4.18, H_E = 0.67$). The $F_{IS}$ estimates of these three populations ranged from moderate to high, from 0.014 in Sundarbans to 0.34 in northern India (Table 5). However, a probable explanation for a high $F_{IS}$ in the northern and peninsular populations is the Wahlund effect due to allele frequency differences in different subpopulations. Mitochondrial diversity indices were also higher in peninsular ($h = 1.0, \pi = 0.002$) and northern India ($h = 0.82, \pi = 0.001$) in comparison to Sundarbans, ($h = 0.679, \pi = 0.001$, Table 5).

The lower level of genetic variation (nuclear and mitochondrial) in Sundarbans tigers as compared to peninsular and northern populations is most likely due to genetic drift and inbreeding in this small and isolated population. It should be noted that as the inbreeding coefficient is calculated using levels of heterozygosity, it can be biased due to a low sample size. The LD-based estimate of $N_e$ suggested a negative value of effective population size of the Sundarbans tiger population. This might be due to the sample size to effective population size ratio being too small, and therefore the sampling error overcomes the effect of drift within the sample.
Table 5. Genetic diversity indices and effective population size ($N_e$) of Sundarbans, peninsular and northern India tiger populations based on nine microsatellite markers and mtDNA sequences. $N$ = sample size, MNA = mean number of alleles, AR = allelic richness, $H_0$ = observed heterozygosity, $H_e$ = expected heterozygosity, $F_{IS}$ = inbreeding coefficient, $S$ = number of segregating sites, $h$ = haplotype diversity and $\pi$ = nucleotide diversity (Article III).

<table>
<thead>
<tr>
<th>Population</th>
<th>Microsatellites</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>NE (95 % CIs)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sundarbans</td>
<td>13</td>
<td>3.33</td>
<td>3.24</td>
<td>0.491</td>
<td>0.587</td>
<td>0.109</td>
<td>241 (10-infinity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peninsular</td>
<td>73</td>
<td>7.33</td>
<td>4.80</td>
<td>0.492</td>
<td>0.707</td>
<td>0.300</td>
<td>73 (48–128)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern</td>
<td>62</td>
<td>4.88</td>
<td>4.18</td>
<td>0.401</td>
<td>0.674</td>
<td>0.394</td>
<td>48 (30–92)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population</th>
<th>Mitochondrial DNA</th>
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</thead>
<tbody>
<tr>
<td>Sundarbans</td>
<td>8</td>
<td>3</td>
<td>0.679</td>
<td>0.001</td>
<td>8</td>
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<tr>
<td>Peninsular</td>
<td>15</td>
<td>19</td>
<td>1.00</td>
<td>0.002</td>
<td>15</td>
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<tr>
<td>Northern</td>
<td>8</td>
<td>3</td>
<td>0.820</td>
<td>0.001</td>
<td>8</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

4.2.2 Population genetic structure and gene flow on Sunderbans tiger landscape

Alignment of the 2600 bp sequences of Sundarbans tigers ($n = 6$) with the sequences from six tiger subspecies (Luo et al. 2004) revealed three nucleotide substitutions that were specific to the Bengal tiger (Panthera t. tigris) and two that were specific to the Sundarbans tiger. Of these, Sundarbans specific (or unique) substitutions were located in the ND5 gene and were present in all the six Sundarbans tiger samples, while the other substitution in the cytb gene was only found in three samples. The three nucleotide substitutions, specific (diagnostic) to the Bengal tiger but found also in the Sundarbans tiger, indicate that Sundarbans tigers belongs to the Bengal tiger ($P. t. tigris$) subspecies. The Sundarbans tigers comprised of a monophyletic group with other Bengal tigers in the median-joining (M-J) network (Fig. 6a). Further evaluation of Sundarbans specific substitutions with the 1026 bp sequences (cytb, ND2, ND5 & ND6) of other Bengal tiger populations (northern and peninsular) reveals that these haplotypes are unique to the Sundarbans tigers (III). In addition, the M-J network with this 1026 bp data revealed that the haplotypes typical to Sundarbans tigers comprise of a separate group that stems from peninsular India and had further split into two lineages (Fig. 6b). This indicates that the Sundarbans tiger population contains two new closely-
related mitochondrial lineages, which had not been identified hitherto. The lower number of mitochondrial lineages in Sundarbans in comparison to mainland tiger populations suggests that genetic drift and founder effects may have had an extensive impact on the Sundarbans gene pool, even though a small sample size might have contributed to a bias in our results. The absence of the haplotype unique to Sundarbans, in other tiger populations indicates that there has been practically no emigration of females from Sundarbans to peninsular India after its geographical isolation. This is probably because there has been no suitable habitat for tigers to disperse to from Sundarbans.

Fig. 6. a) Median-Joining network created from four mtDNA genes (cytb, ND2, ND5 & ND6; 2600 bp) depicting genetic relationships between the haplotypes found in tigers. a) Haplotypes found in the Sundarbans tigers (black), Bengal tigers (green) and all other five tiger subspecies (yellow, from Luo et al. 2004). b) Haplotypes found in the Bengal tiger populations in the present study and from Mondol et al. (2009). Sundarbans (black), northern (pink), central (yellow) and southern (blue). The sizes of the circles are proportional to the haplotype frequencies (Article III).
Increased anthropogenic development could be another reason, which prevents dispersal, as tigers are known to avoid areas with human disturbance (Johnsingh & Negi 2003, Jhala et al. 2008).

I found significant genetic differentiation between the Sundarbans and mainland tiger populations with both microsatellite and mitochondrial markers (Table 6). The pairwise mitochondrial $\Phi_{ST}$ ranged from 0.116 to 0.250 ($p < 0.05$), while pairwise $F_{ST}$ measured using the microsatellite ranged from 0.069 to 0.070 ($p < 0.001$), between the Sundarbans and mainland tiger populations (Table 6). The significant pairwise $F_{ST}$-values between the Sundarbans and mainland tiger populations suggest reduced gene flow between Sundarbans and the mainland. The level of genetic differentiation was higher for mtDNA as compared to nuclear DNA (Table 6). This kind of pattern might be due to the behavior of female tigers i.e. philopatry and territoriality (Smith & McDougal 1991, Seidensticker et al. 1999), and low or no immigration in Sundarbans. This could also be due to the smaller effective population size $(1/4)$ of mtDNA than that of the nuclear markers $(1/4)$ ($N_E = 10$) (Nei & Tajima 1981, Birky et al. 1983), and consequently the stronger effect of drift on mtDNA.

Table 6. Pairwise $F_{ST}$-values between Sundarbans, Peninsular and Northern India tiger populations using microsatellite (below the diagonal) and mtDNA markers (above the diagonal) (Article III).

<table>
<thead>
<tr>
<th>Bengal tiger populations</th>
<th>Northern</th>
<th>Peninsular</th>
<th>Sundarbans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>0</td>
<td>0.058*</td>
<td>0.250*</td>
</tr>
<tr>
<td>Peninsular</td>
<td>0.030**</td>
<td>0</td>
<td>0.116*</td>
</tr>
<tr>
<td>Sundarbans</td>
<td>0.070**</td>
<td>0.069*</td>
<td>0</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.001

Microsatellite-based Bayesian clustering analysis using STRUCTURE with a large geographical scale (Sundarbans, northern and peninsular) suggested five genetic clusters with the mean $LnP(K) = -2882.64$ (Fig. 7a, III), whereas $\Delta K$, calculated from the output of STRUCTURE results, proposed that the most likely number of clusters is four (Fig. 7a, III). However, many studies have argued that $\Delta K$ does not always produce a proper resolution of population structure (Waples & Gaggiotti 2006, Faubet et al. 2007). Clustering analyses performed with the program TESS gave results similar to STRUCTURE with five genetic clusters. Thus, I found that $K = 5$ would be more appropriate and justifiable. At $K = 5$, Sundarbans individuals were assigned together to a distinct cluster with high with high probability ($q > 0.8)$
and there was no evidence of admixture (Fig. 7 & III). These analysis also suggested that the Sundarbans tiger population is an isolated population (III).

Fig. 7. Individual assignment probabilities of the Bengal tigers analysed using program STRUCTURE that was run for $K = 2$–5 for northern, peninsular and Sundarbans populations. (a) Magnitude of $\Delta K$ (rate of change in the log probability) and $\ln P(K)$ (posterior probability of the data) as a function of $K$ (populations) detected 4 and 5 genetic clusters, respectively. b) Bar plots showing each individual by a bar with the probability of membership to each cluster indicated by different colors (Article III).

In the last 600 to 800 years, an enormous portion of forests around Sundarbans has been destroyed and it is at present under pressure of extensive anthropogenic development (Eaton 1990, Nunn & Kumar 2006). These anthropogenic activities included the conversion of forest areas to cultivated land in 1770, establishment of
historical trading places (Bera & Sahay 2010), extension of agriculture due to irrigation canal development in the Ganga basin (Gazetteers of India 2001), settlement of people and increase of human population density to amongst the highest in India (1437.4 individuals/km²) (Qureshi et al. 2006). These are all likely to have had a strong impact on the present population genetic structuring of the Sundarbans tiger.

4.2.3 Demographic history

Demographic analysis of the Sundarbans tiger using mtDNA and microsatellite markers arrived at the conclusion that the Sundarbans tiger has diverged quite recently from the mainland tiger population. The mtDNA based Bayesian skyline plot analysis found more or less stable growth in the effective population size in the last tens of thousands of years that was, however, interrupted by a decline in the effective population size about 1000–2000 years ago. Similar to the Bayesian skyline plot analysis, Approximate Bayesian Computation analysis (ABC) along with microsatellite data, clearly pointed to the recent divergence of Sundarbans from the mainland (peninsular India). In order to explore the divergence time of Sundarbans, the analysis was performed by grouping the data as suggested by STRUCTURE and TESS analyses. Among the three tested scenarios (Fig. 8), the two most likely scenarios (Scenario 1 and 2) showed high posterior probability values using different validation; scenario 1 using direct estimate (0.537), whereas scenario 2 through the logistic regression (0.471) (Table 7). Both scenarios (scenario 1 and 2) reflected a recent divergence and the founding of the Sundarbans tiger population, approximately 125 and 384 generations ago, i.e. around year 600 and 1900 (generation time for tigers is 4–5 years; Smith & McDougal 1991) before present. Estimates of effective population size of the present Sundarbans tiger population were about 1000 in both of these scenarios. The high effective population size estimate might be due to high genetic variation in the founder population. This is possible because our results suggest that in the recent past, the Sundarbans population was a part of a contiguous peninsular Indian population and it probably kept the signature of a high effective population size like in peninsular India even after it became isolated. Mondol et al. (2009) also reported a large effective population size of peninsular Indian tigers in the recent past ($N_e = 23,280$). Interestingly, both these methods arrived at the conclusion of the divergence of Sundarbans in the last 600–2000 years, which is difficult to elucidate in light of the recent anthropogenic activities. As described in the previous section (4.2.2), the
human activities only began 600 to 800 years ago. Therefore, the causes of divergence of the Sundarbans tiger population could be found in more distant biogeographical changes. There is evidence of a series of climatic change which has severely affected the geography and flora of Sundarbans, leading to a shift in the distribution of different habitat types. Ultimately, these changes also impacted the distribution of the Sundarbans fauna and even the tigers (Bera & Sahay 2010).

Fig. 8. Scenarios tested with DIYABC. Pop 1 = Northern India, Pop 2 = Peninsular India and Pop 3= Sundarbans (Article III).

About 6000 years ago (in the mid-Holocene warm period) the shoreline of the north-eastern Indian peninsula was situated to the west of the present shoreline, which was comparatively closer to the foothills of the Himalayas (III). Therefore, the present Sundarbans area did not exist for terrestrial life until the shoreline had moved eastward, and tigers could arrive from peninsular India. Later on, a shift in
the river Ganga (Najman et al. 2008) brought changes to agricultural land use in the areas adjoining Sundarbans. After that, the anthropogenic activities from 600 to 800 years ago started to impact the tiger habitat. All these factors probably caused the complete isolation of Sundarbans tigers (Johnsingh et al. 2004, Jhala et al. 2011). Moreover, historical evidence also supports the genetic data and recent isolation of Sundarbans tigers from the peninsular tigers (III).

Table 7. Summary of the posterior probabilities of the three tested historical scenarios of the tigers (Article III).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Scenario 1</th>
<th>Scenario 2</th>
<th>Scenario 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct estimate</td>
<td>0.259</td>
<td>0.537</td>
<td>0.204</td>
</tr>
<tr>
<td>Logistic regression</td>
<td>0.471</td>
<td>0.341</td>
<td>0.188</td>
</tr>
</tbody>
</table>

4.2.4 Ecological exchangeability

As proof of the genetic distinctiveness of Sundarbans tigers, other ecological traits of the Sundarbans tigers also reflected differences as compared to other Bengal tiger populations. The level of ecological exchangeability regarding morphology, habitat type, size of prey species, resource competition with other predators and tiger density affirmed that the Sundarbans tiger landscape is ecologically distinct from other Bengal tiger landscapes in India. Having a smaller skull size and almost only half of the body weight of mainland tigers (Barlow et al. 2010) would suggest an adaptive difference between Sundarbans tigers and mainland tigers. This adaptive difference might be due to the differences in the ecological niche of the tigers.

Table 8. Comparison of ecological exchangeability between Sundarbans and mainland Bengal tiger populations in India (Article III).

<table>
<thead>
<tr>
<th>Ecological exchangeability parameter</th>
<th>Sundarbans tiger landscape</th>
<th>Mainland Bengal tiger landscape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Small skull size and body weight of females ~ 80 kg</td>
<td>Large skull size and body weight of female ~ 160 kg</td>
</tr>
<tr>
<td>Prey Species</td>
<td>Small size prey (Chital and Wild Pig)</td>
<td>Large size prey (Sambar and Nilgai)</td>
</tr>
<tr>
<td>Habitat</td>
<td>Mangrove forest</td>
<td>Tropical forest</td>
</tr>
<tr>
<td>Competitor</td>
<td>None</td>
<td>Leopard</td>
</tr>
<tr>
<td>Density</td>
<td>4.3 tigers/100 km²</td>
<td>16 tigers/100 km²</td>
</tr>
</tbody>
</table>
The ecological niche of the mainland tiger, i.e. habitat (tropical forest; Karanth et al. 2004) large-sized prey (Karanth 2003), competition with sympatric species (leopard; Jhala et al. 2011) is different from the niche of Sundarbans tigers, i.e. mangrove habitat (Chakraborti et al. 1992) small-sized prey (Khan 2004) and no competition with other carnivores (Jhala et al. 2011) (Table 8). Sundarbans also has a comparatively lower tiger density (4.3 tigers/100 km²) than the mainland (16 tigers/100 km², Jhala et al. 2011, Table 8). Consequently, this difference in the level of ecological exchangeability reinforces the distinctiveness of the Sundarbans tiger population and advocates that morphological and behavioral changes in Sundarbans tigers might be adaptations to the new mangrove habitat and availability of small-sized prey.

4.3 Estimation of sex-specific gene flow

The estimated amount of male differentiation between Sundarbans and northern tiger populations ($F_{ST(m)} = 0.215$) was lower than the amount of female differentiation ($F_{ST(f)} = 0.250$) and their ratio i.e. $m_m/m_f$ was 1.21 (III & IV). This suggests a slightly higher amount of male gene flow than female gene flow in the past. Applying the method of Hedrick et al. (2013) to estimate the male gene flow and its ratio to female gene flow between Sundarbans and peninsular populations resulted in negative estimates, suggesting that all the assumptions of the Hedrick et al. (2013) method were not valid for this situation.

Equation 7a and 7b of Hedrick et al. (2013) used in the present study were derived on the basis of the following assumptions: populations were in genetic drift-gene flow equilibrium, not influenced by male and female effective population sizes (i.e. effective number of females is equal to the effective number of males), and mean gene flow at equilibrium was assumed to be a function of males, and the overall effective population size is not independent of them. However, out of all the above assumptions used for the diploid nuclear and haploid female gene flow, the assumption that the effective number of females is equal to the effective number of males does not hold for tigers. The reason for the difference is the much higher variance in male reproductive success than in female reproductive success due to differential mortalities (Smith & McDougal 1991, Khan 2004, III & IV).

Therefore, I developed a different approach to estimate the male specific gene flow, which is particularly helpful when the female mediated gene flow ($F_{ST(f)}$) is relatively lower than the mean gene flow ($F_{ST}$), as is the case in the peninsular-Sundarbans data. In this new approach, I included the assumption that the
difference in male and female effective population sizes will be an influential factor during the estimation of sex-specific gene flow, along with other assumptions of Hedrick et al. (2013). An alternative approach to estimate the overall and sex-specific differentiation is presented in the following three recursion equations (Wright 1951).

\[
F_{ST}^{t+1} = \left[ \frac{1}{2N} + \left( 1 - \frac{1}{2N} \right) F_{ST}^t \right] (1 - \overline{m})^2
\]

\[
F_{ST(f)}^{t+1} = \left[ \frac{1}{N_f} + \left( 1 - \frac{1}{N_f} \right) F_{ST(f)}^t \right] (1 - m_f)^2
\]

\[
F_{ST(m)}^{t+1} = \left[ \frac{1}{N_m} + \left( 1 - \frac{1}{N_m} \right) F_{ST(m)}^t \right] (1 - m_{fm})^2
\]

These equations can be used to determine the change in differentiation over time from generation \(t\) to \(t + 1\) and their eventual equilibrium values where \(N\), \(N_f\) and \(N_m\) are the total, female and male effective population sizes respectively, and \(m\) is the mean gene flow. By using this new approach I found that the patterns of genetic differentiation observed in Bengal tigers were consistent with lower male effective population size than female effective population size and that the male effective population size was 33% of the female population size. This estimate was also consistent with the observed tiger data (i.e. \(N_m/N_f = 33\%\)) presented by Smith & McDougal (1991).
5 Conclusion and recommendations for conservation

In this thesis, I studied two populations of Bengal tigers on two landscapes which have been crucial for the species conservation in India and I would like to conclude by recommending conservation measures for them.

5.1 Western Terai Arc Landscape (WTAL)

Of the six landscapes identified for ensuring long term conservation goals in India, I studied WTAL. The present study confirmed moderate genetic variation and at least two genetic clusters in the WTAL Bengal tiger population. A detection of low to moderate gene flow and distinct genetic clusters in WTAL imply that recent anthropogenic changes i.e. increase in human settlements after the malaria eradication in the 1960s, clearance of forest for agriculture, monoculture plantations, industrial setups and development of road and railway networks in WTAL have drastically affected the connecting corridor in WTAL. This has also been claimed in previous studies (Johnsingh & Negi 2003, Harihar et al. 2009a, Harihar & Pandav 2012). The higher gene flow between CTR and adjoining FDs and lower gene flow between CTR and RTR support the observed genetic structure and advocate that the area west of CTR is more affected by anthropogenic pressure than the area east of CTR. The high amount of human habitation depicted by night light intensity in Kotdwar and traffic on Kotdwar–Landsdowne and Kotdwar–Kalagarh highways indicate that the west of CTR (i.e. RTR–CTR corridor) is more affected. Therefore, the present study clearly shows that the region east of CTR has the potential to maintain contiguous populations of tigers and our efforts should be focused on minimizing anthropogenic pressure. I also support the recommendation by Johnsingh & Negi (2003) for a “Greater Corbett” conservation area from the east of the Rajaji–Corbett corridor to the Boar River. Moreover, the present study recommends that conserving the Greater Corbett area and retaining a good quality forest patch while minimizing anthropogenic pressure along the identified corridor C2 (Fig. 2, east of CTR) between Ramnagar and Terai East FD via the north of Haldwani along the Gola river, will enable tigers to move from Ramnagar FD to Terai East FD. It may also facilitate movement towards eastern TAL.

The observed low and asymmetric contemporary migration and sibship relationship between RTR and CTR provide evidence that the RTR-CTR corridor is still functional. Therefore, previously suggested measures (relocation of villages
and industrial sites, habitat encroachment prevention and banning sand and boulder mining in the corridor area) by Johnsingh & Negi 2003, and Harihar & Pandav 2012, should be implemented to facilitate the movement of tigers from CTR to RTR. The implementation of the previously suggested measures has already been considered. Therefore, the present study also recommends the genetic characterization of tigers on this landscape at least once every five years. This would enable researchers to assess the efficacy of the conservation measures implemented by the government and monitor the level of genetic variation and structure.

5.2 Sundarbans Tiger Landscape

This was the first study, which elucidated the genetic status (genetic variation and population structure) of Sundarbans tigers and their genetic and ecological connectivity to other mainland tiger landscapes. Population genetic structure based on mitochondrial and microsatellite analyses clearly indicated that the Sundarbans tiger is the most divergent group of the Bengal tiger. The existing low level of genetic and ecological exchangeability also indicated a high level of distinctiveness of the Sundarbans tiger population as compared to the mainland tiger population.

Therefore, as the Sundarbans tiger population is an isolated, genetically and ecologically differentiated group of the Bengal tiger (*Panthera tigris tigris*), it should be managed as a separate conservation unit (CU). Using the criteria suggested by Ryder (1986), Waples (1991) and Moritz (1994), the Sundarbans tiger population is an ESU, because it is genetically and adaptively distinct and shows reciprocal monophyly. In addition, the Sundarbans tiger population meets the Crandel* et al.* (2000) criteria for a MU due to the lack of ecological exchangeability and current gene flow. To summarize, the Sundarbans tigers exhibit strong ecological and genetic differences from other Bengal tiger populations. However, there was evidence of recent historical connectivity with the peninsular Indian tiger population. There is no knowledge of the current gene flow between Sundarbans populations in India and Bangladesh. Based on these results, I suggest that under the adaptive evolutionary conservation (AEC, Fraser & Bernatchez 2001) approach, the Sundarbans population should be managed as an ESU. The Sundarbans tigers need overarching conservation in order to preserve their unique morphological adaptations and genetic uniqueness for the future. I also recommend further genetic studies with a large sample size from both India and Bangladesh.
References


Bera GK & Sahay VS (2010) In the lagoons of the Gangetic delta. New Delhi, Mittal Publications.


Champion HG & Seth SK (1968) The forest types of India. Nasik India, Manager, Government of India press.


71


Jhala YV, Qureshi Q & Gopal R (2008) Status of the Tigers, Copredators, and Prey in India. New Delhi, National Tiger Conservation Authority and Dehradun, Wildlife Institute of India.


75
Maruyama AT & Fuerest PA (1985) Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675–689.


Semwal RL (2005) The Terai Arc Landscape in India, securing protected areas in the face of global change. New Delhi, WWF India.


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CONSERVATION GENETICS OF THE BENGAL TIGER (PANTHERA TIGRIS TIGRIS) IN INDIA