Metapopulation dynamics of tiger (*Panthera tigris tigris*) in the Terai-Arc landscape, India

Thesis submitted for the award of the degree of

Doctor of Philosophy

in

WILDLIFE SCIENCE

by

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to

Saurashtra University
Rajkot -360005 (Gujarat)

Under the supervision of

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भारतीय वन्यजीव संस्थान
Wildlife Institute of India

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DECLARATION

I hereby declare that the work conducted under the thesis entitled "**Metapopulation dynamics of tiger (*Panthera tigris tigris*) in the Terai-Arc landscape, India**", is a record of original research work, done by me and subsequently submitted for the award of the degree of doctor of Philosophy in Wildlife Science to Saurashtra University, Rajkot. This research work has been carried out under the guidance and supervision of Dr. Samrat Mondol, Scientist-E and co-supervision of Dr. Bivash Pandav, Scientist-F Wildlife Institute of India, Dehardun. The work has not formed the basis for the award of any other degree, diploma or any other qualification. I also declare that the thesis embodies my own work, analysis, observation and understanding and the particulars given in it are true to the best of my knowledge.

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CERTIFICATE

This is to certify that the thesis by **Mr. Suvankar Biswas** entitled "**Metapopulation dynamics of tiger (*Panthera tigris tigris*) in the Terai-Arc landscape, India**" is an original and independent research work submitted to the **Saurashtra University, Rajkot (Gujarat)**, for the award of the degree of **Doctor of Philosophy in Wildlife Science**.

Mr. Suvankar Biswas has put more than six semesters of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

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I certify that the research work was appreciated by all who were present, and the comments made by the faculty and researchers have been appropriately included in the thesis.

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CERTIFICATE OF PLAGIARISM CHECK

It is certified that the Ph.D. thesis titled **"Metapopulation dynamics of tiger (*Panthera tigris tigris*) in the Terai-Arc landscape, India"** submitted by Mr. Suvankar Biswas has been examined by us for plagiarism check as per UGC (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulations. The following inferences are drawn from this check:

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CONTENTS

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Introduction

1.1 Background and review of literature:

The tiger (*Panthera tigris*) exemplifies one of the major conservation efforts globally. Once distributed across ~30 present-day nations ranging from Armenia to Indonesia and the Russian far east to India covering a variety of habitats (Hemmer, 1987), their distribution and numbers have drastically reduced to less than 4000 wild individuals at the beginning of the twentieth century largely due to habitat loss and human persecution (Dinerstein et al., 2007; Sanderson et al., 2006). Despite country-specific conservation efforts, the wild tiger population size was found to be >3200 individuals in 2010 (Walston et al., 2010), leading to a commitment from the heads of 13 tiger-range countries to double their tiger number by 2022 (Global Tiger Recovery Program, 2011). As majority of the remaining tigers persisted as small, and often isolated populations (Ranganathan et al., 2008; Sanderson et al., 2006) conservation strategies mostly focused on landscape-based approaches to attain recovery goals where habitat improvement, enhanced protection measures, prey augmentation were targeted (Dinerstein et al., 2007; Karanth and Sunquist, 1995; Sanderson et al., 2006; Wikramanayake et al., 2011). Several priority landscapes were identified as 'Tiger Conservation Landscapes' (TCLs) (Sanderson et al., 2006), where identification of key source populations and consolidation and improvement of surrounding habitats were emphasized (Gubbi et al., 2017, 2016; Joshi et al., 2016). Given that ~80% of global wild tiger population living in most wide variety of habitats were found in India at that time (Goodrich et al., 2015), the success of the tiger recovery plan and the future survival of the species was mostly depended on India's conservation actions. In 2019, India announced the news of doubling its tiger

number since 2006 (population estimate of 1411 (1165-1675) in 2006 to 2967 (2603-3346) in 2018) within its existing habitats (Jhala et al., 2020). However, these increasing tiger populations also face enormous challenges from increasing human density, rapid urbanisation, expanding agriculture, aggressive infrastructure development and economic growth (Gubbi et al., 2012; Mondal and Nagendra, 2011), and their future persistence will depend on the balance between the developmental demands and conservation requirements.

Managing wide-ranging, territorial species like tiger at landscape level requires in-depth understanding of key source populations (Walston et al., 2010), identification of potential tiger habitats surrounding these source populations and ensuring habitat connectivity within the landscape (Gubbi et al., 2016; Joshi et al., 2016). At local level, measures of population dynamics including changes in recruitment and mortality, differential rates of immigration and emigration across sex and age classes, population turnover rates etc. are essential, whereas at landscape scale most emphasis should be given on habitat connectivity to enhance gene flow and reduce the risks from inbreeding (Brook et al., 2002; Norén et al., 2016; Sharma et al., 2013). Both of these are of critical importance in the Indian scenario as most of the available protected land (~5% as per the Wildlife Protection Act 1972) is extremely fragmented (Wildlife Institute of India, 2020) and maintaining connectivity by identifying and managing the critical corridors will play the key role in future tiger conservation (Walston et al., 2010; Wikramanayake et al., 2004). However, generating such detailed information is challenging, time consuming and resource intensive at any scale. In the Indian subcontinent, long-term ecological studies have

already identified priority landscapes (TCLs) for tiger conservation (for example, Western Ghats, central India, north-eastern India, Sundarbans and the alluvial Terai flood plains in the Himalayan foothills) that support high potential tiger densities and relatively larger populations (Jhala et al., 2020). Earlier studies has helped us to understand source-sink population dynamics at landscape-scale in Western Ghats (Gubbi et al., 2017; Reddy et al., 2019; Wikramanayake et al., 2011) and central Indian landscapes (Joshi et al., 2013; Rathore et al., 2012; Sharma et al., 2013; Thatte et al., 2018; Wikramanayake et al., 2011; Yumnam et al., 2014) along with Terai habitats of Nepal (Thapa et al., 2018, 2017), but such information is missing from the Indian part of the Terai-Arc landscape (TAL), where population connectivity and their relation with available habitat is poorly understood.

1.2 Terai-Arc landscape:

The Terai-Bhabhar habitats, otherwise called as Terai-Arc landscape extends over 49000 sq. km., with ~15000 sq. km. moist-deciduous type forest covers along the base of the Himalayas of both India and Nepal. The Indian part of the landscape includes 13 legally designated wildlife protected areas however, fragmented into nine Tiger Habitat Blocks (THBs) (Table 1.1), along with five protected areas in Nepal. This entire region is a global priority tiger conservation landscape comprising of five potential source sites (Rajaji, Corbett, Pilibhit, Dudhwa and Valmiki Tiger Reserves) (Table 1.1 and Figure 1.1) and a number of managed forests together retaining about 22% of the Indian tiger populations (Jhala et al., 2020). Recent studies have recognized that about 25000 sq. km. of potential tiger habitat supports five subpopulations with poor connectivity among them (Johnsingh et al., 2004;

Wikramanayake et al., 2004). Further, monitoring research on some of the core areas (e.g. Corbett Tiger Reserve, Rajaji Tiger Reserve) reveal high density for both tiger (up to 14/ 100 sq. km. (Bisht et al., 2019)) as well as prey species (>80/sq. km. (Harihar et al., 2009)). Active efforts to manage regional dispersal corridors and forests outside reserve boundaries have helped recover tiger populations in this landscape in the recent past (Harihar et al., 2009; Johnsingh et al., 2004; Wikramanayake et al., 2004). Photographic and sign survey monitoring has also identified functional corridors between some of the protected areas (Bivash Pandav, *in review*). Most of the important ecological research and conservation efforts in this landscape has focused on selected protected areas as extending them to a landscape level is logistically challenging. This thesis focused to add deeper understanding of populations at a large, contiguous landscape.

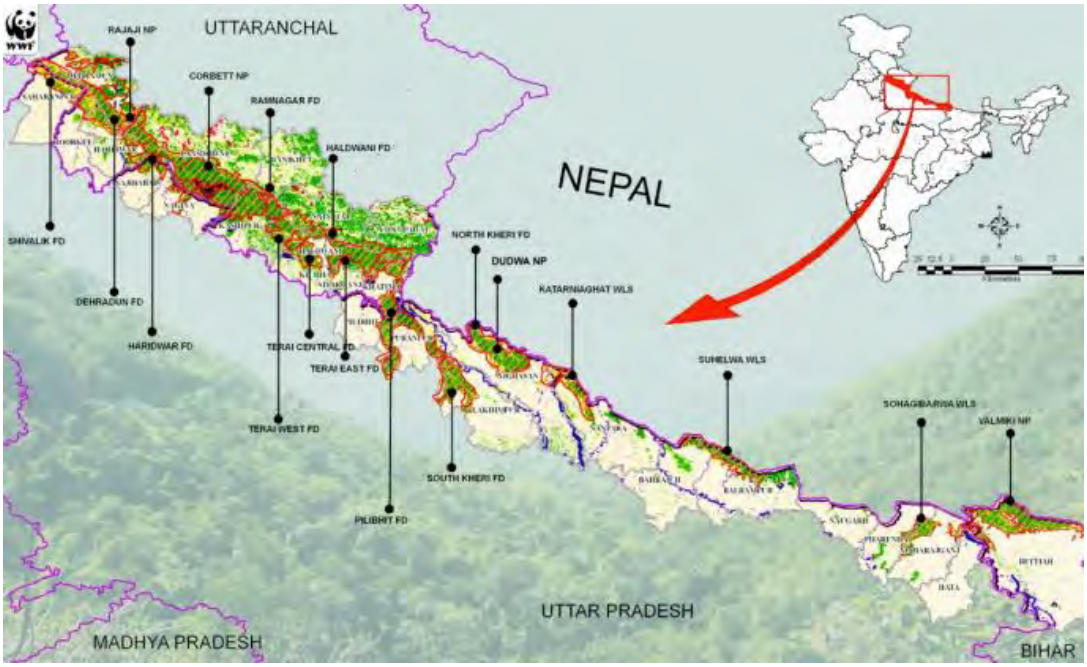

Figure 1.1: Details description of the Terai-Arc landscape

Studying the tiger source-sink population dynamics in relation to current habitat scenarios would be crucial to set the site-specific future management strategies for successful tiger conservation mitigation. In this thesis, a combination of intensive field surveys, non-invasive genetic tools and GIS modelling were used to assess the tiger population connectivity across the TAL. More specifically, genetic diversity, population structure, demographic changes, source and sink populations and the habitat features which help to retain such population dynamics were investigated.

1.3 Objectives:

This thesis has three objecives which have formed three separate chapters.

Chapter 1: Population structure and demographic history of tigers in the Terai-Arc landscape, India.

Chapter 2: Source-sink dynamics of tigers across different protected and non-protected habitats in the Terai-Arc landscape, India.

Chapter 3: Assessment of landscape connectivity of tigers in the Terai-Arc landscape, India.

1.4 Research Questions:

To address above mentioned objectives this thesis has seven specific questions, which are:

2. Identification of population structure if any in the tiger population of TAL

3. Assessment of demographic history of tigers in TAL

4. Identification of genetic migrants across various tiger populations

5. Quantification of magnitude and direction of gene flow between different tiger populations

6. Identification of least-cost pathways of tiger dispersal

7. Modelling the corridor conductance of tiger dispersal

8. Identification of bottlenecks within tiger dispersal corridors

1.5 Thesis Structure:

The future persistence of the increasing global tiger poulations within limited habitats and increasing anthropogenic pressures demands landscape level conservation strategies. However, in India significant tiger populations occupied non-PAs within a landscape (Jhala et al. 2020). This thesis highlighted the conservation importance of non-PAs in long term tiger persistence which strongly demand active management attentions.

The 1st Chapter identified population structure and assessed demographic history of tigers in the Indian part of Terai-Arc landscape using field surveys and DNA-based approaches.

The 2nd Chapter identified genetic migrants and quantified magnitude and direction of gene flow between various tiger populations of TAL using field surveys and DNA-based approaches.

The 3rd Chapter identified the least-cost pathways and modelled the corridor conductances of tiger dispersal, further identified bottlenecks within such corridors across TAL using RS-GIS tools.

The conclusion emphasized the key findings and draw attention to the critical areas where immediate conservation attention is needed.

This is the first study of this kind in this landscape, which provided a deeper understanding of tiger genetic diversity, population structure, past demographic change, functional dispersal, source-sink dynamics and corridor connectivity. These results helped to identify the critical management changes required to ensure effective long-term mitigation efforts, as well as a timely index of the success of such efforts.

Table 1.1: Information of tiger populations, including habitat block details, state, protection status and corridors across TAL

SL No	THB	State	PA, FD, SFD and Corridors	Tiger presence (Unique tigers) (Reference)
1	THB I	HP	PA: Simbalbara NP	No (a)
			Corridor: Yamuna river	No (b)
		HR	PA: Kalesar WLS	No (a)
		UP	FD: Shivalik FD	No (b)

		UK	PA: Rajaji TR (western part)	Yes (2) (a)
			FD: Dehradun FD	No (b)
			Corridor: Kansrau-Barkot, Chilla-Motichur	No (b), No (b)
		UP	PA: Amangarh TR	Yes (20) (a)
			SFD: Najibabad SFD	Yes (5) (e)
2	THB II	UK	PA: Rajaji TR (eastern part), Jhilmil Jheel CR, Corbett TR, Pawalgarh CR	Yes (37) (a), Yes (2) (e), Yes (231) (a), Yes (c)
			FD: Haridwar FD, Lansdowne FD, Terai West FD, Ramnagar FD, Terai Central FD	No (b), Yes (34) (a), Yes (39) (a), Yes (37) (a), Yes (5) (a)
			Corridors: Rajaji-Corbett, Kalagarh, Kosi river, Boar river, Nihal-Bhakra, Gola river	Yes (b), Yes (b), Yes (b), Yes (b), Yes (b), No (b)
3	THB III	UK	PA: Nandhaur WLS	Yes (c)
			FD: Haldwani FD, Champawat FD, Terai East FD	Yes (22) (a), Yes (9) (a), Yes (23) (a),

			Corridor: Kilpura-Khatima-Surai	Yes (d)
		UP	SFD: Pilibhit SFD	Yes (2) (e)
4	THB IV	UP	PA: Pilibhit TR, Kishanpur WLS (part of Dudhwa TR)	Yes (57) (a), Yes (33) (a)
			FD: North Kheri FD, South Kheri FD	No (a, e), Yes (2) (a)
			SFD: Shahjahanpur SFD	No (b),
			Corridor: Lagga Bagga-Shuklaphanta-Tatarganj, Kishanpur-Dudhwa	Yes (b), Yes (b),
5	THB V	UP	PA: Dudhwa NP (part of Dudhwa TR)	Yes (20) (a)
			Corridor: Dudhwa-Katerniaghat	No (b)
6	THB VI	UP	PA: Katerniaghat WLS (part of Dudhwa TR)	Yes (29) (a)
7	THB VII	UP	PA: Suhelwa WLS	No (a)
8	THB VIII	UP	PA: Sohagibarwa WLS (western part)	Yes (a)
9	THB XI	UP	PA: Sohagibarwa WLS (eastern	Yes (a)

	part)	
BR	PA: Valmiki TR	Yes (33) (a)

THB- Tiger habitat block, PA- Protected area, FD- Forest division, SFD- Social forestry division, TGB- Tiger genetic block, NP- National park, WLS- Wildlife sanctuary, TR- Tiger reserve, CR- Conservation reserve, HP- Himachal Pradesh, HR- Haryana, UK- Uttarakhand, UP- Uttar Pradesh, BR- Bihar.

a= Jhala et al., 2020, b= Johnsingh et al., 2004, c= Jhala et al., 2015, d= Anwar and Borah, 2020, e= This study

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Chapter 1:

Population structure and demographic history of tigers in the Terai-Arc landscape, India

2.1 Introduction:

Large carnivores are a vital part of healthy ecosystems. As top predators, they play a critical role in maintaining their structure and diversity and thus actively shape ecological interactions in biological communities (Estes et al., 2011; Terborgh et al., 2001). Yet, growing anthropogenic pressures especially habitat loss, prey depletion, poaching and human-carnivore conflicts are creating severe negative impact on their persistance (Schipper et al., 2008; Cardillo et al., 2006). Large carnivores are particularly at risk because of their high trophic level, low population densities, high cub mortality rates, large home range sizes and human persecution. Currently, predatory carnivores within the families Felidae, Canidae and Ursidae are among the most threatened species globally (Schipper et al., 2008; Cardillo et al., 2006; Ceballos et al., 2005). The survival of their remaining populations depends on future landscape-level management plans involving ecological, demographic and genetic approaches to study these rare, elusive species across their distributions. In the family Felidae, tigers lost ~90% population and ~93% of habitat all across the range (Sanderson et al., 2006). Therefore, tigers have been the prime focus of conservation in the past few decades. Only five out of eight known genetic subspecies of tigers are surviving now, with current estimates was found to be >3200 adult individuals (Goodrich et al., 2015). Out of which India alone holds ~80% of the world's tiger population (Goodrich et al., 2015) that resides in 8-25% of the remaining global tiger habitat (Jhala et al., 2020). Therefore, this existing tiger population and the habitats of India hold the key to the future of the species conservation.

In the Indian subcontinent, long-term ecological studies have already identified priority landscapes for tiger conservation (for example, Western Ghats, central India, north-eastern India, Sundarbans and the Terai-Arc landscape in the Himalayan foothills) that inhabits relatively larger tiger population and as well as densities (Jhala et al., 2020). Terai-Arc landscape (TAL), is the only prime tiger habitat found along the foothills of Himalayas in northern India (Johnsingh et al., 2004; Wikramanayake et al., 2004). It holds the majority of the remaining tiger population of northern India and the entire tiger population of Nepal within its fragmented habitats (Jhala et al., 2020). The Indian part of the TAL retains 22% of the current Indian wild tiger population (Jhala et al., 2020) within its 15000 sq. km. of potential forested habitat (Johnsingh et al., 2004). These tiger populations are restricted within its isolated 11 protected areas and few managed forests (Johnsingh et al., 2004; Wikramanayake et al., 2004). Additionally, in the past the tiger population of TAL had undergone a severe decline due to bounty, trophy and private hunting and more recently illegal hunting or poaching, habitat fragmentation, prey depletion and conflict with humans (Chanchani et al., 2014; Harihar et al., 2009; Johnsingh et al., 2004; Kanagaraj et al., 2011; Rangarajan and Shahabuddin, 2006; Thapa et al., 2017). Additionally, the northern part of India along with Nepal is very conducive for poaching events, due to its proximity to the demanding countries of tiger body parts especially China. Furthermore, this area has Corbett and Rajaji Tiger Reserves which persist one of the highest turnover rates of tiger along with tiger presence in outside protected areas, making this population more prone to poaching and conflict (Jhala et al., 2020; Bisht et al., 2019; Harihar et al., 2009).

The ecology, spatial distribution and population dynamics of Indian tigers have been relatively well studied (Gopal et al., 2010; Harihar and Pandav, 2012; Jhala et al., 2011, 2020, 2015; Johnsingh and Negi, 2003; Karanth et al., 2003), until recently little was known about the genetic makeup and diversity of historical and present tiger populations. Several recent genetic studies in India have described tiger genetic variations and population structure (Kolipakam et al., 2019; Singh et al., 2017; Sharma et al., 2009), past demography (Mondol et al., 2009a), population estimation (Mondol et al., 2009b) and dispersal (Joshi et al., 2013; Reddy et al., 2019, 2012; Sharma et al., 2013a; Thatte et al., 2018; Yumnam et al., 2014). Understanding such patterns of genetic variation, population structure, demographic changes and local population dynamics is of critical importance in evaluating the evolutionary potential and to prioritize conservation efforts. Population genetics of central Indian and Western Ghats tiger populations are relatively well studied using non-invasive molecular approach (Reddy et al., 2019, 2017; Yumnam et al., 2014; Joshi et al., 2013; Sharma et al., 2013a) but till date, only one site-specific study was conducted in TAL (Singh et al., 2017). This study investigated tiger population genetics in the western TAL and therefore, the population genetic measures of extant tiger populations in the entire TAL is poorly understood.

The natural history and ecology of tigers, prey estimates and disturbance patterns are fairly well studied in some of the national parks and protected reserves in the TAL (Bisht et al., 2019; Chanchani et al., 2016a, 2014; Harihar et al., 2014, 2009; Harihar and Pandav, 2012; Jhala et al., 2020; Johnsingh et al., 2004; Joshi et al., 2016; Seidensticker, 1976; Smith, 1993; Sunquist, 1981; Thapa et al., 2017). The

considerable conservation efforts and ecological data available for this region make it the ideal location to conduct genetic studies for a deeper understanding of population genetic measures in one of the few large remaining strongholds of wild tiger populations. This chapter aimed to 1) measure the genetic diversity persists in the extant tiger population 2) identify the population structure and 3) assess the demographic changes in the remaining tiger populations in this landscape using field survey and non-invasive genetic analyses.

2.2 Methodology:

2.2.1 Study area and faecal sample collection:

TAL is 900 km long and 50 to 60 km wide, covering approximately 28000 km^2 of forested habitat in northern India and southern part of Nepal along the foothills of Himalayas (Johnsingh et al., 2004), consisting one of the prime tiger habitats throughout its distribution (Wikramanayake et al., 2004) (Figure 2.1). This landscape categorizes into three major habitat types, the hilly region of Shivalik and Churia hills, pebbly-bouldery foothills known as Bhabar, dominated by Sal (*Shorea robusta*) and the low flood land of Terai, dominated by swamp grasslands and permanently moist reed swamps (Champion and Seth, 1968; Johnsingh et al., 2004). Indian TAL retains 15000 sq. km. of potential tiger habitat encompassing the north Indian states of Haryana (westernmost), Himachal Pradesh, Uttarakhand, Uttar Pradesh and Bihar (easternmost) (Figure 2.1) (Johnsingh et al., 2004). As recent as 2003, areas like west of river Yamuna to the west of the river Ganga almost lost its value as a prime tiger habitat especially due to growing anthropogenic activities and habitat disconnectivity (Johnsingh et al., 2004). Despite heavy habitat fragmentation, prey depletion,

poaching remaining parts of Uttarakhand, Uttar Pradesh and Bihar states, starting from east of river Ganga to Valmiki Tiger Reserve still supporting 22% of current wild Indian tiger populations (646 (567-726)) (Jhala et al., 2020) and identified as a 'Global priority' tiger conservation landscape (TCL) (Sanderson et al., 2006). These tiger populations are primarily restricted within its 11 protected areas boundaries but significant populations are outside of these protected areas (Jhala et al., 2020). Therefore, the field survey was conducted in all protected areas, forest divisions and social forestry divisions across the landscape, starting from Rajaji and Corbett Tiger Reserves, Pawalgarh and Jhilmil Jheel Conservation Reserves, Nandhour Wildlife Sanctuary, Lansdowne, Haridwar, Ramnagar, Haldwani, Champawat, Terai West, Terai Central and Terai East Forest Divisions of Uttarakhand state and Amangarh and Pilibhit Tiger Reserves, Dudhwa National Park, Kishanpur, Katerniaghat, Suhelwa and Sohagibarwa Wildlife Sanctuaries and North Kheri and South Kheri Forest Divisions and Najibabad, Shahjahanpur and Pilibhit Social Forestry Divisions of Uttar Pradesh and Valmiki Tiger Reserve of Bihar between December 2014 to May 2018 (Figure 2.1). Sampling was mostly conducted between November-May i.e. winter and summer seasons every year.

During the field survey, majority of the known and potential tiger trails were walked all across the TAL and looked for large carnivore faecal samples. The total effort included ~9500 km of foot survey for faecal sampling. All the faecal samples were categorized into tiger/leopard through morphological features such as size, shape, diameter as well as associated field signs (track and scrape marks, spray scent, etc.). A dry sampling approach was used for faecal sampling, described in Biswas et al.,

(2019) for the entire study. GPS coordinates were noted during sample collection and further kept the collected samples in dry, covered boxes for a maximum period of one month in the field station before they were shipped to WII and stored in -20 °C freezers until further processing.

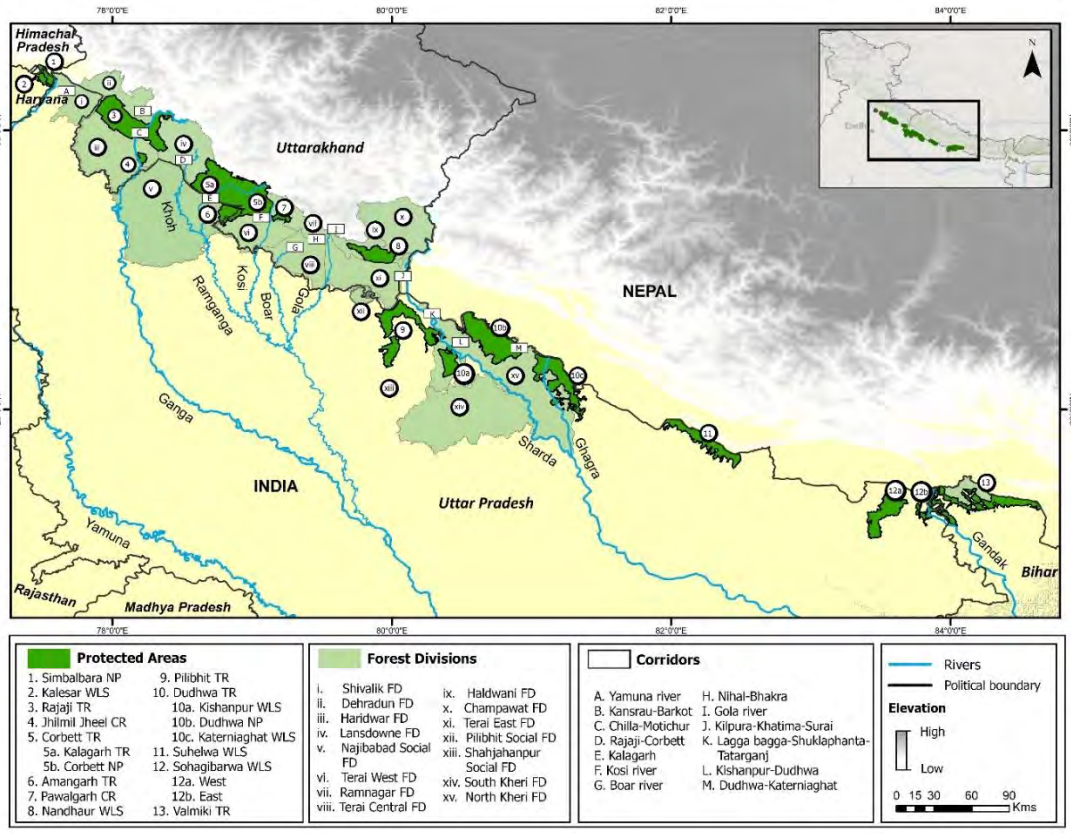

Figure 2.1: Detail description of the Indian part of the Terai-Arc landscape which spread over 13 protected areas, 12 forest divisions and three social forestry divisions and retains ~650 adult free-ranging tigers

2.2.2 Laboratory experiments:

DNA extraction: DNA was extracted from all the faecal samples using a modified Qiagen kit protocol (Biswas et al., 2019). The outer layer of faeces was swabbed with a PBS-soaked sterile cotton swab to collect the top layer of host cells. Each faeces were swabbed twice to maximize the DNA amount and the swabs were stored

in sterile microcentrifuge tubes at -20 °C freezer. Initially, only one of the swabs was used for DNA extraction with QIAmp DNA tissue kit (QIAGEN Inc.) using a slightly modified protocol. Samples were lysed overnight in ATL buffer and proteinase K (25 µl of 20 mg/ml conc.). In brief, swabs were removed after digestion and the remaining protocol was followed as mentioned in the kit. DNA was eluted in 100 µl of TE buffer (pH 7.8) twice. For every set of samples (n=22) two extraction negatives were included to monitor contamination. The extraction of the second swab was conducted after species confirmation and for only tiger samples and used further in downstream laboratory experiments.

Species confirmation: For species identification, three separate methods using two primer mix were used which are described in Maroju et al., (2016); Mondol et al., (2015); Mukherjee et al., (2007) (Table 2.1). This approach of multiple primer mixing has given unambiguous species identification from the field-collected faecal samples. The polymerase chain reaction (PCR) reactions were carried out in 10 µl reaction volumes containing 4 µl of Qiagen master mix (QIAGEN Inc.), 3 µl of BSA, 1.2 µl of primer mix and 2 µl of template DNA. The PCR conditions were included an initial denaturation (95 °C for 15 min); 50 cycles of denaturation (95 °C for 30 sec); annealing (50 °C for 30 sec)); extension (72 °C for 30 sec); followed by a final extension (72 °C for 15 min). PCR products were run in 2% agarose gels and pictures were manually visualized for species identification.

Table 2.1: Details of mitochondrial markers used for tiger faeces confirmation

Sl. No.	Primer	Amplicon length (base pair)	Reference
1	Tig490	225	Mukherjee et al., 2007
2	Tig509	164	Mukherjee et al., 2007
3	TigParND2	110	Mondol et al., 2015
4	TigParND4	85	Mondol et al., 2015
5	TSP	162	Maroju et al., 2016

Nuclear DNA data generation using multilocus microsatellite markers: A panel of 13 microsatellite loci were used for unambiguous individual identification of tiger (Table 2.2) (Mondol et al., 2015; Menotti-Raymond et al., 1999). However, these markers were standardized as multiplex reactions of 4-5 loci in each reaction based on allele size and annealing temperature, making it more useful for individual identification. PCR reactions with respective multiplex primer mixtures were carried out in 10 µl reaction volumes containing 4 µl of Qiagen multiplex master mix (QIAGEN Inc.), 3 µl of BSA, 1.2 µl of primer mix (0.2 µM final concentration) and 3 µl of template DNA. The PCR conditions included an initial denaturation (95 °C for 15 min); 45 cycles of denaturation (94 °C for 30 sec); annealing (Ta for 30 sec); extension (72 °C for 30 sec); followed by a final extension (72 °C for 15 min) in an ABI thermocycler. PCR products were checked in 2% agarose gel and 1µl of PCR product is mixed with 9 µl mix of Hi-Di formamide and 500 LIZ. The mixtures were denatured at 95 °C for 4 min; chilled at 4 °C before putting them in the ABI 3500XL DNA fragment analyzer for genotyping. To validate the generated genotyped data, allele bins were created for each locus used for tiger individual identification and all alleles were called using these bins through program GENEMARKER 2.6.7.

(SOFTGENETICS INC. USA). This process is useful in reducing human errors in allele calling and making the entire data uniform.

Table 2.2: Details of microsatellite markers used in this study

Sl. No.	Locus	Repeat length	Reference
1	FCA090	2	Menotti-Raymond et al., 1999
2	msFCA506	2	Mondol et al., 2015
3	FCA672	2	Menotti-Raymond et al., 1999
4	FCA304	2	Menotti-Raymond et al., 1999
5	FCA628	2	Menotti-Raymond et al., 1999
6	FCA232	2	Menotti-Raymond et al., 1999
7	FCA230	2	Menotti-Raymond et al., 1999
8	FCA279	2	Menotti-Raymond et al., 1999
9	FCA441	4	Menotti-Raymond et al., 1999
10	FCA069	2	Menotti-Raymond et al., 1999
11	msFCA453	4	Mondol et al., 2015
12	msF115	4	Mondol et al., 2015
13	msHDZ170	2	Mondol et al., 2015

2.2.3 Data quality assessment:

To validate the genotyping results, each locus was amplified and scored four times, and only those loci having 75% or more same allele calls were used for downstream analyses (Mondol et al., 2009b). allelic dropout and false allele (FA) rates were

quantified manually as the number of allelic dropouts or FAs over the total number of amplifications, respectively (Broquet and Petit, 2004), as well as using program MICROCHECKER version 2.2.3 (Van Oosterhout et al., 2004). Following this, any allele having less than 10% frequency across all amplified samples were rechecked for allele confirmation. ARLEQUIN 3.1 (Excoffier et al., 2005) was used to determine Hardy Weinberg equilibrium and linkage disequilibrium for all the loci.

2.2.4 Population genetic analyses:

Individual identification and molecular sexing: Identical genotypes from multiple samples (or recaptures) were detected using the 'Identity analysis' module of program CERVUS (Marshall et al., 1998). $P_{ID(sibs)}$ (Probability of identity for siblings) was calculated using GIMLET (Valiere, 2002). $P_{ID(sibs)}$ refers to the probability that two random siblings within the dataset of a population will have a similar genotype at multiple loci. $P_{ID(sibs)}$ refers to the probability of misidentification of two siblings as one individual as both of them have similar genotype at multiple loci.

Molecular sexing was performed for individual tigers, using two sex chromosome markers Amelogenin (Pilgrim et al., 2005) and SRY (DeCandia et al., 2016). PCR reactions were carried out in 10 µl volumes containing 4 µl of Qiagen master mix (QIAGEN Inc.), 3 µl of BSA, 1 µl of primer mix (3 µM concentration) and 2 µl of template DNA. The PCR conditions included an initial denaturation (95 °C for 15 min); 50 cycles of denaturation (95 °C for 30 sec); annealing (Ta for 30 sec); extension (72 °C for 30 sec), followed by a final extension (72 °C for 15 min) in an ABI thermocycler. PCR products were run in 3% agarose gel and sex identification

was carried out visually through sex-specific banding patterns. For validation, the entire process was repeated twice and considered samples providing consensus results.

Population structure or sub-division analysis: Hidden population sub-divisions identification was carried out using multivariate analysis and multiple Bayesian individual-based clustering analyses. 1) Multivariate analysis like discriminant analysis of principal component (DAPC) was performed using *adegenet* package in R as an alternative of individual Bayesian clustering (IBC) for a better understanding of between group's genetic differentiation than within groups (Jombart et al., 2008). It has advantages over other IBC analyses while investigating the wild, natural population, as it does not assume populations to be in Hardy-Weinberg equilibrium (HWE) or linkage equilibrium (LE) (Jombart et al., 2010, 2008). The true population sub-division was identified by calculating the Bayesian information criterion (BIC). 2) In individual Bayesian clustering (IBC) analyses to understand population sub-divisions in a landscape-level TESS2.3.1 (Chen et al., 2007) programme was used. In addition to the DAPC analysis, TESS has an advantage in population clustering analysis of long-ranging species like tiger as it considers geographic locations of each individual while investigating the population sub-divisions. TESS was run with an admixture model with 60000 sweeps and 10000 burns in. 0.6 was set for the interaction parameter with 10 independent runs starting from 2 to 10 for each K value (Vergara et al., 2015). The true population sub-divisions were identified by calculating the average deviance information criterion (DIC) value for each K.

Genetic differentiation estimation: Indices of overall genetic differentiation (pairwise F_{st}) was estimated using GenAlEx version 6.5 (Peakall and Smouse, 2012). The divisions were based on cumulative results obtained from population spatial clustering analyses. The F_{st} value ranged from 0 to 1, where 0 indicates the highest level of genetic differentiation between two sub-populations and 1 represents the highest level of similarity. Additionally, to the pairwise F_{st} calculation, compression of expected heterozygosity (or G_{st}) between all tiger subpopulations was calculated in GenAlEx version 6.5 (Peakall and Smouse, 2012).

Genetic diversity indices: The quantification of genetic diversity present in an extant tiger population is crucial to understand its' evolutionary potential. Therefore, the overall genetic diversity of the existing tiger populations of TAL and each sub-populations, identified in spatial clustering analyses were measured by calculating the mean number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic size range (ASR) using ARLEQUIN 3.1 (Excoffier et al., 2005).

Demography analyses: Demographic analyses were performed using genetic data of individual tigers across different genetic sub-populations. These sub-populations were selected based on the results of population structure analyses. A combination of qualitative and quantitative approaches was used to infer past demography. Both the qualitative approaches were based on allele summary statistics and quantitative approach was a likelihood-based Bayesian approach used to detected past population changes of tigers. In the qualitative approaches the Ewens, Watterson, Cornuet and Luikart and the Garza-Williamson index or M ratio (Garza and Williamson, 2001)

methode were used implemented in program BOTTLENECK (Cornuet and Luikart, 1996) and ARLEQUIN 3.1 (Excoffier et al., 2005) respectively. Further in the quantitative analysis likelihood-based Bayesian approach was used, implemented in the program MSVAR 1.3 (Storz and Beaumont, 2002).

a) *The Ewens, Watterson, Cornuet and Luikart (EWCL) approach:*

In this approach two summary statistics of the allele frequency spectrum were used across different mutational models to detect population size changes. The used summary statistics were number of allele (N_A) and expected heterozygosity (H_E). The expected distribution of H_E for a demographically stable population was obtained by performing simulations under three mutation models: infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model (TPM) and the outcomes were then compared to the real data values. Changes of mutation-drift equilibrium and neutrality were detected using this method, which further can be explained by any shift from the null model. The shift includes selection and population changes (growth or decline). More importantly, consistent results from independent loci could be assigned to demographic events and not selection. Two different (5% and 30%) multi-step mutation events were used in TPM model, for tigers.

b) *The Garza-Williamson index/M ratio approach:*

In this approach two summary statistics of the allele frequency spectrum, were used to detect the population size changes, using number of alleles (N_A) and the allelic size range (ASR). The basic principle of this approach is if the reduction of number

of alleles is higher than the reduction of the allelic size range then suggested a reducing population. Thus, in recently declined populations the ratio between the number of alleles and the allelic size range will be smaller than in an equilibrium populations.

c) *The Storz and Beaumont approach:*

This approach is an extension of Beaumont's approach (Beaumont, 1999) that assumes a stable population of size N1 started to change (either decrease or increase) Ta generations ago to the current population size N0. This change in the population size is assumed to be at an exponential scale under the stepwise mutation model (SMM), at a rate y=2N0m, where m is the mutation rate per locus per generation. to To estimate the posterior probability distribution this method uses the information from the full allelic distribution in a coalescent framework and allow the quantification of effective population sizes N0 and N1, rather than their ratio (as in Beaumont, 1999) along with time (T) since the population started changing. The prior distributions for N0, N1, T and m (mutation rate) are expected to be log-normal in this approach. The prior (or hyperpriors) distributions were used to calculate the mean and the standard deviations of these prior log-normal distributions. To generate samples from the posterior distribution a Markov Chain Monte Carlo (MCMC) algorithm is used of these parameters. Multiple runs for this approach were performed using wide uninformative priors. The prior distributions were kept large for minimal effect towards the posterior distributions variances. 2 million iterations were performed for each run. The generation time for tigers is known to be about 4-5

years (Mondol et al., 2009a) and therefore, a five-year generation time was used for all analyses.

2.3 Results:

2.3.1 Faecal sampling:

13000 sq. km. of remaining tiger habitat staring from Rajaji Tiger Reserve of Uttarakhand in the west to Valmiki Tiger Reserve of Bihar in the east along the Himalayan foothills were sampled and 1608 faecal samples were collected using dry sampling method between December 2014 to May 2018 (Figure 2.2). Upon collection, each of the faecal samples were marked with GPS coordinates, date, location and probable species and further kept in dry and darkroom maximum for one month in the field and later transferred to the laboratory and stored at -20 °C freezer till DNA extraction (Biswas et al., 2019). The faecal sampling covered all the tiger inhabiting 11 protected areas, 10 forest divisions and three social forestry divisions in TAL.

2.3.2 Laboratory experiment:

DNA extraction, species confirmation and nuclear data generation: Out of 1608 faecal samples DNA was extracted from 1524 samples (94.78%). The outer layer of the remaining 84 samples (5.22%) were disturbed due to fungal infection therefore, excluded from DNA extraction. Using tiger specific primer mix 743 faecal samples were identified as tiger positive (Figure 2.3). Genotyped data was generated from 553 tiger samples using 13 microsatellite markers and the remaining 190 tiger

samples were excluded as they did not qualify the minimum cut off amplification success (≥ 9 loci).

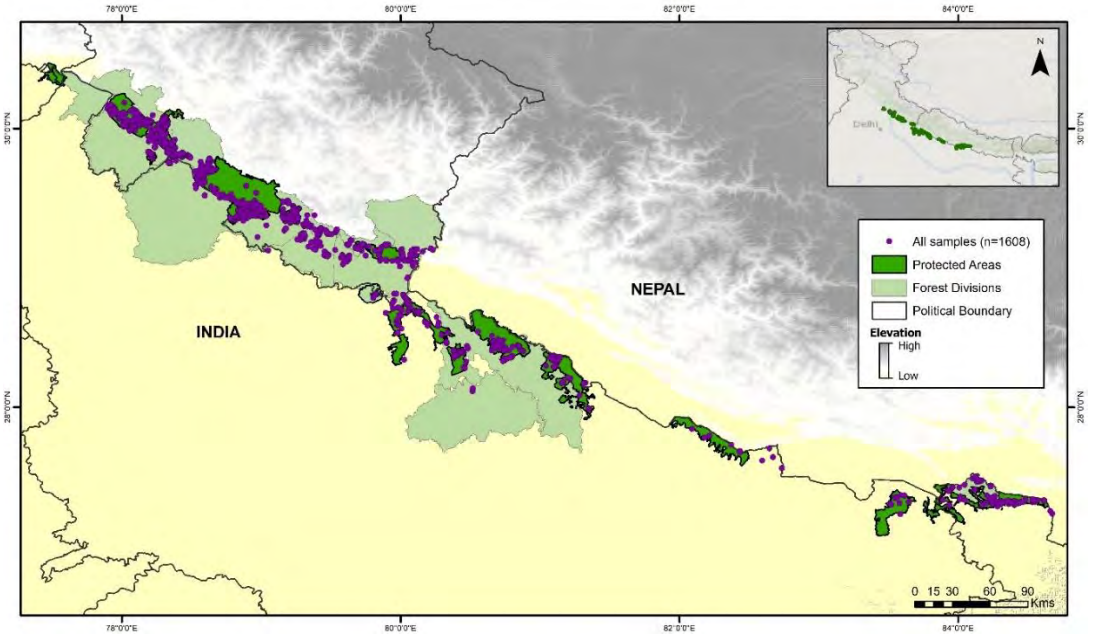

Figure 2.2: Map showing large carnivore faecal samples collected during study period covering 11 protected areas, ten forest divisions and three social forestry divisions in the Indian part of Terai-Arc landscape

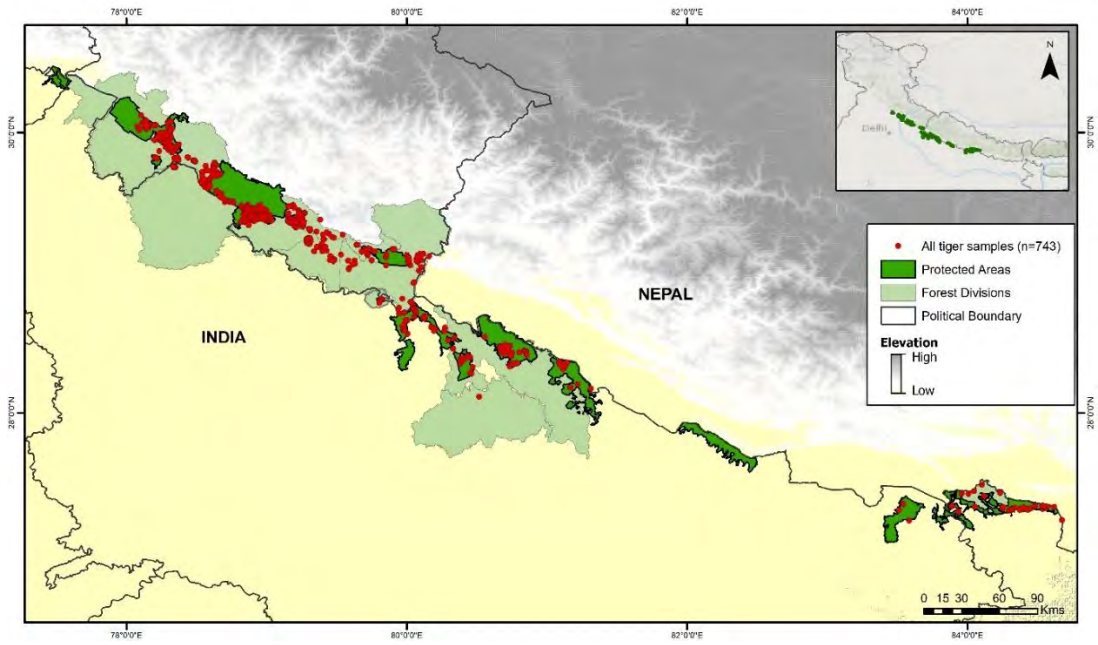

Figure 2.3: Map showing confirmed tiger scat locations identified in this study covering 10 protected areas, nine forest divisions and two social forestry divisions in the Indian part of Terai-Arc landscape

2.3.3 Data quality assessment:

No large-scale signatures of allele drop out or null alleles were found. The mean allelic dropout and false allele rates as 0.01 and 0.03, respectively (Table 2.3), indicating an overall low genotyping error rate across these marker panels. None of the used loci were deviated from the Hardy-Weinberg equilibrium and no signature of strong linkage equilibrium were found between any pair of loci. Locus-wise mean success rate across all samples ranged between 53-97% (see Table 2.3).

2.3.4 Population genetic analyses:

***Individual identification, molecular sexing and overall genetic diversity*:** Out of 553 confirmed tiger samples, 219 individual tigers were identified using CERVUS software which covers 34% of the total tiger population in TAL (Jhala et al., 2020) (Figure 2.4). Of all the identified unique individuals (n=219), ~71% were from Uttarakhand (n=156, 35% of the state's population of 442 (393-491)), whereas ~20% were from Uttar Pradesh (n=44, 25% of the state's population of 173 (148-198)) and ~9% from Bihar (n=19, 61% of the state's population of 31 (26-37)) (Jhala et al., 2020). Tiger presence was confirmed in 10 protected areas, nine forest divisions and two social forestry divisions (Figure 2.3). Samples collected from Suhelwa Wildlife Sanctuary, North Kheri Forest Division and Shahjahanpur Social Forestry Division, did not show any tiger presence upon testing (Figure 2.3).

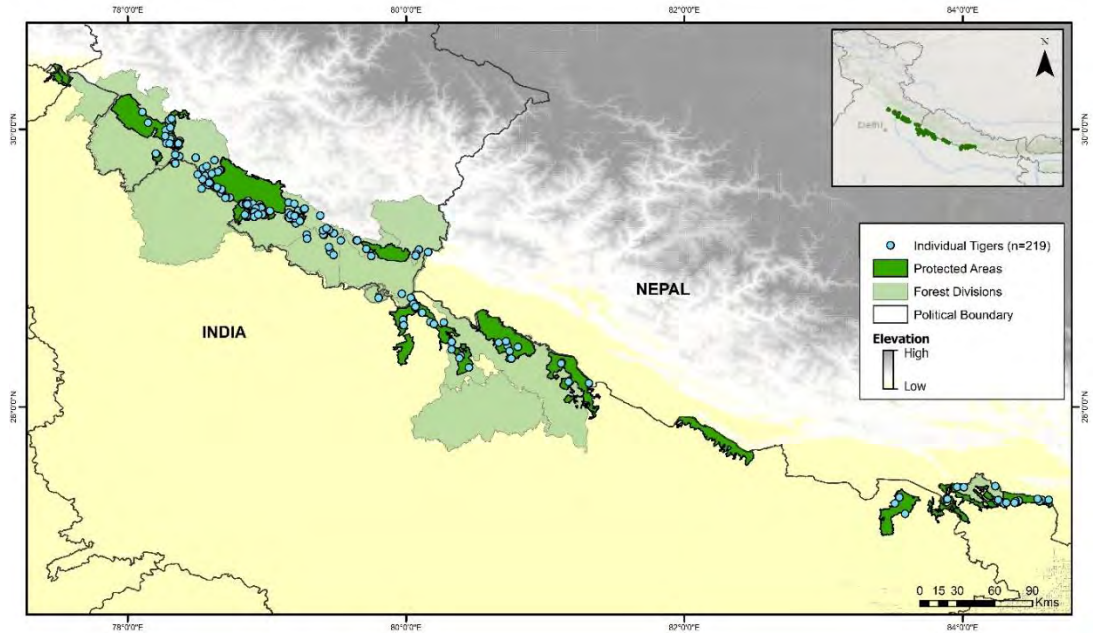

Figure 2.4: Map showing individual tiger locations identified in this study, covering 10 protected areas, nine forest divisions and two social forestry divisions in the Indian part of Terai-Arc landscape

88% success rate was achieved in molecular sexing among 219 unique tigers (n=193, 89 males and 104 females, respectively). Interestingly, 54 of these males were found inside protected areas and 35 individuals were from non-protected areas (forest divisions and social forestry divisions), whereas 59 females were from protected areas and 45 from outside protected areas, indicating that both sexes were using non-protected habitats across TAL.

All 13 loci were polymorphic for TAL tigers with mean number of alleles, observed heterozygosity and allelic size range were found to be 14.46±2.67, 0.35±0.17 and 45.38±10.96, respectively (Table 2.3). The cumulative $P_{ID(sibs)}$ and $P_{ID(unbiased)}$ values ($2.42*10^{-6}$ and $9.13*10^{-17}$, respectively) suggest a statistically strong result for unambiguous tiger individual identification (Table 2.3).

Population structure analysis: Using 219 individual unrelated genetic data multivariate analysis DAPC identified six genetic subpopulations (K=6) across TAL, using optimum Bayesian Information Criterion (BIC). When examined closely, it has been found that Uttarakhand state holds four of these six genetic ancestries as geographically mixed populations, whereas the remaining two ancestries are roughly separated in states of Uttar Pradesh and Bihar, respectively, making three genetic subpopulations across TAL (Figure 2.5). Subsequently, these major three tiger genetic subpopulations were described as 'Tiger Genetic Blocks (TGBs)' while reporting the results.

Table 2.3: Genetic diversity and genotyping error details of tiger population in TAL using 13 microsatellite markers

Locus	N_A	ASR	H_E	H_O	$P_{ID(sibs)}$	ADR	FA rate	Success rate (%)
FCA090	19	38	0.88	0.31	3.14e-01	0.01	0.03	77
msFCA506	12	38	0.85	0.26	1.04e-01	0.00	0.05	59
FCA672	15	42	0.87	0.58	3.33e-02	0.00	0.03	94
FCA304	14	42	0.73	0.17	1.37e-02	0.00	0.02	92
FCA628	20	54	0.83	0.41	4.72e-03	0.01	0.02	53

FCA232	16	32	0.70	0.27	2.04e-03	0.00	0.02	97
FCA230	13	52	0.82	0.12	7.22e-04	0.01	0.02	94
FCA279	16	36	0.87	0.51	2.29e-04	0.00	0.03	91
FCA441	14	60	0.77	0.54	8.83e-05	0.01	0.03	75
FCA069	16	40	0.79	0.30	3.24e-05	0.01	0.01	79
msFCA453	12	72	0.71	0.62	1.37e-05	0.01	0.01	94
msF115	09	48	0.60	0.08	6.86e-06	0.01	0.01	56
msHDZ170	16	36	0.86	0.33	2.24e-06	0.04	0.04	83
Mean	**14.76 ± 2.83**	**45.38± 10.96**	**0.79 ± 0.08**	**0.35 ± 0.16**		**0.01**	**0.02**	**80.3**

N_A- No. of alleles, ASR- Allelic size range, H_E- Expected heterozygosity, H_O- Observed heterozygosity, $P_{ID(sibs)}$- Cumulative probability of identity of siblings, ADR- Allelic dropout rate, FA- False allele.

Overall, these TGBs roughly correspond to the tiger population in the states of Uttarakhand (TGB I), Uttar Pradesh (covering mostly TGB II and small parts of TGB I and III) and Bihar (TGB III), respectively (Figure 2.5). The samples collected (n=171) from the Rajaji Tiger Reserve (western side) to Nandhaur Wildlife Sanctuary (eastern side) in Uttarakhand formed the first genetic subpopulation (TGB I). The TGB I retains the highest tiger population in TAL (Jhala et al., 2020) (Figure 2.5). This block represents six protected areas, eight forest divisions and one social forestry division (Johnsingh et al., 2004). The TGB II includes the samples collected (n=26) from Pilibhit Social Forestry Division (western side) to Katarniaghat Wildlife Sanctuary (eastern side) in Uttar Pradesh (Figure 2.5). This block retains two protected areas, one forest division and one social forestry division (Johnsingh et al., 2004). Finally, the samples collected (n=22) from Sohagibarwa Wildlife Sanctuary (western side) in eastern Uttar Pradesh and Valmiki Tiger Reserve (eastern side) in Bihar, together formed the TGB III and included only two protected areas (Figure 2.5).

Another population sub-division analysis using the Bayesian approach in TESS software also suggested K=4-5 using optimum BIC criterion. In TESS both the K (4 and 5) values showed similar population sub-divisions and identified three TGBs in TAL when compare with DAPC (Figure 2.6).

Tiger populations of Haldwani Forest Division and Terai East Forest Division showed mixed ancestry with TGB I and TGB II, therefore, it is considered as a

transition zone of tiger movement between TGB I and TGB II in this linear landscape.

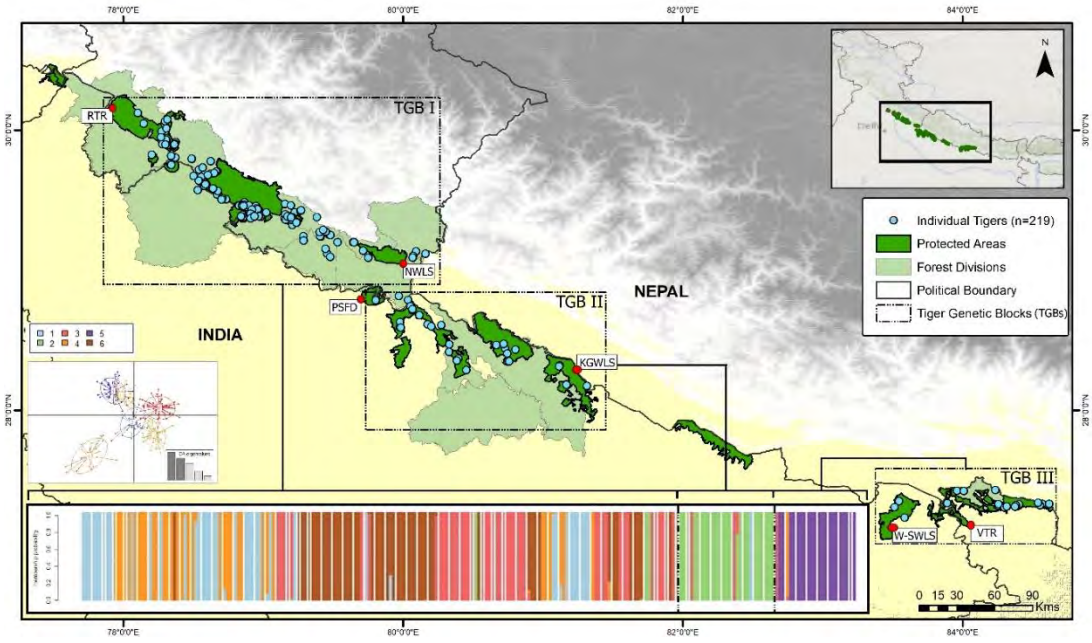

Figure 2.5: Results of DAPC analysis using adegenet package showed three genetic subpopulations of tigers, identified as TGB I, TGB II and TGB III (Tiger Genetic Blocks) in the Indian part of Terai-Arc landscape. Blue, orange, brown and pink colours represented the TGB I, where green and purple colours represented TGB II and TGB III respectively

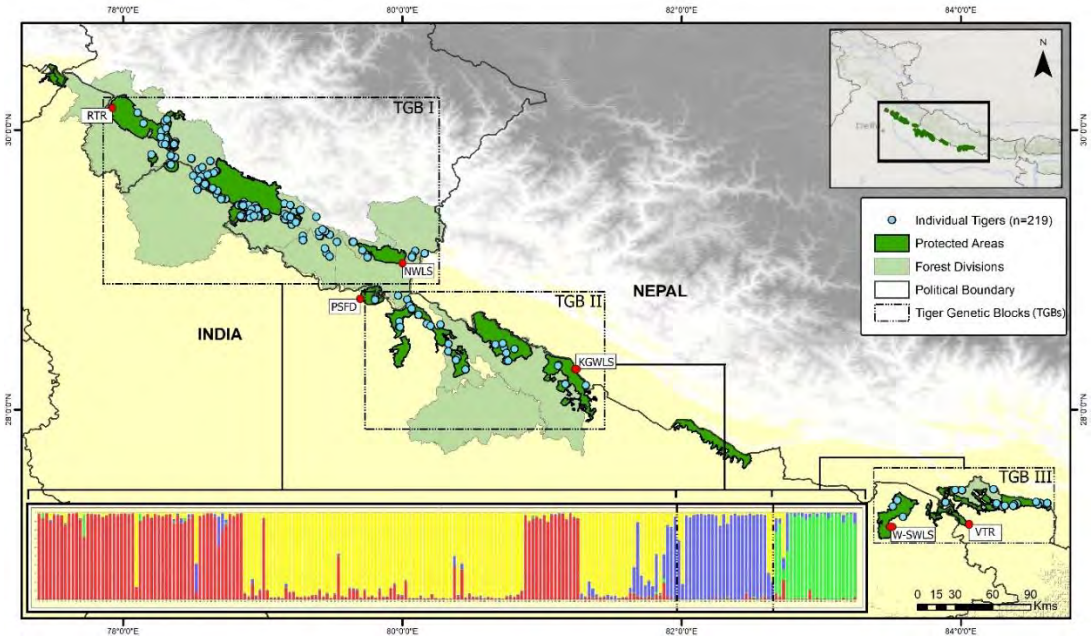

Figure 2.6: Results of cluster analysis using TESS software also identified TGB I, TGB II and TGB III (Tiger Genetic Block) in the Indian part of Terai-Arc landscape. Red and yellow colours represented the TGB I, where blue and green colours represented TGB II and TGB III respectively

Genetic differentiation estimation: Subsequent analyses revealed that the tiger populations of these three genetic blocks of TAL (TGB I, TGB II and TGB III) are genetically differentiated (F_{st} and G_{st}) at low, but significant levels. The F_{st} value among these populations ranged between 0.079-0.107, whereas the G_{st} value between 0.067-0.087 (Table 2.4).

Table 2.4: Genetic differentiation (pairwise F_{st} and G_{st}) for three tiger subpopulations in the TAL. The upper diagonal presents the pairwise G_{st} values whereas the lower diagonal presents the pairwise F_{st} values

	TGB I (N=173)	**TGB II (N=24)**	**TGB III (N=22)**
TGB I (N=173)	--	0.078*	0.067*
TGB II (N=24)	0.089*	--	0.087*
TGB III (N=22)	0.079*	0.107*	--

* p value = 0.001

Genetic diversity indices of each subpopulation: Further, the overall genetic diversity of each subpopulation was calculated and it was found that the N_A is highest in tiger populations of TGB I (10.15±3.06), followed by TGB III (09.08±2.24) and TGB II (07.00±2.11) respectively. However, the H_O and ASR of TGB III (0.43±0.20 and 38.00±14.29) were higher when compared to TGB I

(0.34±0.20 and 24.46±07.37) and TGB II (0.29±0.12 and 24.77±09.30), respectively (Table 2.5).

Demographic changes in all three subpopulations: Since for peninsular India, there was a drastic population decline around 200 years ago probably due to the colonial era (Mondol et al., 2009a). So, to understand what the northern population has gone through in the past and if it had followed the same history, qualitative and quantitative approaches were used.

Both the qualitative approaches found indication of bottleneck. Depending on the selected mutation models the EWCL approach showed maximum nine loci with heterozygote excess, suggested a loss of rare alleles through population decline for all three TGBs (Table 2.6). These results were similar to the M-ratio approach that showed low ratio between N_A and the ASR in all the TGBs. M-ratio across all three TGBs ranged from 0.25 (SD 0.11) to 0.40 (SD 0.13) indicating signatures of the population bottleneck (Table 2.6).

Due to low sample sizes quantitative approach in the MSVAR were not performed for TGB II and TGB III. However, in TGB I a consistent results were obtained using the models with exponential decline scenarios. The results showed the posterior distributions for log (N1) are always higher than log (N0), suggesting population decline for tigers across the western TAL (Table 2.6 and Figure 2.7). Further quantification suggested that the current effective population size is distinctly lower (12%) than the historical effective population size, with TGB I lost approximately 88% of its tiger population (Table 2.6 and Figure 2.7). Subsequent analysis also

Table 2.5: Comparison of genetic diversity between three tiger genetic blocks (TGB I, TGB II, TGB III) identified in TAL

	TGB I (n=173)				TGB II (n=24)				TGB III (n=22)			
Locus	**N_A**	**ASR**	**H_E**	**H_O**	**N_A**	**ASR**	**H_E**	**H_O**	**N_A**	**ASR**	**H_E**	**H_O**
FCA090	12	28	0.85	0.31	08	18	0.70	0.29	09	28	0.78	0.37
msFCA506	11	24	0.83	0.21	08	16	0.72	0.42	08	34	0.84	0.38
FCA672	13	28	0.87	0.62	10	24	0.88	0.27	12	42	0.90	0.63
FCA304	11	28	0.73	0.14	05	20	0.62	0.30	05	10	0.73	0.32
FCA628	13	30	0.79	0.39	09	36	0.89	0.43	09	54	0.79	0.59
FCA232	11	22	0.68	0.20	07	20	0.53	0.43	11	32	0.81	0.71
FCA230	09	26	0.80	0.10	06	18	0.72	0.04	10	52	0.76	0.36
FCA279	13	24	0.86	0.53	09	22	0.59	0.20	11	32	0.77	0.73
FCA441	11	40	0.75	0.57	06	36	0.74	0.31	07	60	0.83	0.53
FCA069	03	26	0.71	0.33	10	28	0.89	0.30	09	32	0.84	0.15
MsFCA453	04	08	0.65	0.70	05	20	0.69	0.38	10	60	0.82	0.33
MSF115	10	16	0.44	0.03	05	48	0.74	0.35	05	24	0.67	0.06
MsHDZ170	05	18	0.85	0.35	03	16	0.24	0.08	12	34	0.90	0.36
Mean	**10.15 (3.06)**	**24.46 (7.37)**	**0.75 (0.11)**	**0.34 (0.20)**	**07.00 (2.11)**	**24.77 (9.33)**	**0.69 (0.17)**	**0.29 (0.12)**	**9.08 (2.24)**	**38.00 (14.29)**	**0.80 (0.06)**	**0.43 (0.20)**

revealed that tiger population of TGB I (western TAL) had faced the decline about 160-200 years ago (Table 2.6 and Figure 2.7).

Table 2.6: Detail results of qualitative and quantitative demographic analyses of Tiger Genetic Block I

Demography analyses			**TGB I (n=160)**
Method	Analysis type		
EWCL method (BOTTLENECK)	Qualitative	IAM	Heterogygosity excess for 09 loci
		SMM	Heterogygosity excess for no loci
		TPM	Heterogygosity excess for 04 loci
Garza-Williamson index (M ratio)	Qualitative		0.34 (SD 0.12)
Storz-Beaumont method (MSVAR)	Quantitative		Decline- 88% Time- ~160 years

2.4 Discussion:

An extensive survey was carried out in all the potential tiger habitats, which covers all protected areas (six tiger reserves, three national parks, two conservation reserves and six wildlife sanctuaries) and non-protected areas (10 forest divisions and three social forestry divisions) (Figure 2.1) of the Terai-Arc landscape (TAL). Tiger

presence was confirmed in most of the existing tiger habitats in TAL. These areas include already known tiger reserve and forest divisions (Jhala et al., 2020) along with unreported areas in this landscape (Figure 2.3). The field surveys corroborated with early reports of tiger absence beyond the western part of Rajaji Tiger Reserve, Suhelwa Wildlife Sanctuary, and remaining areas between Dudhwa Tiger Reserve and Sohagibarwa Wildlife Sanctuary (Figure 2.3) (Jhala et al., 2020; Johnsingh et al., 2004), possibly due to high anthropogenic pressure and habitat loss.

The remaining forested habitat of Suhelwa Wildlife Sanctuary is an isolated forest patch in India but potential connectivity present with Banke National Park of Nepal. Additionally, Suhelwa Wildlife Sanctuary is also severely affected by anthropogenic pressures. An earlier study estimated severe human pressure along the southern boundary of the sanctuary along with extensive cattle usage (Johnsingh et al., 2004). High human population both from India (southern boundary) and Nepal (northern boundary) sides and grazing pressure leads to poor prey base in Suhelwa Wildlife Sanctuary. Therefore, with poor habitat quality and prey base, Suhelwa Wildlife Sanctuary showed the lowest frequency of occurrence of tigers (Jhala et al., 2020; Chanchani et al., 2016b; Johnsingh et al., 2004) and hence no tiger sign was detected during the current study. Reduction in anthropogenic pressures from both sides and improve the connectivity with Banke National Park are the only way Suhelwa Wildlife Sanctuary could bring back its lost tiger population. Similarly, no tiger sign was confirmed from the North Kheri Forest Division of Uttar Pradesh due to heavy habitat destruction, prey depletion due to high human activities in the recent past and thus resulted in low tiger frequency of occurrence (Johnsingh et al., 2004). With

reference to earlier study by Johnsingh et al., (2004), no tiger sign was detected in the degraded forest of Shahjahanpur Social Forestry Division.

Unfortunately, with the increasing human populations the anthropogenic pressures like habitat destruction, prey depletion are the major limiting factors of tigers to reclaim their lost habitats, which eventually has a negative effect on the overall biodiversity in this highly productive grassland ecosystem of earth.

Interestingly, this study confirmed tiger presence (using direct sighting, pug mark and confirmed tiger faeces) in some of the previously tiger unreported areas of Haridwar Forest Division, Najibabad Social Forestry Division and Pilibhit Social Forestry Division (Johnsingh et al., 2004; Jhala et al., 2020). This expansion in tiger habitat occupancy is possibly due to increasing tiger dispersals to newer areas driven by a recent increase in their population in this landscape (Jhala et al., 2020). Tigers are territorial and high resource-demanding (in terms of both food and space) animals and such dispersal events are thus normal (Smith, 1993). In the Indian subcontinent, such dispersal events have been reported from other landscapes also (Northwest India (Reddy et al., 2012), Central India (Yumnam et al., 2014; Joshi et al., 2013; Sharma et al., 2013b), Nepal-TAL (Thapa et al., 2018, 2017)). Interestingly, both male and female tigers were found beyond the protected areas including relatively marginal habitats of forest divisions and social forestry divisions indicating both-sex dispersal events in TAL, as found in other habitats (Central India (Yumnam et al., 2014), Nepal-TAL (Thapa et al., 2018)).

The genetic analyses measured the moderate level of genetic diversity in remaining tiger populations (Table 2.3) and the presence of three genetic subpopulations

(named as Tiger Genetic Blocks or TGBs) in TAL (Figures 2.5 and 2.6). This was an unexpected result considering the population size (~650 tigers) (Jhala et al., 2020) and the distribution of tiger presence in the majority of the protected and non-protected areas, except some parts of the central and the eastern TAL (Figures 2.5 and 2.6). The easternmost subpopulation or TGB III covering Sohagibarwa Wildlife Sanctuary and Valmiki Tiger Reserve is physically separated from the central and western TAL, without any forest connectivity (Johnsingh et al., 2004) (Figure 2.5 and 2.6). Tigers from Valmiki Tiger Reserve have also been reported earlier as genetically unique (Kolipakam et al., 2019). Even in the Nepal part of TAL, three genetic subpopulations have been reported those are connected with TGB II and TGB III (Thapa et al., 2018), indicating genetic discontinuity across transboundary sections of TAL in this region. However, the pattern of genetic discontinuity between TGB I and TGB II within the seemingly continuous habitat of central and western TAL was surprising (Figure 2.5 and 2.6). Similar patterns of tiger population structure have been earlier reported from other tiger landscapes in the subcontinent (for example, Central Indian landscape (Sharma et al., 2013b), Western TAL (Singh et al., 2017), Nepal-TAL (Thapa et al., 2018)). Even in the same TAL landscape Bhatt et al., (2020) has reported two leopard genetic subpopulations, possibly driven by landscape features. When compared with this study, it makes ecological sense that TAL leopards showed less population structure than tigers (three TGBs) as they are more habitat generalist and even found in human-dominated areas (Athreya et al., 2010). Unlike the other major tiger landscapes in India, both of these sympatric species face one common problem related to this linear landscape where one-dimensional space (resulting in restricted movement opportunities) combined with

very high human density makes the entire landscape extremely vulnerable to fragmentation at a relatively small temporal scale (Johnsingh et al., 2004). Results indicate that the genetic separation between TGB I and II is driven by human-disturbance mediated loss of corridor functionality leading to population differentiation at small, but significant levels (Table 2.4).

Further, results obtained in this study strongly suggest that a large single tiger population of TGB I and a relatively small population of TGB III but connected with multiple populations of Nepal (i.e. Chitwan National Park and Parsa Wildlife Reserve) hold relatively higher genetic diversity than the isolated and moderate size population of TGB II (Table 2.5). Therefore, the detailed understanding of population connectivity especially the source-sink dynamics of these tiger populations have become extremely critical to maintaining high genetic diversity and stable populations for the long-term persistence of tigers in the TAL.

In demographic analyses, both results obtained from qualitative (bottleneck and M-ratio analyses) and quantitative (Storz-Beaumont analysis) approaches strongly suggested a severe tiger population decline of ~88% over the past ~160 years in western TAL (Table 2.6 and Figure 2.7). This results strongly concurrent with the historical hunting records of tigers all across the Indian subcontinent (Rangarajan and Shahabuddin, 2006). Nearly 500 years ago Mughals started hunting large carnivores especially Asiatic lions (*Panthera leo parsica*) and tigers (*Panthera tigris tigris*) for trophies (Rangarajan and Sivaramakrishnan, 2014).

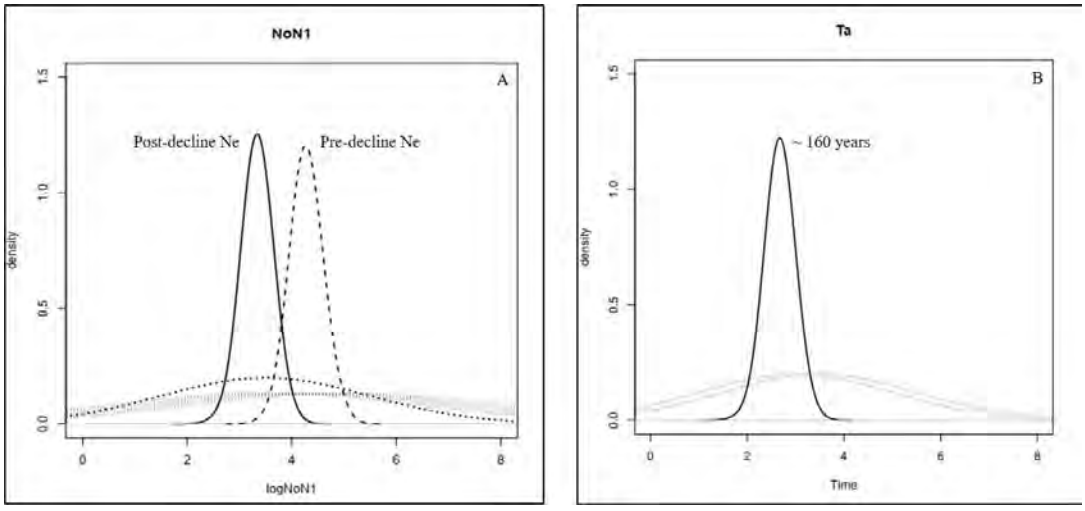

Figure 2.7: Demographic history of tigers of the western Terai-Arc landscape. Results of 13 microsatellite loci showed the posterior distributions for tiger population size changes of Tiger Genetic Block I using Storz and Beaumont approach. In A the posterior distributions of ancestral and present effective population sizes were represented by dashed and solid lines. The dotted line showed the priors. B represents the posterior distribution for the time since the tiger population decline started for Tiger Genetic Block I

However, Asiatic lions were more preferred than tigers for Mughals until British colonized India around 250 years ago (Rangarajan and Sivaramakrishnan, 2014). By that time Asiatic lions became rare and tigers became a major target of trophy hunting (Rangarajan and Sivaramakrishnan, 2014). Historical record imparts over 80,000 tigers were killed especially by the high-ranked Bristish officials and royal families as trophies and bounties all across the Indian subcontinent within a period of 50 years (1875-1925) (Rangarajan and Shahabuddin, 2006). This large-scale tiger hunting started during the Mughal era (~500 years ago) and accelerated during the British era (~250 years ago) coincided with the results obtained in this chapter. However, a higher magnitude of population decline of tigers was reported in Mondol et al., (2009a), where peninsular Indian population including central Indian and Western Ghats landscapes faced 98% decline over the last ~200 years. The decrease

in population decline rates probably explains by the logistical constrain to conduct large-scale hunting in the hilly region of western TAL. A similar pattern of variations in population decline rate was also reported in Bhatt et al., (2020) for leopards, where the hilly region of Western Ghats faced a lower rate of population decline compare to the central Indian landscape. However, the results strongly corroborate that Indian tiger population faced 88-98% decline over the past ~160-250 years because of rampant hunting. Furthermore, other sympatric, endangered species in the Asian region (for example Orangutan- ~210 years, (Goossens et al., 2006); Giant panda- ~250 years, (Zhu et al., 2010)) also showed a similar pattern of population decline when compared. Other wide-ranging carnivores across the globe (for example European wolves (Aspi et al., 2006); African wild dog- (Marsden et al., 2012); Eurasian badgers- (Frantz et al., 2014)) too faced similar population decline period like tigers.

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Chapter 2:

Source-sink dynamics of tigers across different protected and non-protected habitats in the Terai-Arc landscape, India

3.1 Introduction:

Tiger (*Panthera tigris*) typifies large feline carnivores across the world and are major targets of present-day conservation efforts. The current global wild tigers faced ~90% decline in the past ~200 years and estimates of range from 3000–3500 individuals. These tiger populations are restricted to only 7% of their historical range (Morell, 2007; Sanderson et al., 2006) and 41% of the remaining range has declined in the past couple of decades alone (Dinerstein et al., 2007), indicating the acceleration of both recent population and habitat loss. Globally most of the remaining tiger populations now persist in relatively small populations (between 20-100 individuals) that are confined to often spatially isolated, patchy forests (Ranganathan et al., 2008; Sanderson et al., 2006). In the Indo-Pacific region that retains most of the primary tiger habitats, 68% of reserves are less than 300 sq. km and 90% are less than 1000 sq. km (Dinerstein and Wikramanayake, 1993), are inadequate to support large tiger populations (Wikramanayake et al., 1998). To recover this doomed tiger populations, in 2010, the leaders of 13 tiger residing countries had set a 12 years-long goal of doubling the wild tiger population across their distribution (Global Tiger Initiative, 2011). To achieve such an ambitious goal, the recent conservation strategy has emphasized landscape-based conservation that could potentially support viable populations for the future survival of tigers (Sanderson et al., 2006; Walston et al., 2010; Wikramanayake et al., 2011). Ecological studies have identified a few conservation units (also called 'Tiger Conservation Landscapes' (TCLs)) across the distribution which could support viable tiger populations (Sanderson et al., 2006). In India, Sanderson et al., (2006)

suggested that conservation efforts must prioritize the tiger populations residing in larger landscapes like the Western Ghats, central India, north-eastern India, Sunderbans and the alluvial flood plains in the Himalayan foothills that support high potential tiger densities and relatively larger populations (Jhala et al., 2020).

In 2019, India which currently harbors ~80% of the global wild tiger population (Goodrich et al., 2015), announced the news of doubling its tiger number since 2006, within its existing habitats (Jhala et al., 2020) which confirms the site-specific management success. However, the source sites within the identified TCLs are the primary habitats that experienced such population recovery (Jhala et al., 2020), thereby necessitating the need for landscape-wide conservation strategies through the protection of source sites and maintaining connectivity with adjoining sink habitats (Sanderson et al., 2006). Therefore, studying the tiger source-sink population dynamics would be crucial to set the site-specific future strategies for successful tiger conservation mitigation. Studies carried out in India have helped us to understand such dynamics for tiger populations within the Western Ghats (Reddy et al., 2019) and central Indian landscapes (Joshi et al., 2013; Sharma et al., 2013a; Thatte et al., 2018; Yumnam et al., 2014). However, no such study has focused on tigers in the Indian part of the Terai-Arc landscape (TAL), and the relation between source and sink populations is poorly understood.

The 15000 sq. km. forested habitats of TAL retains 22% (~650 tigers) of current Indian tiger population primarily restricted within its 10 protected areas, nine forest divisions and two social forestry divisions (See Chapter 1; Jhala et al., 2020) (Figure 3.1). The tiger population of TAL is structured which confirmed restricted gene flow

between few of the identified Tiger Habitat Blocks (THBs), however, majority of them still maintain genetic connectivity (See Chapter 1). Additionally, the population sizes of each of the protected areas, forest divisions and social forestry divisions also varies (Jhala et al., 2020) which drive the gene flow to maintain source-sink dynamics. This chapter aims to understand the tiger population connectivity in a form of source-sink dynamics within various protected areas and non-protected areas. More specifically, 1) identification of genetic migrants and 2) quantification of magnitude and direction of gene flow across various tiger population in TAL using non-invasive molecular analyses.

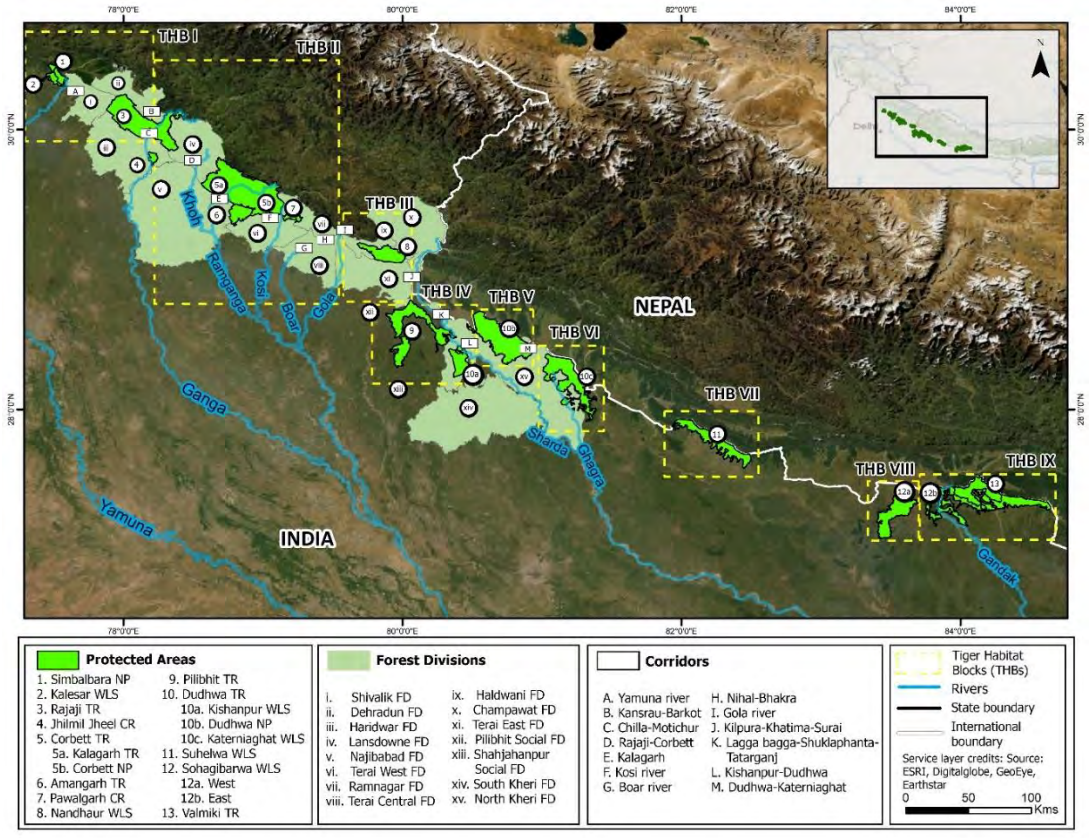

Figure 3.1: Detail description of the Indian part of the Terai-Arc landscape. The forested habitats are fragmented and formed nine major blocks and connected through 13 tiger dispersal corridors

3.2 Methodology:

3.2.1 Data accumulation:

In this chapter source-sink dynamics of tigers were investigated using multilocus (13 loci) genetic data, generated from 219 (~34% of total population, Jhala et al., 2020) unique tigers across TAL. These genetically identified individual tigers represent tiger population of six tigers reserves, one wildlife sanctuary, nine forest divisions and two social forestry divisions across TAL (Table 3.1). In Chapter 1, these genetic data from TAL were used to understand the genetic diversity, population structure and demographic history of tiger. Analyses measured the moderate level of genetic diversity in the remaining tiger populations (Table 2.3) and strongly suggested a severe tiger population decline of ~88% over the past ~160 years in western TAL (Table 2.6 and Figure 2.7). Additionally, genetic analyses confirmed the presence of three genetic subpopulations (named as Tiger Genetic Blocks or TGBs) in TAL (Figure 2.5 and 2.6).

Table 3.1: Details of individual tigers identified in various protected and non-protected areas. Genetic data from these 219 tigers were used in source-sink analyses

Area	Unique tigers captured by Jhala et al., (2020)	Unique tigers captured in this study	Sampling percentage (%)
Rajaji Tiger Reserve	37	27	73

Haridwar Forest Division	-	02	-
Najibabad Social Forestry Division	-	05	-
Lansdowne Forest Division	34	26	76
Corbett Tiger Reserve	231	41	18
Amangarh Tiger Reserve	20	10	50
Terai West Forest Division	39	19	49
Ramnagar Forest Division	37	23	62
Haldwani Forest Division	23	04	17
Champawat Forest Division	09	05	56
Nainital Forest Division	04	-	-
Terai Central Forest Division	05	06	83
Terai East Forest Division	23	03	13

Pilibhit Social Forestry Division	-	02	-
Pilibhit Tiger Reserve	57	10	18
Dudhwa Tiger Reserve	82	12	15
South Kheri Forest Division	02	02	100
Sohagibarwa Wildlife Sanctuary	05	03	60
Valmiki Tiger Reserve	33	19	58
Total	***641***	***219***	***34***

3.2.2 Identification of tiger source-sink populations:

The identified TGB I, II and III roughly correspond to the tiger population in the states of Uttarakhand (western TAL- including six protected areas, eight forest divisions and one social forestry division), Uttar Pradesh (central TAL- covering two protected areas, one forest division and one social forestry division) and Bihar (eastern TAL- included only two protected areas), respectively (Figure 2.5 and 2.6). Furthermore, analyses also confirmed genetic connectivity between various protected areas, forest divisions and social forestry divisions within each of the identified TGBs, however, disrupted between TGBs at landscape level in TAL. Therefore, in

this chapter the tiger source-sink dynamics were assessed within three TGBs. Assessing tiger connectivity directly (through radio-telemetry or camera trapping) at a landscape level is challenging due to their secretive behaviour, and logistical constraints resulting in infrequent information (Janečka et al., 2007). Therefore, in this chapter indirect approache was addopted to understand population connectivity of tigers in TAL. A number of specific genetic analyses were conducted to evaluate source-sink dynamics (using directional gene flow and migrant analyses as proxies of population connectivity) involving all the TGBs within this landscape. More specifically, two different analyses were performed focusing on 'detection of genetic migrants' and 'assessment of rate and direction of gene flow' between all the protected areas, forest divisions and social forestry divisions within each TGBs (see Chapter 1 for more details).

Identification of genetic migrants: To detect tiger dispersals, two different approaches were used that use allele frequencies to detect migrant individuals. First, prior population information was used in the USEPOPINFO option implemented in STRUCTURE 2.3.2 (Pritchard et al., 2000) to detect first-generation migrants in the sampled populations. The number of clusters (K between 1-10) was detected based on 10 independent runs with 500,000 iterations and a burn-in of 50,000 assuming an admixture model. The membership coefficient (q) was considered above 0.9 as a realistic cut-off value to assign an individual to a population (Joshi et al., 2013). Different migration rates (MIGPRIOR 0.01, 0.05, 0.1) were assigned as a sensitivity test. the analysis was ran with two separate datasets: a) individuals grouped as populations according to their sampling locations and b) individuals grouped as

genetic clusters from the initial run to evaluate the consistency of the results across different genetic groups created.

Further, the 'Migrant detection' function was used in program GENECLASS 2.0 (Piry et al., 2004) to identify the first-generation migrant tigers. Here a Bayesian approach was used as described by Rannala and Mountain, (1997) along with the resampling method of Paetkau et al., (2004) for likelihood computation (L_home/L_max). The run parameters included 10000 simulated individuals with a threshold alpha value of 0.01 (Type 1 error) (Paetkau et al., 2004). This method allows detection of migrants even when the overall differentiation between populations is low. Apart from first-generation migrant detection, individual assignments was performed in GENECLASS using Bayesian criterion of Rannala and Mountain, (1997) in combination without resampling with rest of the parameters same as described above.

Quantification of directional gene flow: The magnitude and direction of recent gene flow was measured using the Bayesian MCMC based approach implemented in BAYESASS v.3.0.4 (Wilson and Rannala, 2003), with run parameters of 1000000 iterations and 100000 burn-in. This approach is generally used to detect recent gene flow (5-7 generations) between populations (Smith and Mcdougal, 1991).

3.3 Results:

3.3.1 Population connectivity:

Gene flow and tiger source-sink populations within TGBs: The individual tiger genetic data was analysed to understand the tiger source-sink population dynamics

(which also confirmed connectivity among populations) within each of the three already identified TGBs (see Chapter 1 for more details) in TAL. Within the TGB I, 'genetic migrant detection' and gene flow analysis identified two major habitat complexes with genetic connectivity: The Rajaji-Lansdowne-Haridwar region and the Corbett-Ramnagar-Terai Forest Divisions-Haldwani region. In the Rajaji-Lansdowne-Haridwar habitat complex five first-generation migrant tigers were detected (Table 3.2): two migrants each from Lansdowne Forest Division to Rajaji Tiger Reserve and Haridwar Forest Division and one from Rajaji Tiger Reserve to Lansdowne Forest Division (Table 3.2). Further Bayesian analyses show higher rates of gene flow between Lansdowne Forest Division to Rajaji Tiger Reserve and Rajaji Tiger Reserve to Haridwar Forest Division, whereas Najibabad Social Forestry Division shows signatures of immigration from both Rajaji Tiger Reserve and Lansdowne Forest Division (Figure 3.2). Combining this information, both Rajaji Tiger Reserve and Lansdowne Forest Division were identified as source tiger populations and Haridwar Forest Division and Najibabad Social Forestry Division are sink populations in this habitat complex, respectively (Table 3.2 and Figure 3.2).

Similarly, in the Corbett-Ramnagar-Terai Forest Divisions-Haldwani habitat complex 12 first-generation migrant tigers were detected (Table 3.2), showing extensive genetic connectivity among the sampled areas within the habitat complex. Three migrants were detected from Ramnagar Forest Division to Corbett Tiger Reserve and two migrants to Haldwani Forest Division (Table 3.2). One migrant was detected tiger from Terai West Forest Division to Ramnagar Forest Division (Table 3.2). Two migrants were detected from Terai Central Forest Division to Terai West

Forest Division, one migrant to Ramnagar Forest Division and one to Champawat Forest Division (Table 3.2). One migrant individual was also detected from Haldwani Forest Division to Terai East Forest Division (Table 3.2). In addition, one migrant individual was detected from Najibabad Social Forestry Division to Amangarh Tiger Reserve (Table 3.2). Bayesian analyses show higher gene flow from Corbett Tiger Reserve to adjoining Najibabad Social Forestry Division and Amangarh Tiger Reserve (Figure 3.2). Corbett Tiger Reserve also shows a high rate of bidirectional gene flow with Ramnagar Forest Division and Terai West Forest Division. Similarly, Terai West Forest Division is highly interconnected with Amangarh Tiger Reserve, Terai Central Forest Division and Ramnagar Forest Division (Figure 3.2). The other areas such as Ramnagar Forest Division and Champawat Forest Division, Haldwani Forest Division and Ramnagar Forest Division, Terai Central Forest Division and Terai East Forest Division and Haldwani Forest Division and Terai East Forest Division have lower rates of gene flow among them (Figure 3.2). Combined together, this information suggests that Corbett Tiger Reserve is the main source population for Ramnagar Forest Division, Amangarh Tiger Reserve, Terai West Forest Division and Najibabad Social Forestry Division, whereas Ramnagar Forest Division is the source tiger population for Haldwani Forest Division, Champawat Forest Division and Terai East Forest Division (Table 3.2 and Figure 3.2).

Within the two major tiger habitat complexes in TGB I (Rajaji-Lansdowne-Haridwar and Corbett-Ramnagar-Terai Forest Divisions-Haldwani) connectivity is maintained through the Najibabad Social Forestry Division, which acts as a sink population for

both Corbett Tiger Reserve and Lansdowne Forest Division (Table 3.2 and Figure 3.2).

Table 3.2: Results of detection of first-generation migrant analyses in Tiger Genetic Block I (TGB I) using GENECLASS and STRUCTURE

Sampled location	**Migrant from**	**GENECLASS likelihood computation (L_home/L_max)**	***p* Resident**	**STRUCTURE USEPOPINFO (MIGPRIOR 0.05)**
		TGB I		
Rajaji TR	Lansdowne FD	3.645	0.003	NA
Rajaji TR	Lansdowne FD	3.695	0.004	NA
Lansdowne FD	Rajaji TR	8.089	0.000	NA
Haridwar FD	Lansdowne FD	NA	NA	0.936
Haridwar FD	Lansdowne FD	NA	NA	0.937
Terai West FD	Terai Central FD	7.816	0.001	NA
Terai West FD	Terai Central FD	5.653	0.006	NA

Ramnagar FD	**Terai West FD**	**6.329**	**0.006**	**0.945**
Ramnagar FD	Terai Central FD	6.307	0.004	NA
Haldwani FD	**Ramnagar FD**	**2.347**	**0.006**	**0.960**
Champawat FD	Terai Central FD	4.993	0.000	NA
Terai East FD	Haldwani FD	2.374	0.000	NA
Corbett TR	Ramnagar FD	NA	NA	0.828
Corbett TR	Ramnagar FD	NA	NA	0.820
Corbett TR	Ramnagar FD	NA	NA	0.922
Amangarh TR	Najibabad SFD	NA	NA	0.995
Haldwani FD	Ramnagar FD	NA	NA	0.994

TR- Tiger Reserve, FD- Forest Division, SFD- Social Forestry Division, NP- National Park, WLS- Wildlife Sanctuary

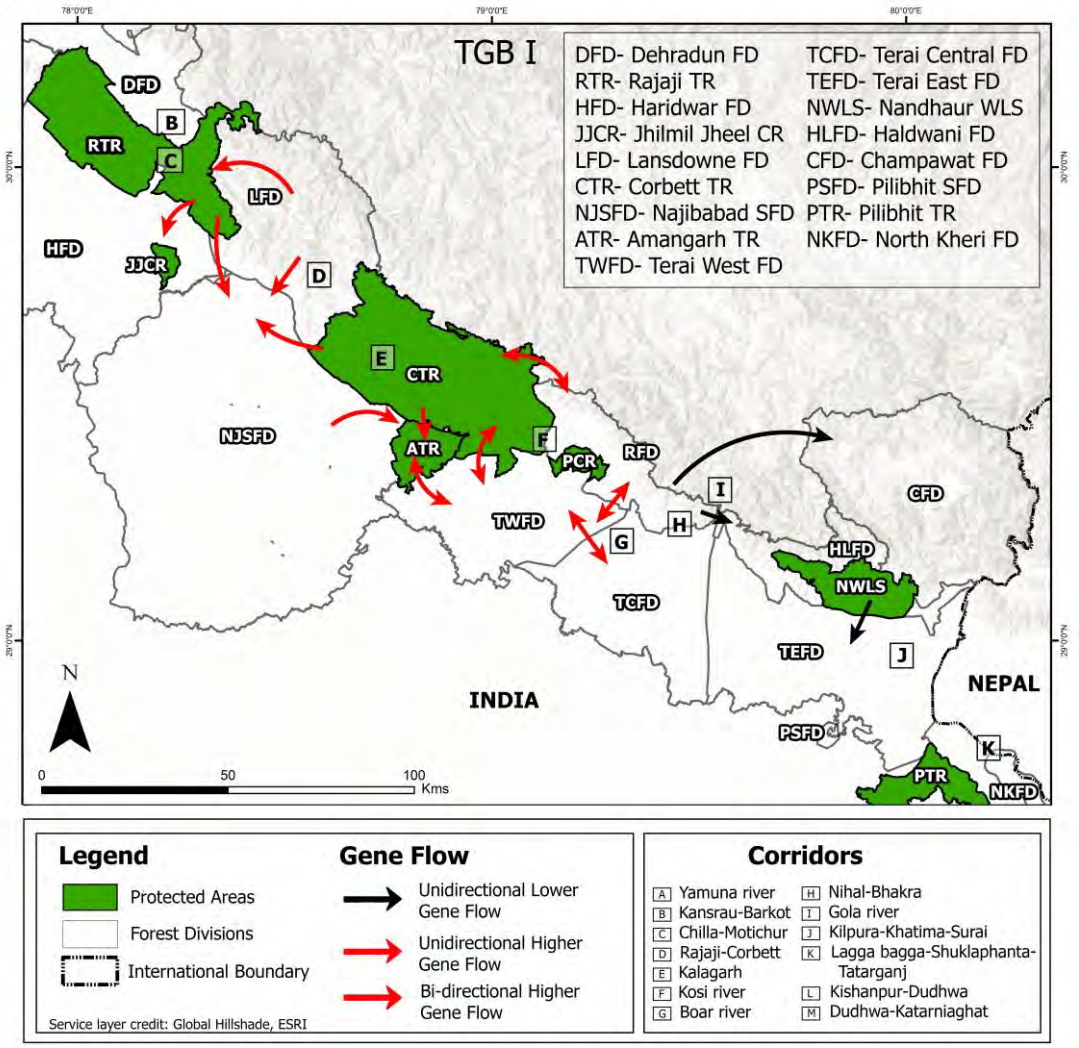

Figure 3.2: Detail understanding of magnitude and direction of tiger gene flow in the Tiger Genetic Block I, identified in this study. Majority of the forest divisions acting as corridors and facilitating tiger gene flow between various protected areas

In TGB II, the tiger habitats are comparatively more fragmented than TGB I and 10 first-generation migrant tigers were detected (Table 3.3). Two migrants were detected from Pilibhit Tiger Reserve to Dudhwa National Park (part of Dudhwa Tiger Reserve) and one migrant to Katarniaghat Wildlife Sanctuary (part of Dudhwa Tiger Reserve) (Table 3.3). Two migrants were also found from Dudhwa National Park to Pilibhit Tiger Reserve, one migrant to Kishanpur Wildlife Sanctuary (part of

Dudhwa Tiger Reserve) and two migrants to South Kheri Forest Division (buffer zone of Dudhwa Tiger Reserve) (Table 3.3). Further, two migrants were detected from Kishanpur Wildlife Sanctuary to Pilibhit Social Forestry Division (Table 3.3). Higher gene flows were measured from Pilibhit Tiger Reserve to Dudhwa National Park, Kishanpur Wildlife Sanctuary, South Kheri Forest Division and Pilibhit Social Forestry Division (Figure 3.3). Bidirectional gene flows were also estimated among Dudhwa National Park, Kishanpur Wildlife Sanctuary and Katarniaghat Wildlife Sanctuary, along with unidirectional gene flow to adjoining South Kheri Forest Division (Figure 3.3). This information suggests Pilibhit Tiger Reserve and Dudhwa Tiger Reserve are the major source tiger populations, and South Kheri Forest Division and Pilibhit Social Forestry Division are the sink populations in the TGB II (Table 3.3 and Figure 3.3).

Table 3.3: Results of detection of first-generation migrant analyses in Tiger Genetic Block II (TGB II) using GENECLASS and STRUCTURE

Sampled location	**Migrant from**	**GENECLASS likelihood computation (L_home/L_max)**	***p* Resident**	**STRUCTURE USEPOPINFO (MIGPRIOR 0.05)**
		TGB II		
Pilibhit TR	Dudhwa NP	4.293	0.008	NA
Pilibhit TR	Dudhwa NP	4.259	0.010	NA

Kishanpur WLS	Dudhwa NP	2.647	0.000	NA
Katarniaghat WLS	**Pilibhit TR**	**3.773**	**0.000**	**0.981**
Pilibhit SFD	Kishanpur WLS	NA	NA	0.985
Pilibhit SFD	Kishanpur WLS	NA	NA	0.986
Dudhwa NP	Pilibhit TR	NA	NA	0.942
Dudhwa NP	Pilibhit TR	NA	NA	0.985
North Kheri FD	Dudhwa NP	NA	NA	0.984
North Kheri FD	Dudhwa NP	NA	NA	0.989

TR- Tiger Reserve, FD- Forest Division, SFD- Social Forestry Division, NP- National Park, WLS- Wildlife Sanctuary

Finally, within TGB III two first-generation migrants were detected from west Sohagibarwa Wildlife Sanctuary to Valmiki Tiger Reserve (Table 3.4). This result was surprising as within this habitat block Valmiki Tiger Reserve retains a larger tiger population (Jhala et al., 2020). However, the Bayesian analysis results indicated higher gene flow from Valmiki Tiger Reserve and east Sohagibarwa Wildlife Sanctuary to west Sohagibarwa Wildlife Sanctuary (Figure 3.4). Combined together, Valmiki Tiger Reserve-east Sohagibarwa Wildlife Sanctuary identified as the source population for west Sohagibarwa Wildlife Sanctuary in TGB III.

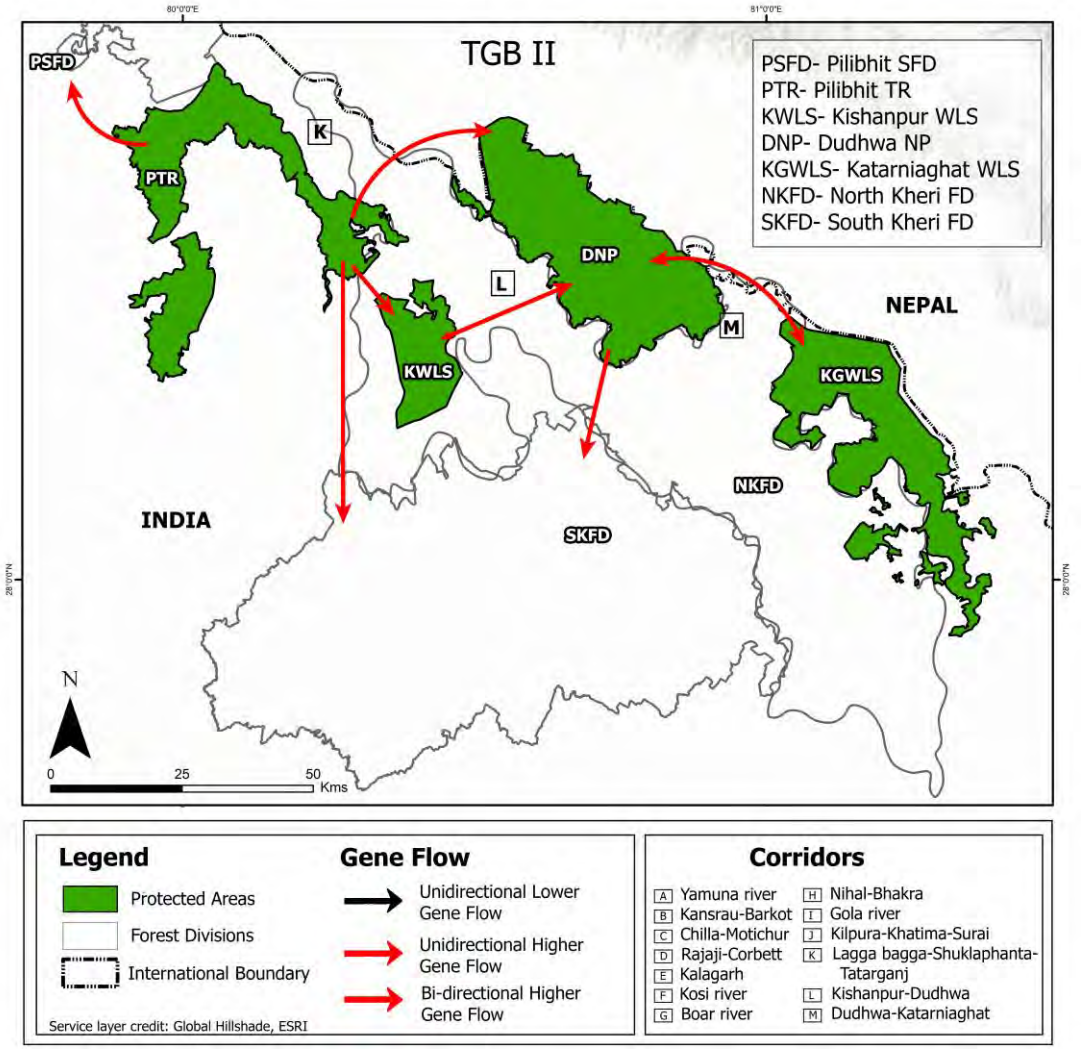

Figure 3.3: Detail understanding of magnitude and direction of tiger gene flow in the Tiger Genetic Block II. Forest divisions acting as corridors and facilitating tiger gene flow between various protected areas

Table 3.4: Results of detection of first-generation migrant analyses in Tiger Genetic Block III (TGB III) using GENECLASS and STRUCTURE

Sampled location	**Migrant from**	**GENECLASS likelihood computation (L_home/L_**	***p* Resident**	**STRUCTURE USEPOPINFO (MIGPRIOR 0.05)**

		max)		
		TGB III		
Valmiki TR	Sohagibarwa WLS	5.204	0.001	NA
Valmiki TR	Sohagibarwa WLS	2.799	0.007	NA

TR- Tiger Reserve, FD- Forest Division, SFD- Social Forestry Division, NP- National Park, WLS- Wildlife Sanctuary

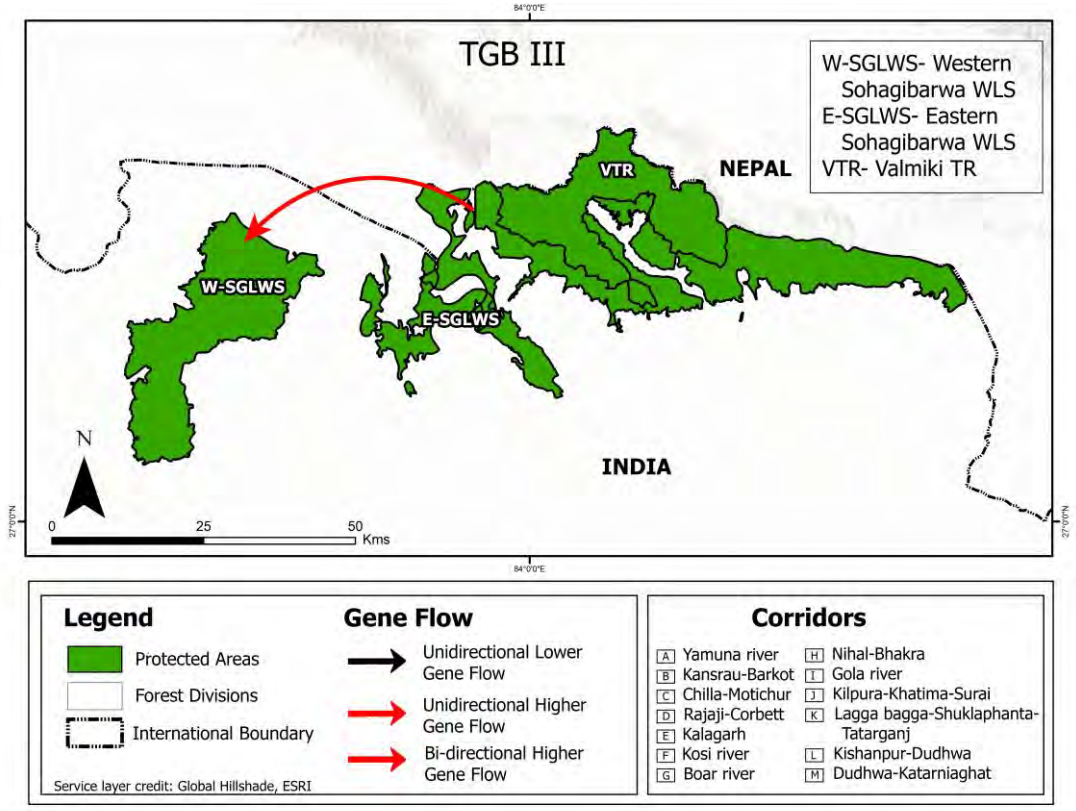

Figure 3.4: Detail understanding of magnitude and direction of tiger gene flow in the Tiger Genetic block III, identified in this study

3.4 Discussion:

Due to extensive sampling, multilocus genetic data were generated from 219 tigers (~34% of total population) from the Terai-Arc landscape (TAL). Using these genetic data further, analyses identified tiger source-sink dynamics and fine-scale population connectivity patterns among all protected areas (six tiger reserves, three national parks, two conservation reserves and six wildlife sanctuaries) and non-protected areas (nine forest divisions and two social forestry divisions) (Tables 3.2, 3.3, 3.4 and Figures 3.2, 3.3, 3.4) of the three identified Tiger Genetic Blocks (TGBs) in TAL. Tiger source and sink populations were identified, involving detection of genetic migrants and quantification of directional gene flow to understand the fine-scale genetic connectivity within each TGBs.

Analyses with tiger genetic data from TGB I identified both migrant individuals (n=17) and signatures of high gene flow among majority of the protected areas and other surrounding non-protected areas (Table 3.2 and Figure 3.2), indicating functional connectivity within this region. Further, confirmed Rajaji and Corbett Tiger Reserves and Lansdowne and Ramnagar Forest Divisions as source populations where Amangarh Tiger Reserve, Haridwar, Terai West, Haldwani, Champawat and Terai East Forest Divisions and Najibabad Social Forestry Division as sink populations. Recent census report suggest the increase of the tiger numbers in the identified source populations and also corroborate with the genetic results (Jhala et al., 2020). The combine results of functional connecitivity between four source and seven sink populations suggest that the complex, undulating forested Shivalik and Bhabhar habitat found in this region has helped in tiger movements as

hypothesized by Johnsingh et al., (2004). Similar pattern was also identified in Western Ghats and central Indian landscapes where tigers were reported to use rough terrain for dispersal and avoided human habitations (Reddy et al., 2019, 2017). In addition, this area also retains the largest number of tigers, mostly within the protected areas and recent increase in tiger population (Jhala et al., 2020) has probably resulted in migration of the surplus individuals to newer areas. Therefore, the terrain complexity is crucial to maintain the future tiger connectivity in TGB I. The source-sink dynamics analyses results also corroborate this pattern, where results found that majority of the sink populations in TGB I are in the flat, Terai region in the southern part (Figure 3.2). The Terai habitat also supports very high human density and known to support poor tiger populations in recent past (Johnsingh et al., 2004).

Similarly, in TGB II, genetic analyses identified both migrant individuals (n=10) and signatures of high gene flow among two protected areas and other surrounding non-protected areas (Table 3.3 and Figure 3.3), indicating functional connectivity within this region. Further, confirmed Pilibhit and Dudhwa Tiger Reserves as source populations where tiger number has increased significantly (Jhala et al., 2020), and South Kheri Forest Division and Pilibhit Social Forestry Division as sink populations in TGB II. The combine results of functional connecitivity between two source and two sink populations showed, Terai habitat dominated TGB II (where majority of the tiger populations are found within protected areas) good connectivity (Figure 3.3). The results indicated that mostly the protected areas are source population and forest divisions/social forestry divisions are the sink populations (Figure 3.3), showing a

contrasting pattern from TGB I where even forest divisions were identified as source populations (like Lansdowne and Ramnagar Forest Divisions) (Figure 3.2). Recent study also identified protected areas as source populations and able to maintain functional connectivity through non-protected habitats in the Terai habitat of Nepal-TAL (Thapa et al., 2018). Therefore, the protected areas were identified as crucial habitats for future tiger persistence in Terai habitats of TGB II.

Further, In TGB III, genetic analyses identified both migrant individuals (n=2) and signatures of unidirectional gene flow among two protected areas (Table 3.4 and Figure 3.4), indicating functional connectivity. Further, confirmed the relatively larger tiger population of Valmiki Tigre Reserve and eastern Sohagibarwa Wildlife Sanctuary (Jhala et al., 2020) was the source whereas the western Sohagibarwa Wildlife Sanctuary was the only available sink habitats (Figure 3.4). Overall, the genetic analyses identified lowest both migrant individuals and signatures of gene flow in TGB III (eastern TAL) possibly due to unavailability of potential habitats and low population sizes. Similarly low genetic connectivity was also observed in the small and isolated tiger population of Sunderban Tiger Reserve in eastern India (Singh et al., 2015). However, recent tiger dispersal from adjoining Chitwan National Park and Parsa Wildlife Reserve of Nepal-TAL helped Valmiki Tigre Reserve and subsequently the eastern Sohagibarwa Wildlife Sanctuary to increase their population sizes (Jhala et al., 2020; Thapa et al., 2018) and act as source population for western Sohagibarwa Wildlife Sanctuary. Therefore, maintaining connecitivity with larger populations of Chitwan National Park and Parsa Wildlife Reserve of Nepal-TAL

became crucial for future persistence of the smaller tiger population of TGB III in TAL.

An earlier study in the central Indian landscape showed tigers lost gene flow due to loss of corridor connectivity (Sharma et al., 2013b). Results of this chapter showed tigers still maintain gene flow among majority of the remaining habitats across TAL, however, gene flow is lost between TGB I and II and TGB II and III possibly due to habitat disconnectivity and growing anthropogenic pressures (Johnsingh et al., 2004). The existing corridors (identified by Johnsingh et al., 2004) are playing a crucial role in maintaining such tiger gene flow within all three TGBs. Similarly, tiger populations of Nepal-TAL also showed gene flow within three identified subpopulations through stepping stone habitats (Thapa et al., 2018). These results confirmed, corridors are facilitating the functional connectivity to maintain tiger metapopulations in TAL. Therefore, the careful management of these corridors is the uttermost important management actions to maintain the overall integrity of tiger population in TAL.

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Chapter 3:

Assessment of landscape connectivity across the Terai-Arc landscape, India

4.1 Introduction:

Habitat loss due to burgeoning anthropogenic pressures is one of the major factor for drastic decline in tiger's population size and distribution range globally (Wolf and Ripple, 2017). Contrasting, in India tiger population is increasing since past decade within the remaining habitats, especially in the isolated protected areas, indicating site-specific management success (Jhala et al., 2020). Currently, India retains ~80% of the global tiger population (Goodrich et al., 2015) within the 8-25% of the remaining global tiger habitat (Jhala et al., 2020). These tiger habitats primarily reside in Shivalik hills and Gangetic plains or Terai-Arc landscape (TAL), central Indian and Eastern Ghats, Western Ghats, north east hills and Brahmaputra plains and Sundarbans landscapes (Jhala et al., 2020). However, the long-term persistence of these isolated populations depends on maintaining gene flow among those isolated protected areas through dispersal corridors at a landscape level and retain ecologically, demographically and genetically viable populations.

Corridors mostly reside outside protected areas and often faced severe habitat loss due to increasing human density, rapid urbanisation, expanding agriculture, aggressive infrastructure development and economic growth (Gubbi et al., 2012; Mondal and Nagendra, 2011). Despite severe habitat loss studies have identified such corridors still facilitating gene flow in central Indian (Joshi et al., 2013; Sharma et al., 2013; Thatte et al., 2018; Yumnam et al., 2014) and Western Ghats landscapes (Reddy et al., 2019). Terai-Arc landscape (TAL) which retains 22% of the current Indian tiger population (Jhala et al., 2020) and also maintain source sink dynamics

(Chapter 2) still, no such effort was taken to understand the relationship between corridor connectivity and gene flow and poorly understood.

Terai-Arc landscape (TAL) is one of the last remaining prime tiger habitats along the foothills of Himalayas, covering 28000 sq. km. of forested habitat in the northern and southern parts of India and Nepal respectively. The Indian TAL is 900 km long and 50 km wide linear landscape, starts from river Yamuna in the west to Valmiki Tiger Reserve in the east covering 15000 sq. km. of tiger habitat in the states of Haryana, Himachal Pradesh, Uttarakhand, Uttar Pradesh and Bihar (Figure 4.1). Haryana and Himachal Pradesh lost their tiger population where Uttarakhand state holds the third largest tiger population and Uttar Pradesh and Bihar hold moderate tiger populations across India (Jhala et al., 2020). Terai (or low land) majorly contains highly fertile alluvial clay which comes from the Himalayas through the river networks and supports one of the finest grassland habitats (Johnsingh et al., 2004). After the eradication of malaria, this fertile land also attracted millions of human beings in the last century for settlements, which resulted into the conversion of grassland into the agricultural field, thus accelerated the habitat fragmentation (Johnsingh et al., 2004; Smith et al., 1998). The earlier study divided this landscape into nine Tiger Habitat Blocks (THBs) based on habitat fragmentation and disturbance (Johnsingh et al., 2004) (Figure 4.1). Johnsingh et al., (2004) confirmed poor tiger occurrence all across the THB I, which covers Simbalbara National Park of Himachal Pradesh, Kalesar Wildlife Sanctuary of Haryana, Shivalik Forest Division of Uttar Pradesh and Kalsi and Narendranagar Forest Divisions and western part of Rajaji Tiger Reserve of Uttarakhand (Figure 4.1). THB II starting from east of river Ganga to

west of river Gola holds one of the finest habitats as well as tiger populations, which includes Corbett and eastern parts of Rajaji Tiger Reserves, Lansdowne, Haridwar Ramnagar, Terai West, Terai Central Forest Divisions of Uttarakhand and Amangarh Tiger Reserve and Najibabad Social Forestry Division of Uttar Pradesh. In THB II, Corbett Tiger Reserve alone holds the highest tiger density (14/100 sq. km.) across all the remaining wild populations of all the sub-species (Bisht et al., 2019). Areas between east of river Gola to the west of river Sharda, include Haldwani, Terai East and Champawat Forest Divisions of Uttarakhand form the THB III (Figure 4.1). Despite of fragmented habitats tiger populations of THB I, II and III together formed first genetic subpopulation or Tiger Genetic Block I (TGB I) and confirmed uninterrupted gene flow (Table 4.1) (also see Chapters 1 and 2 for more details). One range (Surai) of Terai East Forest Division of Uttarakhand and entire Pilibhit Tiger Reserve and Kishanpur Wildlife Sanctuary (part of Dudhwa Tiger Reserve) of Uttar Pradesh form the THB IV which remain connected with Shuklaphanta Wildlife Reserve of Nepal through Lagga bagga corridor (Thapa et al., 2017) (Figure 4.1). Entire Dudhwa National Park (part of Dudhwa Tiger Reserve) and the adjoining buffer areas form the THB V (Figure 4.1). Katerniaghat Wildlife Sanctuary (part of Dudhwa Tiger Reserve) alone forms THB VI, where it maintains connectivity with Bardia National Park of Nepal through Khata corridor (Thapa et al., 2017) (Figure 4.1). The entire tiger populations of THB IV, V and VI together formed second genetic subpopulation or TGB II and confirmed uninterrupted gene flow among the THBs (Table 4.1) (also see Chapters 1 and 2 for more details). Further isolated forest patches of Suhelwa and western part of Sohagibarwa Wildlife Sanctuaries of Uttar Pradesh form the THB VII and VIII respectively, where the tiger population

has deteriorated in the recent past (Jhala et al., 2020; Johnsingh et al., 2004) (Figure 4.1). Towards the eastern end of the TAL, eastern part of Sohagibarwa Wildlife Sanctuary of Uttar Pradesh and the Valmiki Tiger Reserve (only tiger reserve of Bihar), form the THB IX. This is an isolated tiger population in India but fortunately has connectivity with Chitwan National Park and Parsa Wildlife Reserve of Nepal (Thapa et al., 2017), which helped Valmiki Tiger Reserve to recover its' tiger population recently (Jhala et al., 2020). Finally, THB VIII and IX together formed third genetic subpopulation or TGB III and confirmed constant gene flow (Table 4.1) (also see Chapter 1 and 2 for more details).

Table 4.1: Information of tiger genetic blocks, including habitat block details, protected and non-protected areas

SL No	**THB**	**PA, FD, SFD and Corridors**	**TGB**
1	THB I	PA: Simbalbara NP	
		PA: Kalesar WLS	
		FD: Shivalik FD	
		PA: Rajaji TR (western part)	TGB I
		FD: Dehradun FD	
		Corridor: Kansrau-Barkot, Chilla-Motichur	TGB I
2	THB II	PA: Amangarh TR	

		SFD: Najibabad SFD	
		PA: Rajaji TR (eastern part), Jhilmil Jheel CR, Corbett TR, Pawalgarh CR	
		FD: Haridwar FD, Lansdowne FD, Terai West FD, Ramnagar FD, Terai Central FD	
3	THB III	PA: Nandhaur WLS	
		FD: Haldwani FD, Champawat FD, Terai East FD	
		SFD: Pilibhit SFD	
4	THB IV	PA: Pilibhit TR, Kishanpur WLS (part of Dudhwa TR)	
		FD: North Kheri FD, South Kheri FD	TGB II
		SFD: Shahjahanpur SFD	
5	THB V	PA: Dudhwa NP (part of Dudhwa TR)	TGB II
6	THB VI	PA: Katerniaghat WLS (part of Dudhwa TR)	
7	THB VII	PA: Suhelwa WLS	
8	THB VIII	PA: Sohagibarwa WLS (western part)	TGB III
9	THB XI	PA: Sohagibarwa WLS (eastern part)	

PA: Valmiki TR

THB- Tiger habitat block, PA- Protected area, FD- Forest division, SFD- Social forestry division, TGB- Tiger genetic block, NP- National park, WLS- Wildlife sanctuary, TR- Tiger reserve, CR- Conservation reserve.

Results obtained from Chapter 1 and 2 strongly suggested the tiger populations of TAL still maintained regional gene flow however, restricted at a landscape scale. Earlier study also described 13 structural corridors, which have the potential to facilitate tiger dispersal between these fragmented THBs (Figure 4.1). Despite several anthropogenic impacts including habitat fragmentation, prey depletion, poaching and grazing pressure this landscape still holds ~650 adult tigers (Jhala et al., 2020). Active efforts to manage regional dispersal corridors and forests outside reserve boundaries and the transboundary connectivity with Nepal have helped to recover tiger populations in this landscape in the recent past (Chanchani et al., 2016; Jhala et al., 2020). These tiger populations primarily restricted within its 10 protected area's boundaries but significant populations are outside of these protected areas (Chapter 1; Jhala et al., 2020) (Figure 2.4). The future survival of the tiger populations in TAL depends on keeping the corridors functional for tiger dispersal and maintain more than one metapopulation. However, to date, no effort has been made to assess the functionality of these dispersal corridors across TAL, and a detailed understanding of the habitat connectivity and the source-sink dynamics is critical for this landscape. Additionally, identify the landscape features which hinder and facilitate tiger dispersal to maintain connectivity and the source-sink dynamics in this landscape also crucial to investigate. The future persistence of tigers in TAL will

depend on the balance between the developmental demands and conservation requirements. This chapter focuses to 1) identify landscape features which hinder or facilitate tiger dispersal in this landscape, 2) identify least-cost pathways (LCPs), 3) measure the corridor conductance, 4) model the critical corridors of tiger dispersal among various tiger habitats and finally, 5) assess the genetic exchange as a measure of functionality of the 13 known corridors across TAL using field sampling, non-invasive genetic approaches, remote sensing (RS) and geographical information system (GIS) tools. These multidisciplinary approaches identified the current status of corridor functionality to guide appropriate management decisions to maintain source-sink dynamics, which will ensure the long-term sustenance of tigers in this fragmented and human-modified landscape.

4.2 Methodology:

4.2.1 Corridor connectivity analyses:

Results of Chapter 1 and 2 strongly suggest the tiger populations of TAL is structured (Figure 2.5 and 2.6 in Chapter 1) and functional connectivity is restricted at landscape scale, however, still maintain regional connectivity (Tables 3.1, 3.2, 3.3 and Figures 3.2, 3.3, 3.4 in Chapter 2). Existing dispersal corridors facilitate such regional functional tiger connectivity within three TGBs (Figures 3.2, 3.3, 3.4 in Chapter 2). To understand habitat connectivity corridors were modelled between

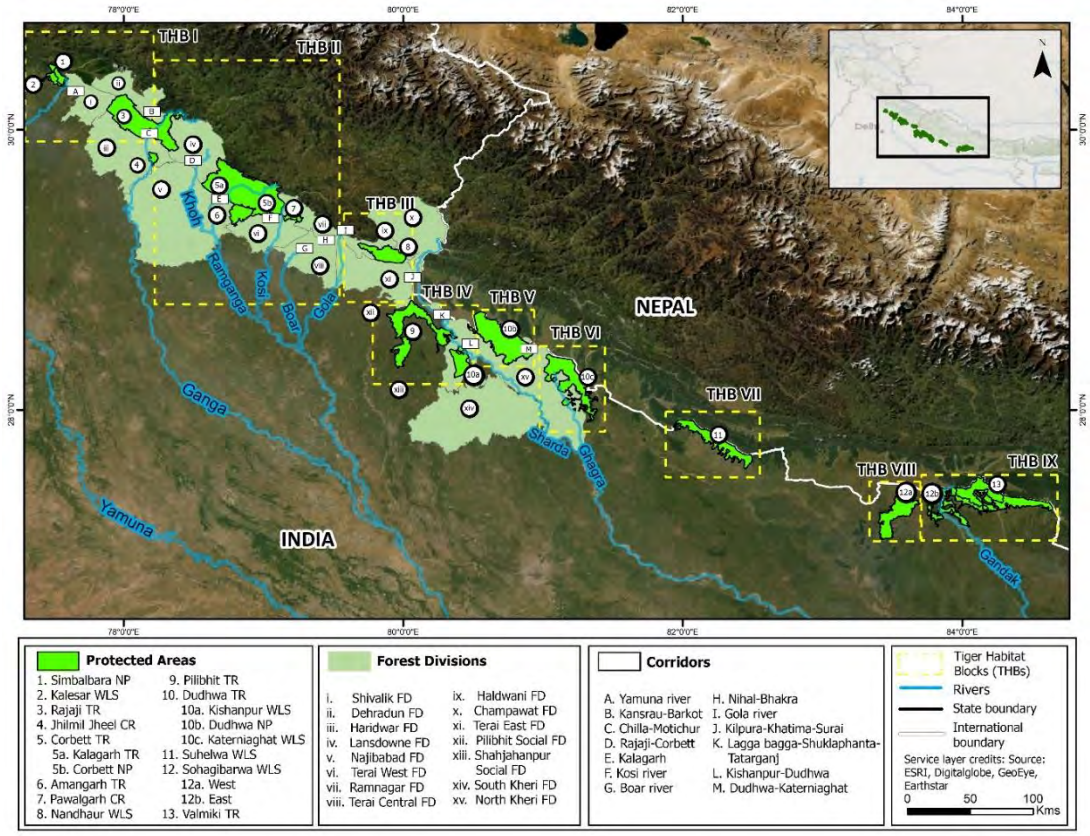

Figure 4.1: Detail description of the Indian part of the Terai-Arc landscape. The forested habitats are fragmented and formed nine major blocks and connected through 13 tiger dispersal corridors

protected areas using genetically confirmed tiger presence points (Figure 2.3) and environmental variables (Table 4.2) in Circuitscape v.4.0 (McRae et al., 2016) across TAL. Further, corridor bottlenecks were identified using Pinchpoint mapper and finally, least-cost pathways were modelled using Linkage mapper toolbox V2.0 in ArcGIS 10.7 (www.esri.com).

Habitat permeability layer was developed to understand potential tiger connectivity in TAL, using the maximum entropy modelling approach in the program MaxEnt v 3.4.1 (Philips and Dudı´k, 2008). Habitat permeability layer and focal nodes (regions between which connectivity is to be modelled) are the primary input data for

connectivity analyses, indicating the difficulty experienced by an individual in moving across a landscape (McRae et al., 2016). Tiger presence points and environmental variables were used in MaxEnt v 3.4.1 (Philips and Dudı´k, 2008) to develop a habitat permeability layer, where each pixel is a proxy of the likelihood that individuals will move through that cell (i.e. conductance). After removing the spatial cluster between genetically confirmed tiger point locations (total 743 tiger presence points were confirmed in Chapter 1), a total 465 presence points along with five environmental variables were used based on tiger's ecology and habitat requirements, viz. distance to forest cover, protected areas, water, road, and settlements (Table 4.2). For running the model, the presence points were splited as training (70%) and testing (30%) during cross-validate run with 10 replicates and rest of the settings were kept as default. The average of all outputs (n=10 runs) were used as the habitat permeability layer for Circuitscape. Subsequently, export to Circuitscape (http://www.jennessent.com/arcgis/Circuitscape_Exp.htm) tool in ArcGIS 10.7 was used to export the focal nodes and habitat permeability layer to ASCII raster layers of equal extents, cell sizes, and spatial references.

A total of 21 focal nodes (primarily protected areas) (Table 4.3) and habitat permeability layer were used in Circuitscape v.4.0 (McRae et al., 2016) to measure the corridor conductance across TAL. Weightages were provided to each of the focal nodes based on tiger source-sink populations as identified in the Chapter 2 (Table 3.1, 3.2, 3.3 and Figure 3.2, 3.3, 3.4). The node weightages were set in four categories that ranged from 0 (nodes with no tiger presence), 0.25 (nodes that act as sink populations), 0.50 and 0.75 (nodes those are moderate-level source populations)

and 1 (nodes those are primary source populations) (Table 4.3) during analyses. Subsequently, based on conductance value the output corridors were classified into three categories: low functioning (0-0.30), medium functioning (0.31-0.70) and high functioning (0.71-1.0).

To identify the corridor bottlenecks, 'Pinchpoint mapper' in Linkage mapper toolbox V2.0 were used in ArcGIS 10.7. The habitat permeability layer was converted into a resistance surface using SDM toolbox (Brown, 2014) in ArcGIS 10.7 (www.esri.com) for running the analysis. Finally, the least-cost pathways (LCPs) were modelled for tiger dispersal using Linkage mapper toolbox V2.0 in ArcGIS 10.7.

Table 4.2: Environmental covariates used in this study to generate habitat permeability map

Source file	**Covariates used**	**Source**	**Unit**
Forest cover	Distance from forest	Forest Survey of India	Meter
Protected area	Distance from protected areas	World database of protected areas	Meter
Road network	Distance from road	Diva-GIS	Meter
Settlement	Distance from settlement	Columbia-Village data	Meter
River network	Distance from river	Diva-GIS	Meter

Table 4.3: Details (name and weightage) of nodes used in Circuitscape analysis to measure the corridor conductance across the Indian part of Terai-Arc landscape

SL	Name of the node	Protection status of nodes	Weightage (0-1)
1	Kalesar-Simbalbara complex	Protected area	0
2	Rajaji TR-western part	Protected area	0.50
3	Rajaji TR-eastern part	Protected area	1
4	Jhilmil Jheel CR	Protected area	0.25
5	Najibabad SFD	Non-protected area	0.25
6	Corbett TR	Protected area	1
7	Pawalgarh CR	Protected area	1
8	Nandhaur WLS	Protected area	0.75
9	Pilibhit SFD	Non-protected area	0.25
10	Pilibhit TR	Protected area	0.75
11	Pilibhit TR-southern part	Protected area	0.25
12	Kishanpur WLS	Protected area	0.75
13	Dudhwa NP	Protected area	0.75
14	Katarniaghat WLS	Protected area	0.25

15	Suhelwa WLS	Protected area	0
16	Sohagibarwa WLS-western part	Protected area	0.25
17	Sohagibarwa WLS-eastern part	Protected area	0.75
18	Sohagibarwa WLS-southern part	Protected area	0
19	Valmiki TR-western part	Protected area	0.75
20	Valmiki TR-central part	Protected area	0.75
21	Valmiki TR-eastern part	Protected area	0.75

TR=-Tiger Reserve, CR- Conservation Reserve, SFD- Social Forestry Division, WLS- Wildlife Sanctuary, NP- National Park

4.3 Results:

4.3.1 Corridor connectivity across TAL

Habitat permeability analyses indicated certain habitat variables such as distance to forest cover and protected areas are the main governing factors of tiger dispersal (Table 4.4). Out of the 13 earlier-described corridors (Johnsingh et al., 2004), twelve showed conductance for tiger dispersal (Table 4.5 and Figure 4.2).

Table 4.4: Habitat permeability analysis quantified the contribution of habitat variables in tiger dispersal in the fragmented habitats of the Indian part of Terai-Arc landscape

Environmental variables	**Contribution (%)**

Distance from forest cover	66.8
Distance from protected areas	23.8
Distance from settlements	4.6
Distance from river network	3.3
Distance from road network	1.5

In the western end of TAL, corridor connecting Simbalbara-Kalesar complex with western part of Rajaji Tiger Reserve (Yamuna river corridor) did not show any signatures of corridor conductance (Table 4.5 and Figure 4.2). However, the remaining corridors showed high, medium and low conductances (Table 4.5 and Figure 4.2). Corridors connecting, Chilla range of eastern Rajaji Tiger Reserve with Motichur range of western Rajaji Tiger Reserve (Chilla-Motichur corridor), eastern Rajaji Tiger Reserve with Corbett Tiger Reserve (Rajaji-Corbett), Corbett Tiger Reserve with Pawalgarh Conservation Reserve (Kosi river corridor) showed high conductances, corridor connecting Nandhaur Wildlife Sanctuary with Pilibhit Tiger Reserve (Kilpura-Khatima-Surai corridor) showed medium conductance and corridors connecting Kansrau range of western Rajaji Tiger Reserve with Barkot range of Dehradun Forest Division (Kansrau-Barkot corridor), Terai West Forest Division with Terai Central Forest Division (Boar river corridor), Terai Central Forest Division with Ramnagar Forest Division (Nihal-Bhakra corridor) and Pawalgarh Conservation Reserve with Nandhaur Wildlife Sanctuary (Gola river

corridor) showed low conductances of tiger connectivity in TGB I (Table 4.5 and Figure 4.2). Similarly, in TGB II, corridors connecting Kishanpur Wildlife Sanctuary with Dudhwa National Park (Kishanpur-Dudhwa corridor) and Dudhwa National Park with Katarniaghat Wildlife Sanctuary (Dudhwa-Katarniaghat corridor) showed medium conducntances and corridor connecting Pilibhit Tiger Reserve with Dudhwa National Park (Lagga bagga-Shuklaphanta-Tatarganj corridor) showed low conductance of tiger connectivity (Table 4.5 and Figure 4.2).

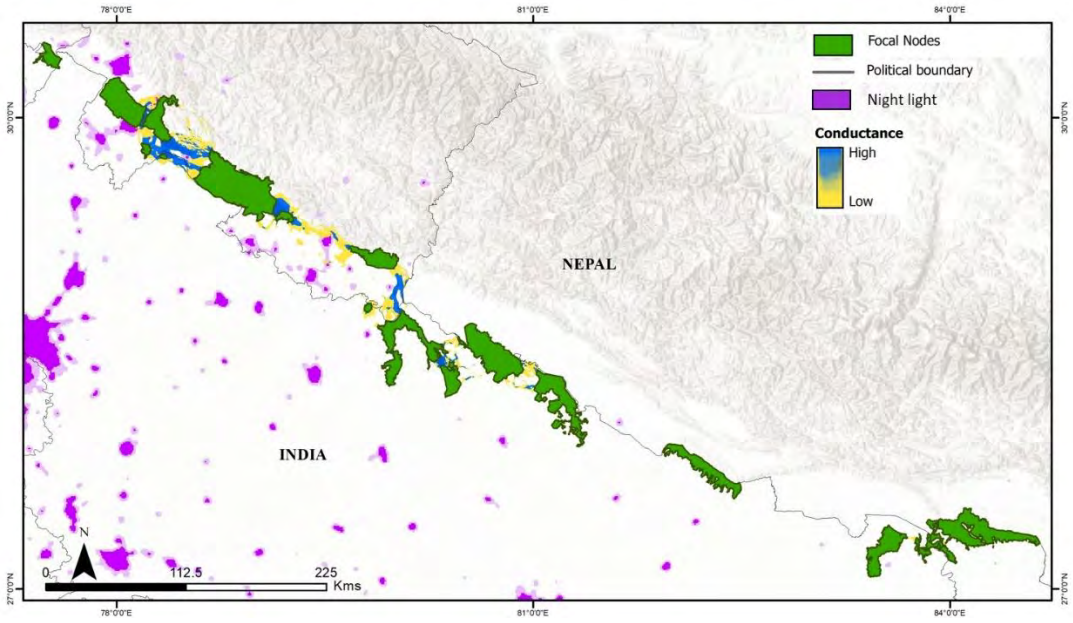

Figure 4.2: Corridor conductance of tiger dispersal was modelled using Circuitscape software in the fragmented habitats of the Indian part of Terai-Arc landscape

In addition, seven new tiger dispersal corridors were identified in TAL. The first corridor (high conductance) connects eastern Rajaji Tiger Reserve with Najibabad Social Forestry Division through Lansdowne Forest Division (Table 4.5 and Figure 4.2). The second corridor (high conductance) connects Jhilmil Jheel Conservation Reserve with Najibabad Social Forestry Division through Haridwar Forest Division

(Table 4.5 and Figure 4.2). The third corridor (high conductance) connects Corbett Tiger Reserve with Najibabad Social Forestry Division (Table 4.5 and Figure 4.2) making these areas very well connected with each other within TGB I. In TGB II, two new corridors (high conductance) were identified, where the first one connected the northern and southern part of Pilibhit Tiger Reserve, and the second one connected Pilibhit Tiger Reserve to Kishanpur Wildlife Sanctuary through North Kheri Forest Division, respectively (Table 4.5 and Figure 4.2). Finally, two new corridors were identified in TGB III, the first one (low conductance) connecting western and eastern part of Sohagibarwa Wildlife Sanctuary and the easternmost corridor (high conductance) connecting two parts of Valmiki Tiger Reserve, respectively (Table 4.5 and Figure 4.2).

Table 4.5: Details of least-cost pathways, corridor conductance and critical corridors of tiger dispersal identified across the TAL

SL	Corridors identified between	Length of the Least-cost pathways (km)	Conductance of corridors	Critical corridors
1	Simbalbara-Kaleser complex and Rajaji TR (western part)	41.5	NA	NA
2	Kansrau (western part of Rajaji TR) and Barkot (Dehradun FD)	No	Low	No

3	Chilla (eastern part) and Motichur (western part) ranges of Rajaji TR	3.9	High	Yes
4	Rajaji TR (eastern part) and Jhilmil Jheel CR	9.5	High	No
5	Rajaji TR (eastern part) and Najibabad SFD	9.9	High	No
6	Jhilmil Jheel CR and Najibabad SFD	9.5	High	No
7	Rajaji TR (eastern part) and Corbett TR	36.6	High	Yes
8	Corbett TR and Najibabad SFD	31.2	High	Yes
9	Corbett TR and Pawalgarh CR	1.9	High	Yes
10	Terai West and Terai Central FDs	NA	Low	No
11	Terai Central FD and Ramnagar FDs	NA	Low	No
12	Pawalgarh CR and Nandhaur	53.8	Low	Yes

	WLS			
13	Nandhaur WLS and Pilibhit SFD	32.2	NA	NA
14	Nandhaur WLS and Pilibhit TR	38.6	Medium	Yes
15	Pilibhit SFD and Pilibhit TR	14	NA	NA
16	Northern and southern parts of Pilibhit TR	1.4	High	Yes
17	Pilibhit TR and Kishanpur WLS	5.6	High	Yes
18	Pilibhit TR and Dudhwa NP	14.8	Low	No
19	Kishanpur WLS and Dudhwa NP	16.2	Medium	No
20	Dudhwa NP and Katarniaghat WLS	11.5	Medium	No
21	Katarniaghat WLS and Suhelwa WLS	71.2	NA	NA
22	Suhelwa WLS and west Sohagibarwa WLS	120.3	NA	NA

23	Western and eastern Sohagibarwa WLS	7.4	Low	Yes
24	Western and eastern parts of Valmiki TR	0.5	High	Yes

TR- Tiger Reserve, CR- Conservation Reserve, SFD- Social Forestry Division, WLS- Wildlife Sanctuary, NP- National Park

The corridor bottleneck analysis identified 10 critical tiger dispersal corridors in TAL (Table 4.5 and Figure 4.3). Critical corridors were identified in seven high conductance corridors (connecting Chilla range of eastern Rajaji Tiger Reserve with Motichur range of western Rajaji Tiger Reserve, Rajaji Tiger Reserve with Corbett Tiger Reserve, Corbett Tiger Reserve with Pawalgarh Conservation Reserve, Corbett Tiger Reserve with Najibabad Social Forestry Division, northern part of Pilibhit Tiger Reserve with southern part of Pilibhit Tiger Reserve, Pilibhit Tiger Reserve with Kishanpur Wildlife Sanctuary, western part of Valmiki Tiger Reserve and eastern part of Valmiki Tiger Reserve), one medium (connecting Nandhaur Wildlife Sanctuary with Pilibhit Tiger Reserve) and two low conductance corridors (connecting Pawalgarh Conservation Reserve with Nandhaur Wildlife Sanctuary and western Sohagibarwa Wildlife Sanctuary with eastern Sohagibarwa Wildlife Sanctuary) across TAL (Table 4.5 and Figure 4.3).

Finally, 21 least-cost pathways (LCPs) were identified between 21 focal nodes used (including 13 protected and two non-protected areas) across TAL (Table 4.5 and Figure 4.4). All the 13 protected and two non-protected areas confirmed corridor connectivity for tiger dispersal. Further, the longest LCP of 120 km was measured

between Suhelwa and Sohagibarwa Wildlife Sanctuaries and shortest LCP of 0.5 km was measured between western and eastern parts of Valmiki Tiger Reserve in the eastern TAL (Table 4.5 and Figure 4.4).

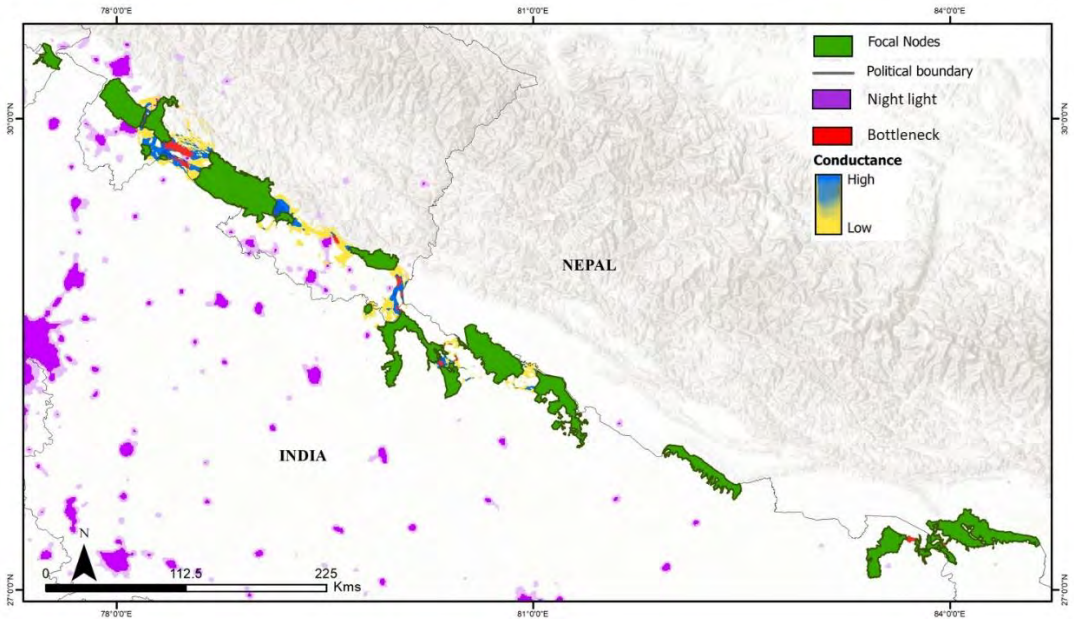

Figure 4.3: Bottlenecks were identified within 10 conductance corridors using Linkage mapper toolbox in ArcGIS

4.4 Discussion:

The Circuitscape analyses provided strong support and possible explanations to the genetic connectivity patterns observed in this landscape (see Chapter 2). The Circuitscape analyses overall identified a total of 10 high, three medium and six low conductance tiger corridors across TAL (Table 4.5 and Figure 4.2), all of them are outside protected areas (Figure 4.2). Despite of high anthropogenic pressures in non-protected areas, 15 of the 19 identified tiger corridors still facilitate genetic connecitivity and maintain source sink dynamics of tigers (Table 3.1, 3.2, 3.3 and

Figure 3.2, 3.3, 3.4 in Chapter 2) and further, identified as functional corridors, whereas the remaining four corridors were identified as non-functional in TAL.

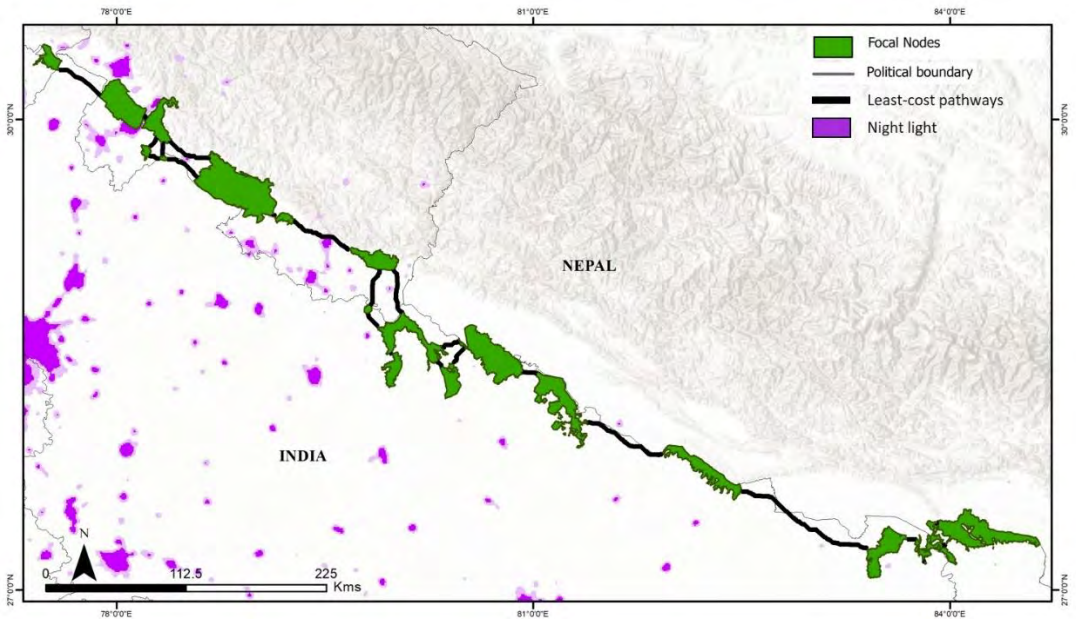

Figure 4.4: 21 least-cost pathways were modelled for tiger dispersal using Linkage mapper toolbox in ArcGIS across 21 focal nodes

In TGB I, nine corridors were identified as functional corridors and facilitate tiger source-sink dynamics. These corridors connect, eastern part of Rajaji Tiger Reserve with Jhilmil Jheel Conservation Reserve and Najibabad Social Forestry Division, Jhilmil Jheel Conservation Reserve with Najibabad Social Forestry Division, Rajaji Tiger Reserve with Corbett Tiger Reserve, Corbett Tiger Reserve with Najibabad Social Forestry Division and Pawalgarh Conservation Reserve, Terai West Forest Division with Terai Central Forest Division, Terai Central Forest Division with Ramnagar Forest Division and Pawalgarh Conservation Reserve with Nandhaur Wildlife Sanctuary (Table 4.5 and Figure 4.2). Similarly, in TGB II four functional corridors were identified which connect, Pilibhit Tiger Reserve with Kishapur

Wildlife Sanctuary and Dudhwa National Park, Kishapur Wildlife Sanctuary with Dudhwa National Park and Dudhwa National Park with Katarniaghat Wildlife Sanctuary. Further, in TGB III, two functional corridors were identified which connects, western part of Sohagibarwa Wildlife Sanctuary with eastern part of Sohagibarwa Wildlife Sanctuary and western part of Valmiki Tiger Reserve with eastern part of Valmiki Tiger Reserve.

Despite medium conductance, first genetic discontinuity was identified between TGB I and II is possibly due to loss of functionality of the Kilpura-Khatima-Surai corridor (marked as corridor J in Figure 4.1) (Johnsingh et al., 2004). This corridor structurally connects the Champawat-Haldwani-Terai East Forest Divisions complex of TGB I with Pilibhit-Dudhwa Tiger Reserves complex of TGB II but currently dysfunctional (Figure 4.1). Earlier Johnsingh et al., (2004) reported no tiger use in this corridor, which has been further confirmed by Jhala et al., (2020). The loss of connectivity between TGB I and II is possibly resulting from developmental activities (e.g. highway, railway tracks, canal etc.) and habitat loss (from human settlements and urbanization etc.) along the corridor (Figure 4.2). A similar pattern of restricted gene flow was also identified in the central Indian landscape due to anthropogenic activities through high conductance corridors (Joshi et al., 2013; Yumnam et al., 2014). Some recent evidences suggested tiger dispersal events in the Kilpura-Khatima-Surai corridor (Anwar and Borah, 2020) indicating hope for this corridor but strong management interventions are required to maintain its functionality and ensure one panmictic population between Rajaji Tiger Reserve in the western TAL to Dudhwa Tiger Reserve in the central TAL (Figure 4.2). Next, the

high conductance Chilla-Motichur (marked as corridor C in Figure 4.1) and low conductance Kansrau-Barkot (marked as corridor B in Figure 4.1) corridors that connect eastern and western parts of Rajaji Tiger Reserve and western parts of Rajaji Tiger Reserve and Dehradun Forest Division respectively, were identified as a weak connecting links (Figure 4.2) (Jhala et al., 2020; Johnsingh et al., 2004). Despite showing corridor conductances in Circuitscape analysis there is no functional connectivity through these corridors, possibly due to intense anthropogenic activities and low tiger presence (Harihar et al., 2018; Harihar and Pandav, 2012; Johnsingh et al., 2004). Further, within the TGB I the southern part of Gola river corridor (marked as corridor I in Figure 4.1) connecting Ramnagar-Terai Central Forest Divisions complex with Champawat-Haldwani-Terai East Forest Divisions complex was identified as a weak corridor (Figure 4.2). While the northern part of this corridor showed medium conductance, the southern part is heavily affected by anthropogenic activities like human settlements, urbanization, industrialization and large-scale boulder mining (Johnsingh et al., 2004; Qureshi et al., 2014) leading to low genetic exchange across it (Figure 3.2). Finally, in TGB II, corridor connecting northern and southern parts of Pilibhit Tiger Reserve identified as non functional. Despite showing high conductances in Circuitscape analysis no functional connectivity was measured through this corridor, possibly due to high anthropogenic activities.

Further, one of the most important outputs of this study is the identification of seven critical bottlenecks distributed across the functional tiger corridors (Figure 4.3). Due to the linear geography of the TAL where only one-directional movements are feasible, these corridor bottlenecks demand immediate conservation attention. Except

these identified regions analyses could not find any other alternative paths (high Himalayan area in north and human-dominated Terai flats in south) and thus maintaining functional connectivity through these identified corridors are the only way these tiger populations of TAL could maintain genetic connectivity and metapopulation in near future (Qureshi et al., 2014).

4.5 References:

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Conclusion

5.1 Conclusion

Due to growing anthropogenic pressures in the form of habitat and prey depletion, poaching and human-animal conflicts tiger populations faced ~90% decline in the past ~200 years and restricted within ~7% of their global range (Schipper et al., 2008; Cardillo et al., 2006; Dinerstein et al., 2007; Morell, 2007; Sanderson et al., 2006)**.** However, in India tiger population is increasing since past decade within the remaining habitats, especially in the isolated protected areas, indicating site-specific management success (Jhala et al., 2020). Currently, India retains ~80% of the global tiger population (Goodrich et al., 2015) within the 8-25% of the remaining global tiger habitat (Jhala et al., 2020) and the survival of the remaning populations depend on future landscape-level management plans involving the protection of source sites and maintaining connectivity with adjoining sink habitats (Sanderson et al., 2006). In India majorly protected areas act as primary source habitats where sink habitats mostly are non-protected areas (Sanderson et al., 2006). Several priority landscapes were identified as 'Tiger Conservation Landscapes' (TCLs) (for example, Western Ghats, central India, north-eastern India, Sundarbans and the Terai-Arc landscape in the Himalayan foothills) (Sanderson et al., 2006)**,** where identification of key source populations, consolidation and improvement of surrounding sink habitats and ensuring habitat connectivity within these landscapes were emphasized (Gubbi et al., 2017, 2016; Joshi et al., 2016). In this thesis, the source-sink population dynamics of current tiger populations of the protected areas, non-protected areas and corridor areas of TAL were studied using field surveys and non-invasive genetic tools and habitat connectivity between protected areas through non-protected areas was

identified using RS-GIS modelling. More specifically, spatial distribution, genetic diversity, population structure, demographic changes (Chapter 1), source and sink populations (Chapter 2) and the habitat connecitivty and landscape features which help to retain such population dynamics (Chapter 3) were investigated**.**

In Chapter 1, spatial distribution, genetic diversity, population structure, demographic changes of tigers were investigated using filed surveyes and non-invasive genetic tools. Results identified 219 individual tigers which cover ~34% of the total tiger population in TAL (Jhala et al., 2020) (Figure 3.4). Tiger presence was confirmed in 10 protected areas, nine forest divisions and two social forestry divisions covering previously known and few unreported areas (Figure 3.3). Additionally, results confirmed that both male and female tigers were using protected and non-protected habitats across TAL. The analyses measured the moderate level of genetic diversity in the remaining tiger populations (Table 3.3) and the presence of three genetic subpopulations (named as Tiger Genetic Blocks or TGBs), largely correspond to the western, central and eastern parts of TAL (Figures 3.5 and 3.6). Further, demographic analyses strongly suggested a severe tiger population decline of ~88% over the past ~160 years in western TAL (Table 3.6 and Figure 3.7).

In Chaper 2, tiger source-sink populations were identified within three TGBs using genetic tools. Results identified four source and seven sink populaions and highest functional connectivity was measured among majority of the protected and other surrounding non-protected areas in TGB I (Table 4.2 and Figure 4.2). Similarly, in TGB II, two source and two sink populations were identified and moderate functional connectivity was measured among two protected areas and other

surrounding non-protected areas (Table 4.3 and Figure 4.3). In TGB III, poorest functional connectivity was found among two source and one sink populations of tiger (Figure 4.4).

Finally, in Chapter 3, landscape connectivity was modelled using RS-GIS analyses. Results identified environmental variable such as distance to protected areas is the major limiting factors for tiger distribution in TAL. Further, modelling identified 19 tiger dispersal corridors and out of those, 10 showed high, three showed medium and six showed low conductance of tiger connectivity across TAL (Table 5.5 and Figure 5.2). Despite of high anthropogenic pressures in non-protected areas, 15 of the 19 identified tiger corridors still facilitate genetic connecitivity to maintain source-sink dynamics of tiger populations (Table 4.1, 4.2, 4.3 and Figure 4.2, 4.3, 4.4, 5.2) and further, identified as functional corridors, whereas the remaining four corridors were identified as non-functional in TAL. Further, results confirmed critical bottlenecks distributed across the seven identified functional tiger corridors across TAL **(**Figure 5.3**).**

Considering the linear shape of tiger habitats in TAL, fragmentation has always been a major concern for tiger conservation (Johnsingh et al., 2004; Kanagaraj et al., 2011). Maintaining the integrity of this landscape with very high human density and associated linear developments will remain the most important challenge for long-term persistence of tiger in this globally high priority tiger conservation landscape (Johnsingh et al., 2004). The results on tiger population structure, demography, source-sink dynamics and corridor connectivity thus have critical management implications especially in the backdrop of recent increase in tiger numbers in TAL.

One of the encouraging point is the revealing of only three Tiger Genetic Blocks (TGBs) (Figure 3.5, 3.6) compared to the nine distinct Tiger Habitat Blocks (THBs) (Figure 4.1) described by Johnsingh et al., (2004), indicating considerable amount of genetic exchange within these THBs through 15 identified functional corridors (Figure 4.2, 4.3, 4.4, 5.2). Tiger populations of THB I, II and III together formed TGB I, corfirmed functional connectivity and further suggested that the complex, undulating forested Shivalik and Bhabhar habitat of nine functional corridors has helped in tiger dispersal (Johnsingh et al., 2004), therefore crucial to maintain connectivity. Similarly, functional connecitivty was also confirmed between tiger populations of THB IV, V and VI through four functional corridors and formed TGB II. Tiger population of THB VIII and IX confirmed functional connecitivity through two functional corridors and together formed TGB III. Therefore, these 15 functional corridors were playing a crucial role to maintain tiger connectivity. However, seven of the functional corridors were identified as critical corridors within the three TGBs (Table 5.4 and Figure 5.3) that require urgent attention failing which integrity of TAL will be severely compromised. Further analyses showed that a total of 2707.54 sq. km. habitat as conservation priority area (Table 6.1 and Figure 6.1) and needs appropriate conservation planning to ensure panmictic tiger populations in TAL. Where Uttarakhand and Uttar Pradesh states together hold >99% of the total conservation priority area (Table 6.1 and Figure 6.1). Therefore, appropriate mitigation measures associated with ongoing linear infrastructure developments, roads in particular, are a must in this highly sensitive conservation landscape especially in the Uttarakhand and Uttar Pradesh states (Qureshi et al., 2014). In addition, TGB II and III require transboundary cooperation between India and Nepal

to ensure maintenance of genetic variation and source-sink dynamics in this population.

Another potentially important upcoming tiger conservation challenge in the form of increasing human-tiger interactions was also anticipated. The results of Chapter 1, showed that a significant proportion of both male and female tigers in TAL are found outside protected areas along with few previously unreported non-protected areas (10 forest divisions and three social forestry divisions) (Figure 3.3 and 3.4) (possibly driven by recent increase in tiger population). Further, the combine results of Chapter 2 and 3 strongly suggested that most of the identified sink populations and functional corridors fall outside the protected areas (Figure 4.2, 4.3, 4.4, 5.2), co-existing with significant amount of human density and associated livestock (Jhala et al., 2020). It is likely that the incidences of human-tiger conflict will increase around the sink habitats and corridors in the coming years and active management of such conflict situations will be critical for these tigers living in the marginalized habitats. Further, adequate attention towards habitat and prey management in the forest divisions and social forestry divisions where large number of tigers are found (at least in TGB I and II) will play important role in maintaining connectivity of the entire landscape.

India played a leading role in tiger conservation and has achieved a rare global success in population recovery of a large-bodied, apex carnivore (Jhala et al., 2020). However, the future of these tiger populations depend on appropriate management of the ever-shrinking habitats and maintaining the existing populations as metapopulations. The results from TAL showed the functionality of the existing corridors and point out critical areas where immediate conservation attention is

needed. A focused approach to address such concerns will improve the long-term sustainability of the tiger populations in TAL.

Table 5.1:Details of the critical conservation priority areas identified in this study

SL	Geographic location	Area (sq.km.)	State
1	Connecting western and eastern parts of Rajaji TR (northern block)	9.73	Uttarakhand
2	Connecting western and eastern parts of Rajaji TR (central block)	14.12	Uttarakhand
3	Connecting western and eastern parts of Rajaji TR (southern block)	9.74	Uttarakhand
4	Connecting eastern Rajaji TR, Jhilmil Jheel CR and Najibabad SFD through Haridwar and Lansdowne FDs	345.07	Uttarakhand and Uttar Pradesh
5	Connecting Corbett TR and Najibabad SFD through Lansdowne FD and Najibabad SFD	267.16	Uttarakhand and Uttar Pradesh
6	Connecting Rajaji and Corbett TRs through Lansdowne FD	328.32	Uttarakhand
7	Connecting Corbett TR and Pawalgarh CR	53.54	Uttarakhand

	through Terai West FD		
8	Connecting Corbett TR and Pawalgarh CR through Ramnagar FD	84.66	Uttarakhand
9	Connecting Pawalgarh CR and Nandhaur WLS through Ramnagar, Haldwani and Terai East FDs	405.37	Uttarakhand
10	Connecting Nandhaur WLS and Pilibhit TR through Haldwani and Terai East FDs and Pilibhit SFD	828.77	Uttarakhand and Uttar Pradesh
11	Connecting northern and southern parts of Pilibhit TR	17.4	Uttar Pradesh
12	Connecting Pilibhit TR and Dudhwa NP through North Kheri FD	56.76	Uttar Pradesh
13	Connecting Pilibhit TR and Kishanpur WLS through North Kheri FD	44.88	Uttar Pradesh
14	Connecting Kishanpur WLS and Dudhwa NP through North Kheri FD	34.83	Uttar Pradesh
15	Connecting Dudhwa NP and Katarniaghat WLS through North Kheri FD (northern part)	115.42	Uttar Pradesh
16	Connecting Dudhwa NP and Katarniaghat WLS through North Kheri FD (southern part)	64.46	Uttar Pradesh

17	Connecting western and eastern parts of Sohagibarwa WLS	18.41	Uttar Pradesh
18	Connecting western and eastern parts of Valmiki TR	8.9	Bihar
	Total	**2707.54**	

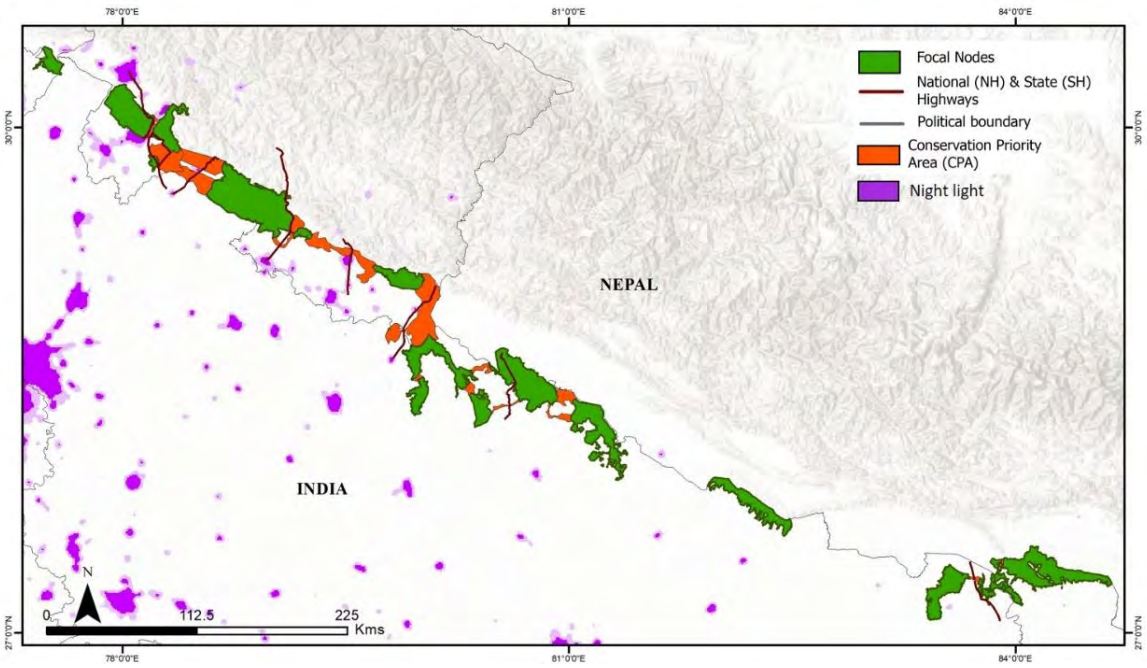

Figure 5.1: Conservation priority areas for the long term persistence of tigers were demarcated all across the Indian part of Terai-Arc landscape

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ANNEXURE- I

Permits and Ethical Clearance:

All required permissions for field survey and faecal sampling were provided by the Forest Departments of Uttarakhand (Permit no: 90/5-6), Uttar Pradesh (Permit no: 1127/23-2-12(G) 105 and 1891/23-2-12) and Bihar (Permit no: Wildlife-589). Due to the non-invasive nature (faecal sample based) of the work, no ethical clearance was required in this study.

ANNEXURE- II

Conference certificates:

NACCB 2020
Participation

THIS CERTIFICATE GOES TO

SUVANKAR BISWAS

for presenting a talk entitled: "Corridor Connectivity Drives Tiger Meta-population Dynamics In The Terai-Arc Landscape, India" in the 2020 North American Congress for Conservation Biology

Karen V. Root

KAREN ROOT

SCB North America President

NACCB 2020

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This is to certify that

Suvankar Biswas

has attended the Student Conference on Conservation Science, 26-28 March 2019,

at the University of Cambridge, Downing Street, Cambridge, UK

and presented a talk entitled

"Metapopulation dynamics of tigers in the Terai Arc landscape, India"

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This is to certify that Mr./~~Ms./Dr~~ SUVANKAR BISWAS participated at the national student conference YETI Baroda 2018 with a/an oral presentation / ~~poster presentation~~ / ~~poster-cum-speed talk~~ titled Understanding the functionality of identified tiger dispersal corridors in the Terai-Arc landscape, India. at The Maharaja Sayajirao University of Baroda, Vadodara from 22nd to 25th January, 2018.

For
Organizing Committee

Head,
Department of Zoology

Dean,
Faculty of Science

A Practive Faeces Collection Protocol for Multidisciplinary Research in Wildlife Science

Research *in* Current Science · June 2019

DOI: 10.18520/cs/v116/i11/1878-1885

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A practive faeces collection protocol for multidisciplinary research in wildlife science

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Faecal samples have become an important non-invasive source of information in wildlife biology and ecological research. Despite regular use of faeces, there is no universal protocol available for faeces collection and storage to answer various questions in wildlife biology. In this study we collected 1408 faeces from ten different species using a dry sampling approach, and achieved 77.49% and 75.25% success rate in mitochondrial and nuclear marker amplifications respectively. We suggest a universal framework to use the same samples to answer different questions. This protocol provides an easy, quick and cheap option to collect non-invasive samples from species living in different environmental conditions to answer multidisciplinary questions in wildlife biology.

Keywords: Non-invasive wildlife research, species biology, dry sampling, variable habitats, field logistics.

NON-INVASIVE samples, especially faeces, have become a regular choice in wildlife biology, population monitoring and ecological research globally. Advantages of faecal sample-based wildlife research include easy collection, access to large sample sizes and spatio-temporal coverage. Historically, large-scale use of faeces in wildlife biology started with dietary analysis of animals[1], but the introduction of advanced molecular tools added a new dimension to non-invasive research. These molecular tools have allowed biologists to examine questions regarding population genetics[2,3], species distribution[4], demography[5,6], evolutionary biology[7] and wildlife forensics[8]. In more recent times, faecal samples have been used to address various questions related to wildlife physiology, including endocrinology and reproductive capacity[9,10], along with parasitology[11,12], disease dynamics[13] and conservation genomics[14]. The sampling and storage demands of various questions in non-invasive wildlife research have led to a gradual development of faecal sampling and storage protocols. Several logistical factors including collector's safety, storage in the field, shipping samples from remote field areas in different environmental conditions, etc. have been considered with a gradual development of these protocols.

Over the years, a number of faeces collection and storage approaches have been used in wildlife research that are broadly classified into three categories: (i) dry sampling (e.g. simple drying[15], silica preservation[16]); (ii) wet sampling (ethanol collection[17], TNE and DMSO buffer[18], DETs solution[19], RNAlater[20]) and (iii) two-step approach[21,22] (Table 1). While all these approaches have been used in wildlife research, they have several logistical limitations making their implementation in the field challenging. For example, sampling with silica beads has advantages in post-collection sample transport and storage[23], but is not cost-effective as it requires large amounts of silica beads to keep the samples moisture-free in humid areas. Similarly, ethanol preservation, the most widely used wet sampling approach is also expensive, requires specific training to collect samples and is often problematic during the shipping of samples from remote areas[21]. Currently no universal sampling protocol is available and limited work has focused on testing faeces sampling and storage protocols to answer different questions in non-invasive wildlife research[17,23,24]. Most of such experiments were conducted on captive animals[15,23,25] or studies were performed under favourable environmental conditions for faecal sampling, where frozen samples were collected from the field[26,27].

Here we describe a simple and cost-effective dry sampling approach for faeces collection and storage that overcomes the above-mentioned limitations and helps in answering different questions in faecal sample-based wildlife research. We followed this sampling approach to collect faecal samples of several carnivore and herbivore species living in different environmental conditions. Following sampling, we conducted molecular species identification and microsatellite amplification to demonstrate the efficacy of this approach for genetic work. Finally we have proposed a universal framework to use the faecal samples for various research purposes. We believe that the simplicity of the approach, ease of sample collection in the field and downstream use of the samples to answer various ecological questions will make this protocol useful in studying cryptic, elusive wild species across different environmental conditions globally.

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Table 1. Details of different faecal sampling protocols and their downstream research use

Sampling approach	Sample collection protocol	Advantages	Disadvantages	Downstream use			
				DNA	Diet	Hormone	Parasite
Wet sampling	Ethanol[17,28,39]	Better amplification success, reduced collector health hazards, easy availability	Expensive, logistical issues during transportation	Yes	Yes	Yes	No
	Queen's College lysis buffer[23]	Cheap ingredients, easy preparation in the laboratory	Logistical issues during transportation	Yes	Yes	No	No
	20% DMSO in TNE buffer[23]	Cheap ingredients, easy preparation in the laboratory	Health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	DETs solution[19]	Cheap ingredients, easy preparation in the laboratory	Health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	RNAlater[20,40]	Easy availability	Expensive, health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	LST buffer[23]	Simple, low cost of preparation	Health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	Formalin[41]	Cheap and easily available	Health hazards to collector, logistical issues during transportation	No	No	No	Yes
Dry sampling	Drierite desiccant[23]	Easy to store, easy transportation	Expensive, required in large quantities	Yes	Yes	Yes	No
	Freeze dry[23]	Easy availability in the laboratory, better amplification success	Difficult to maintain equipment in remote field areas	Yes	Yes	Yes	No
	Oven dry[23]	Easy availability in the laboratory	Difficult to maintain equipment in remote field areas	Yes	Yes	Yes	No
	Silica desiccant[23]	Easy availability, no transport and storage issues	Expensive, required in large quantities	Yes	Yes	Yes	No
	Direct collection[23]	Very cheap, no transport issues, works well for temperate conditions	Not been extensively tested in tropical and subtropical conditions	Yes	Yes	Yes	Yes
Two-step sampling	Dry and wet: ethanol then silica[20,21]	Reduced collector health hazards, easy availability, no transport and storage issues	Expensive, requires a long time to process samples in the field, difficult to implement in remote field areas	Yes	Yes	No	No

Methods

Research permissions

All required permissions for our surveys and collection of biological samples were provided by the Forest Departments of Uttarakhand (Permit no.: 90/5-6 and 978/6-32/56), Uttar Pradesh (UP; Permit no.: 1127/23-2-12(G) and 2233/23-2-12 (G)) and Maharashtra (Permit no.: 09/2016).

Study habitats and species

In this study our focus was to develop a faecal sampling protocol that could be used to answer different ecological questions (DNA, diet, parasite, hormone, etc.) for terrestrial species. To test our protocol, we collected samples from both herbivores (elephants and other wild ungulates) as well as carnivores (both small and large) occupying various habitats ranging from sub-alpine forests of the Lesser Himalayas, dry alpine scrub forests of trans-Himalayas, moist-deciduous forests and swampy grassland of Terai-Arc landscape in northwestern India and dry-deciduous forests of the central Indian landscape. Sampling was conducted during different seasons across the states of Uttarakhand, Uttar Pradesh and Maharashtra, where environmental conditions (ambient temperature, precipitation, humidity, etc.) are varied.

Collection and storage of faecal samples

We adopted a simple, cheap but effective field sampling protocol that involves inexpensive and easily available material. Instead of standard use of absolute ethanol, silica gel, RNA later or other similar approaches, we collected faecal samples in butter paper (wax paper) and stored them individually in sterile zip-lock bags. The samples were stored inside dry, dark boxes in the field till they were transferred to the laboratory (within a maximum time of two months duration in this study). In the laboratory, the samples were stored in –20°C freezers till further processing. All samples were collected with the respective GPS locations and other associated field information. We collected a total of 1408 faecal samples of various carnivore and herbivore species across different habitats between December 2015 and May 2017. During collection, the samples were categorized into their respective groups (large carnivore, small carnivore and herbivore respectively) based on the morphological characteristics (physical characters and signs) in the field. Table 2 provides details of species-wise sample size.

Faecal DNA extraction

To check the DNA quality following this dry sampling approach, we tested two different DNA extraction protocols in the laboratory. Both methods were initially tested with few faecal samples collected from different habitat types before being employed in large-scale sample processing. Our first approach was a slightly modified version of faecal swabbing protocol described in Ball *et al.*[26]. This approach is advantageous over the others as it retains most of the host cells from the top layer and reduces the inhibitors present inside the faecal samples. Frozen faecal samples were thawed at room temperature and the upper layer was swabbed with phosphate buffer saline (PBS) (Sigma-Aldrich, USA) saturated gamma-sterilized cotton applicators (HiMedia, Catalogue no.: PW1136-1x500NO). Each sample was swabbed twice separately and immediately stored in separate 2 ml microcentrifuge tubes in –20°C freezers till further processing. During extraction, 30 µl of proteinase K (20 mg/ml) and 300 µl of ATL buffer (Qiagen Inc., Mississauga, Ontario) were added into each tube containing swab and incubated overnight at 56°C, followed by Qiagen DNAeasy tissue DNA kit extraction protocol. DNA was eluted twice in 100 µl preheated 1× TE buffer. For every set of 22 samples, two extraction negatives were taken to monitor any possible contaminations.

In the second approach, we scraped the top layer of faecal samples with a sterile blade and stored it in 2 ml microcentrifuge tubes for further processing. DNA was extracted using slightly modified QIAamp DNA stool mini kit (Qiagen Inc.) protocol described in Mondol *et al.*[28]. In brief, the scraped faecal layers were lysed overnight at 56°C with a mix of 300 µl of ASL buffer (Qiagen Inc.) and 30 µl of proteinase K (20 mg/ml). Following lysis, the standard stool DNA extraction protocol mentioned in the kit was followed. Final elution was carried out twice with 100 µl preheated 1× TE buffer. All DNA extractions were conducted in an exclusive faecal DNA extraction room.

Molecular data generation

Field-collected non-invasive samples often generate DNA of poor quantity and quality for downstream molecular work[29]. Here we tested the efficacy of sampling and DNA extraction protocols through molecular assignment of species (using mitochondrial DNA markers) and amplification of nuclear DNA (microsatellites) from faecal DNA samples collected in the field during the study.

Species identification (using mitochondrial DNA): We have adopted a number of approaches currently available for assignment of faecal samples to species. These involve both species-specific PCRs as well as sequencing-based methods. Table 2 provides the details of species-specific approaches used for the identification of species. We did not perform molecular species identification for elephants as they were easily identified in the field from their size.

Table 2. Details of faecal sample collection and species-wise success rates in molecular species identification

Targeted order/species	Area/landscape	No. of samples collected	DNA extraction protocol	Species confirmation method	Confirmed species	Species identified samples	Success rate (%)
Large carnivore	Terai-Arc landscape, India	1260	Swab	Species-specific PCR-electrophoresis[42]	Tiger (*Panthera tigris tigris*)	567	75.95
	Terai-Arc landscape, India,		Swab	Species-specific PCR-electrophoresis[42–44]	Leopard (*Panthera pardus fusca*)	259	
	Central Indian landscape		Swab and scrape	Species-specific PCR-electrophoresis[45]	Dhole (*Cuon alpinus*)	126	
	Trans Himalayas		Swab	Carnivore-specific PCR-sequencing[46]	Red fox (*Vulpes vulpes*)	5	
Small carnivore	Lesser Himalayas	21	Swab and scrape	Carnivore-specific PCR-sequencing[46]	Jungle cat (*Felis chaus*)	15	100
			Swab and scrape	Carnivore-specific PCR-sequencing[46]	Leopard cat (*Prionailurus bengalensis*)	6	
Herbivore	Terai-Arc landscape, India	127	Swab	Visual observation	Elephant (*Elephas maximus*)	11	88.98
	Terai-Arc landscape, India		Swab	Ungulate-specific PCR-sequencing[47]	Swamp deer (*Rucervus duvaucelii*)	71	
	Terai-Arc landscape, India		Swab	Ungulate-specific PCR-sequencing[47]	Chital (*Axis axis*)	22	
	Middle Himalayas		Swab	Ungulate-specific PCR-sequencing[47]	Himalayan tahr (*Hemitragus jemlahicus*)	9	
Total		1408				1091	77.49

Table 3. Details of microsatellite marker amplification success rates on species-identified samples

Species confirmed	Species-identified samples	Samples with microsatellite loci data	Microsatellite loci used	Average success rate (%)
Tiger (*P. tigris tigris*)	567	408	13 (refs 43 and 48)	66.23
Leopard (*P. pardus fusca*)	259	159	12 (refs 43 and 48)	62.5
Dhole (*C. alpinus*)	126	126	4 (refs 49 and 50)	62.5
Red fox (*V. vulpes*)	5	5	4 (refs 49 and 50)	69
Jungle cat (*F. chaus*)	15	4	4 (refs 43and 48)	100
Leopard cat (*P. bengalensis*)	6	4	4 (refs 43and 48)	100
Elephant (*E. maximus*)	11	11	3 (ref. 51)	100
Swamp deer (*R. duvaucelii*)	71	71	3 (ref. 52)	100
Chital (*A. axis*)	22	11	3 (ref. 52)	100
Himalayan tahr (*H. jemlahicus*)	9	9	3 (ref. 52)	100
Total	1091	821		75.25

Table 4. Cost comparison between various sampling protocols

Consumables required	Faecal sampling approach and associated cost (INR) per sample			
	Dry sampling (INR) (direct collection)	Dry sampling (INR) (silica method)	Dry sampling (INR) (Drierite desiccant)	Wet sampling (INR) (ethanol preservation)
Zip-lock bag	5	5	5	Not required
Plastic container	Not required	Not required	Not required	50 (Tarsons Products Private Ltd, Kolkata)
Butter paper	5	Not required	Not required	Not required
Silica beads	Not required	325 (Sigma-Aldrich, USA)	50 (W.A. Hammond Drierite Company, Ltd, USA)	Not required
Ethanol	Not required	Not required	Not required	250 (MilliporeSigma, USA)
Cardboard box	25	25	25	Not required
Approximate cost	35	355	80	300

Nuclear DNA (microsatellite) amplification: Nuclear DNA amplification from non-invasive samples is challenging due to poor quantity and quality of DNA[29]. In this study we have also amplified nuclear microsatellite markers from our field-collected and species-identified faecal samples. We used a number of microsatellite markers to test the quality of DNA from field-collected samples from different species (Table 3). Species-wise cumulative amplification success rates for all tested loci were calculated.

Results

We considered species identification and nuclear microsatellite amplification success rates from both swabbing and scraping protocols as efficacy of our faecal sampling approach for non-invasive wildlife genetic research. Initially we tested both approaches with 100 field-collected carnivore faecal samples (50 were swabbed and 50 were scraped) and achieved 100% success rates in species identification. As both approaches resulted in high success rate from field-collected faeces we compared other factors such as cost of consumables, ease of extraction protocol, time required, etc. for both methods and finally adopted the swabbing approach for larger sample size. Subsequently, we swabbed the remaining 1308 faecal samples of different carnivore and herbivore species (Table 2) collected from different habitats across India. We ascertained 10 different species from these field-collected faeces, including four large carnivore species ($n = 957$ samples), two small carnivore species ($n = 21$ samples) and four herbivore species ($n = 113$ samples). Overall, success rate was 75.95%, 100% and 88.98% for large carnivores, small carnivores and herbivores respectively (Table 2). The species identified were tiger (*Panthera tigris tigris*), leopard (*Panthera pardus fusca*), dhole (*Cuon alpinus*), red fox (*Vulpes vulpes*), jungle cat (*Felis chaus*), leopard cat (*Prionailurus bengalensis*), elephant (*Elephas maximus*), swamp deer (*Rucervus duvaucelii*), chital (*Axis axis*) and Himalayan tahr (*Hemitragus jemlahicus*) (Table 2).

Following species identification we amplified multiple nuclear microsatellites for all ten species (Table 3). We successfully amplified 821 of the total 1091 samples of different species, with an average success rate of 75.25% (see Table 3 for species wise details).

Discussion

Here, we describe a simple, quick and cost-effective faecal sampling approach for non-invasive wildlife research. We have tested this method on ten different species that are found in a variety of different habitats. Development of field-suitable sampling and storage protocol is a progressive approach in non-invasive wildlife research as faeces degradation in the wild is accelerated by exposure to various environmental conditions including sunlight (UV), humidity, temperature and rainfall, thus posing a challenge to generate useful information for any target species. In comparison to other studies involving field-sampling and storage protocol standardization[17,22–26], we collected a large number of samples ($n = 1408$) from multiple species covering a wide variety of habitats to test this protocol. Depending on the regions, the samples were stored in field conditions for up to two months before processing in the laboratory. High amplification success in species identification and nuclear marker amplification from field-collected samples indicate the efficacy of the approach for DNA-based research. To the best of our knowledge, no previous studies have used such a large sample size from varied species to test faecal sampling and storage protocol. Given our sampling from a wide variety of habitats and range of species with different ecologies, we believe that this protocol would work well in other species living in different habitats across the globe. This approach is much cheaper than other commonly used sampling protocols (e.g. silica gel, drierite, ethanol, etc.) (Table 4 provides a cost comparison of the widely used protocols), takes less time in the field and does not require specific training of field staff for implementation. However, we strongly suggest appropriate

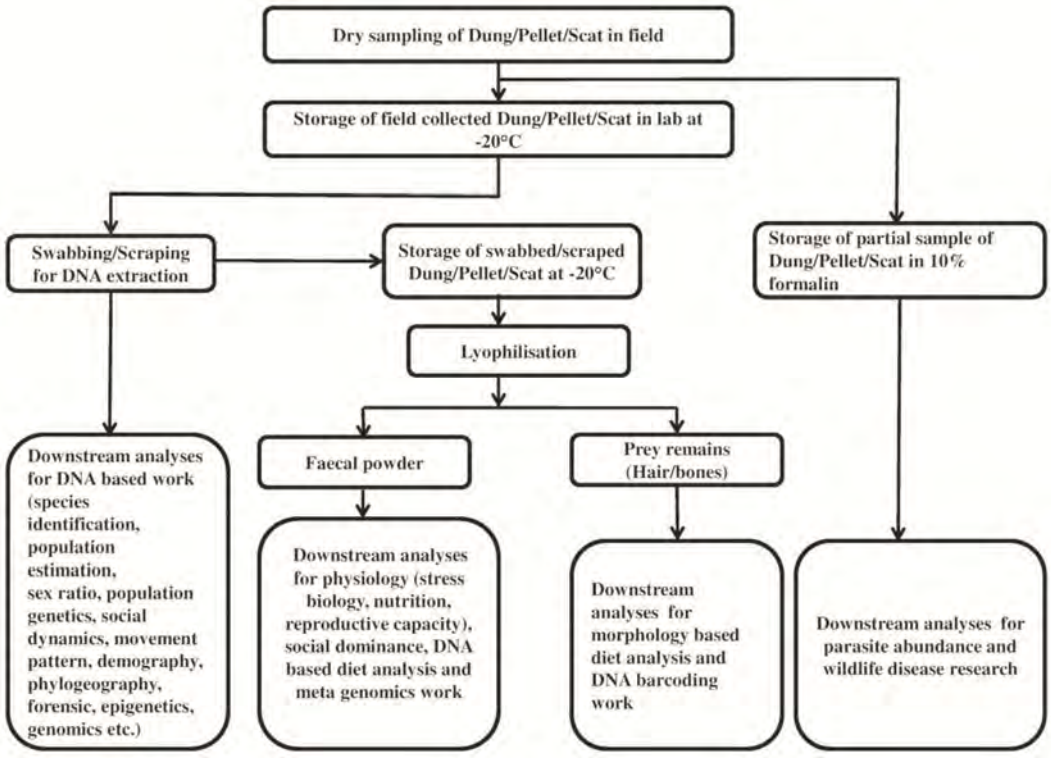

Figure 1. Flowchart showing a framework of various uses of faecal samples collected through the dry sampling approach described in this study.

safety protocols (mask, gloves, protective gears, etc.) during sample collection and processing for dry sampling approaches as exposure to potential pathogens is possible from dry faeces.

Testing two different DNA extraction protocols during this study provided important insights on generating good-quality DNA data from samples of differing quality. We performed swabbing and scraping DNA extraction approaches on a set of 100 carnivore samples. Carnivore scat samples were specifically chosen for standardization as they often yield poor results due to the presence of prey DNA[30]. Given similar success rates achieved from both approaches and considering low cost of consumables, extraction time and ease of the protocol, swabbing was used for the remaining samples. While earlier studies have shown great efficiency of this approach[25–27,31,32], swabbing was mostly conducted with fresh (≤24 h)[19,23], captive[15,25] and frozen (≤0°C)[26,27] faecal samples. Due to higher success rates with a large number of faeces from multiple species in this study, we recommend the use of swabbing approach in future non-invasive genetic research. However, it is also important to point out that the scraping approach would be useful for comparatively older (≥ 2 weeks) faeces where the outer layer is disturbed and for samples collected from dry/dusty regions where swabbing the top layer is not feasible. Though we have tested both approaches with a reasonably large number of carnivore faecal samples, any new study should test both approaches either with a few field-collected samples of the target species, or decide on a specific approach based on the sample conditions and physical characteristics (strata, dryness, availability of faeces top layer, etc.) of the study area.

Another major advantage of this dry sampling approach is the ability to use the same samples to generate additional information apart from DNA data at species/individual levels. We propose a useful framework to showcase different uses of the same samples in addressing various important biological questions in wildlife biology (Figure 1). For example, following swabbing/scraping for DNA, the frozen sample can be lyophilized to separate faecal powder and the remaining prey hairs/plant products[16]. Morphological analyses of hair/plant material can provide information on diet[33,34]. Similarly, the faecal powder could be subsequently used in understanding physiological parameters (stress[35,36], reproductive fitness[9,10,37], social dominance[38] and diet[16]). During field sampling, a part of the faeces can be collected in formalin to study parasite abundance[11]. In conclusion, our dry faecal sampling method provides an easy and cheap option to collect non-invasive samples from terrestrial wild animals. This universal protocol can be used to collect samples from species living in different environmental conditions and answer various questions related to genetics, genomics, physiology, diet, health, etc. Along with other ecological information, these

parameters would help develop informed conservation plans for any target species.

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Assessing tiger corridor functionality with landscape genetics and modelling across Terai-Arc Landscape, India

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Abstract

India led the global tiger conservation initiatives since last decade and has doubled its wild tiger population to 2967 (2603-3346). The survival of these growing populations residing inside the continuously shrinking habitats is a major concern, which can only be tackled through focused landscape-scale conservation planning across five major extant Indian tiger landscapes. The Terai-Arc landscape (TAL) is one of the 'global priority' tiger conservation landscapes holding 22% of the country's wild tigers. We used intensive field-sampling, genetic analyses and GIS modelling to investigate tiger population structure, source-sink dynamics and functionality of the existing corridors across TAL. Genetic analyses with 219 tigers revealed three low, but sigficantly differentiated tiger subpopulations. Overall, we identified Seven source and 10 sink areas in TAL through genetic migrant and gene flow analyses. GIS modelling identified total 19 (10 high, three medium and six low conductance) corridors in this landscape, with 10 being critical to maintain landscape connectivity. We suggest urgent management attention towards 2707 sq. km. non-protected habitat, mitigation measures associated with ongoing linear infrastructure developments and transboundary coordination with Nepal to ensure habitat and genetic connectivity and long-term sustainability of tigers in this globally important landscape.

Keywords: Tiger dispersal, Source-sink dynamics, *Panthera tigris tigris*, Landscape connectivity, Anthropogenic impacts, Priority conservation areas

Introduction

The tiger (*Panthera tigris*) exemplifies one of the major conservation efforts globally. Once distributed across ~30 present-day nations ranging from Armenia to Indonesia and the Russian Far East to India covering a variety of habitats [1], their distribution and numbers have drastically reduced to less than 4000 wild individuals at the begining of the twentieth century largely due to habitat loss and human persecution [2,3]. Despite country-specific conservation efforts, the wild tiger population size was found to be >3200 individuals in 2010 [4], leading to a commitment from the heads of 13 tiger-range countries to double their tiger number by 2022 [5]. As majority of the remaining tigers persisted as small, and often isolated populations [2,6] conservation strategies mostly focused on landscape-based approaches to attain recovery goals where habitat improvement, enhanced protection measures, prey augmentation were targeted [2,3,7,8]. Several priority landscapes were identified as 'Tiger Conservation Landscapes' (TCLs) [2], where identification of key source populations and consolidation and improvement of surrounding habitats were emphasized [9–11]. Given that ~80% of global wild tiger population living in most wide variety of habitats were found in India at that time [12], the success of the tiger recovery plan and the future survival of the species was mostly dependent on India's conservation actions. In 2019, India announced the news of doubling its tiger number since 2006 (population estimate of 1411 (1165-1675) in 2006 to 2967 (2603-3346) in 2018) within its existing habitats [13]. However, these increasing tiger populations now face enormous challenges from increasing human density, rapid urbanisation, expanding agriculture, aggressive infrastructure development and economic growth [14,15], and their future persistance will depend on the balance between the deveopmental demands and conservation requirements.

Managing wide-ranging, terrotorial species like tiger at landscape level requires in-depth understanding of key source populations [4], identification of potential tiger habitats

surrounding these source populations and ensuring habitat connectivity within the landscape [9,10]. At local level, measures of population dynamics including changes in recruitment and mortality, differential rates of immigration and emigration across sex and age classes, population turnover rates etc. are essential, whereas at landscape scale most emphasis should be given on habitat connectivity to enhance gene flow and reduce the risks from inbreeding [16–18]. Both of these are of critical importance in the Indian scenario as most of the available protected land (~5% as per the Wildlife Protection Act 1972) is extremely fragmented [19] and maintaining connectivity by identifying and managing the critical corridors will play the key role in future tiger conservation [4,20]. However, generating such detailed information is challenging, time consuming and resource intensive at any scale. In the Indian subcontinent, long-term ecological studies have already identified priority landscapes for tiger conservation (for example, Western Ghats, central India, north-eastern India, Sundarbans and the alluvial Terai flood plains in the Himalayan foothills) that support high potential tiger densities and relatively larger population sizes [13]. Earlier studies have helped us to understand source-sink population dynamics at landscape-scale in Western Ghats [8,11,21] and central Indian landscapes [17,22–26] along with Terai habitats of Nepal [27,28], but such information is missing from the Indian part of the Terai-Arc landscape (TAL), where population connectivity and their relationship with currently available habitats is poorly understood.

In this paper, we used a combination of intensive field surveys, non-invasive genetic tools and GIS modeling to assess the tiger population connectivity across the TAL. More specifically, we investigated (1) tiger presence and population structure across this landscape covering protected as well as non-protected habitats; (2) population connectivity in terms of tiger source-sink population dynamics in TAL; and (3) identify the critical corridors that helps maintaining the metapopulation dynamics. We addressed these questions by using 219 genetically identified unique tigers across TAL and details from already identified corridors in this landscape [29].

Methods

Research permissions and ethical considerations

All required permissions for field survey and faecal sampling were provided by the Forest Departments of Uttarakhand (Permit no: 90/5-6), Uttar Pradesh (Permit no: 1127/23-2-12(G) and 1891/23-2-12) and Bihar (Permit no: Wildlife-589). Due to the non-invasive nature (faecal sample based) of the work, no ethical clearance was required in this study.

Study area

This study was conducted in the Indian part of the TAL. The TAL is the only prime tiger habitat found along the foothills of Himalayas [20,29]. Covering approximately 28000 km^2 of forested habitat in the northern India and southern part of Nepal, this region is one of the most important global tiger conservation landscapes [20,29]. The Indian TAL is a linear 900 km long and 50 km wide landscape with 15000 km^2 tiger habitat encompassing the north Indian states of Haryana (westernmost), Himachal Pradesh, Uttarakhand, Uttar Pradesh and Bihar (easternmost) (Fig. 1a). Situated at the Himalayan foothills, the habitat supports tropical moist deciduous forests dominated by Sal (*Shorea robusta*), tall Terai swamp grasslands and permanently moist reed swamps [30]. This landscape is identified as a 'Global priority' tiger conservation landscape (TCL) [2] and retains about 22% of India's wild tiger population [13]. As recent as 2003, tigers were reported across this entire landscape [29] but currently found only in the states of Uttarakhand, Uttar Pradesh and Bihar [13].

This transboundary tiger habitat in TAL comprises a network of Protected Areas (PAs) and multiple use Forest Divisions (FDs) that maintain habitat connectivity through forest corridors [20,29]. The first comprehensive landscape-scale study carried out on tiger distribution in Indian TAL by Johnsingh *et al.* (2004) [29] highlighted the issue of habitat fragmentation. The study also identified nine tiger habitat blocks (THBs) and 13 structural corridors that potentially facilitate tiger dispersals (Fig. 1a). Currently this entire landscape has 13 PAs

(including six Tiger Reserves (TRs), 12 FDs and three Social Forestry Divisions (SFDs)). According to the latest tiger population estimation report [13] this landscape hosts 646 (567-726) individual tigers, with a ~33% population increase since the last estimation (n=485 (427-543) [31]). All relevant details for the THBs are provided in Supplementary Table S1.

Field sampling

In this study, we aimed to use both direct and indirect approaches (intensive field sampling, genetic analyses and GIS tools) to assess tiger presence, source-sink dynamics and functional connectivity across the entire tiger habitats in TAL. To achieve this goal at such a large landscape scale, it is important to conduct intensive sampling throughout the target study area. We conducted extensive field surveys covering all the THBs (including both PAs as well as FDs) of Uttarakhand, Uttar Pradesh and Bihar between December 2014 to May 2018 (Supplementary Table S1). During field surveys, a team of 5-6 experienced trackers walked this entire region searching for large carnivore faecal samples along all the potential trails and other habitats in this region. Sampling was mostly conducted between November-May i.e. winter and summer seasons every year. The total effort included ~9500 km of foot survey for faecal sampling.

In the field, we categorized large feline carnivore faeces (tiger and leopard) through morphological features such as size, shape, diameter as well as associated field signs (track and scrape marks etc.). We used a dry sampling approach described in Biswas *et al.* (2019) [32] for the entire study. Samples were collected with GPS coordinates and kept in dry, dark boxes in the field for a maximum period of one month before they were shipped to the laboratory, where they were stored in -20 °C freezers until further processing. During the sampling period, we collected a total of 1608 relatively fresh large carnivore faecal samples from the study area. The locations of the samples are provided in Supplementary Fig. S1.

DNA extraction, species, individual and sex identification

We extracted DNA from all the field-collected faeces using protocol described in Biswas *et al.* (2019) [32]. In brief, we swabbed the top layer of each sample twice with PBS-soaked sterile cotton swabs. Each swab was subsequently lysed overnight at 56 °C with 330 µl ATL buffer and 30 µl of proteinase K (20 mg/ml). Following digestion, swabs were removed and the remaining extraction was followed by the Qiagen DNeasy Tissue Kit (Qiagen Inc., Hilden, Germany) standard protocol. DNA was eluted twice in 100 µl of TE buffer (pH 7.8). For every set of samples (n=22) two extraction negatives were included to monitor contamination.

For species identification, we combined two earlier described tiger-specific molecular markers [33,34]. The PCR reactions were carried out in 10 µl reaction volumes containing 4 µl of Qiagen master mix (Qiagen Inc., Hilden, Germany), 3 µl of BSA, 1 µl of primer mix and 2 µl of template DNA. The PCR conditions included an initial denaturation (95 °C for 15 min); 50 cycles of denaturation (95 °C for 30 sec); annealing (50 °C for tiger and 57 °C for leopard for 30 sec); extension (72 °C for 30 sec); followed by a final extension (72 °C for 15 min). PCR products were visualized in 2% agarose gels and species-specific band patterns were used for unambiguous species identification. Only tiger samples were subsequently used for individual level analyses.

For individual identification, we used an already standardized panel of 13 microsatellite markers described in Mondol *et al.* (2009) [35], Mondol *et al.* (2012) [36]. These markers were amplified as 10 µl multiplex reactions containing 4 µl of Qiagen multiplex master mix (Qiagen Inc., Hilden, Germany), 3 µl of BSA, 1 µl of primer mix (2 µM concentration) and 3 µl of template DNA. The PCR conditions included an initial denaturation (95 °C for 15 min); 45 cycles of denaturation (94 °C for 30 sec); annealing (Ta for 30 sec); extension (72 °C for 30 sec); followed by a final extension (72 °C for 15 min) in an ABI thermocycler. Post-amplification, 1 µl of PCR product was mixed with 9 µl mix of Hi-Di formamide and 500

LIZ (Applied Biosystems, Foster City, CA, USA) and genotyped in ABI 3500XL DNA fragment analyzer. For genotype validation, we performed a modified multiple tube approach [37]. Each locus was genotyped and alleles were scored four independent times and a quality index [35] was calculated. Only loci with 0.75 or higher quality were retained for further analyses. For allele calling, we expanded the earlier tiger microsatellite allele bins for the north Indian population in program GENEMARKER 2.6.7. (Softgenetics Inc., State College, PA, USA) and scored the alleles manually.

We performed molecular sexing for only individually identified tigers using two sex chromosome markers Amelogenin [38] and SRY [39]. PCR reactions were carried out in 10 µl volumes containing 4 µl of Qiagen master mix (QIAGEN Inc.), 3 µl of BSA, 1 µl of primer mix (3 µM concentration) and 2 µl of template DNA. The PCR conditions included an initial denaturation (95 °C for 15 min); 50 cycles of denaturation (95 °C for 30 sec); annealing (Ta for 30 sec); extension (72 °C for 30 sec), followed by a final extension (72 °C for 15 min) in an ABI thermocycler. PCR products were run in 3% agarose gel and sex identification was done visually through sex-specific banding patterns. For validation, we repeated the entire process twice and only samples provided consensus results were considered further.

Data analyses

Microsatellite summary statistics

We calculated average amplification success as the percent positive PCR for each locus, as described by Broquet and Petit (2004) [40]. We quantified allelic dropout and false allele (FA) rates manually as the number of allelic dropouts or FAs over the total number of amplifications, respectively [40], as well as using MICROCHECKER v 2.2.3. [41].

Post data quality assessment, we selected only those samples with good quality data for at least nine loci (out of 13) for further analyses. We identified unique tigers by removing samples with identical genotypes using the 'Identity analysis' module of program CERVUS

[42] and removed the 'genetic recaptures' from the dataset. We used the program GIMLET [43] to calculate the probability of identity for siblings ($PID_{(sibs)}$) and unbiased ($PID_{(unbiased)}$) for all the individuals. Any allele with >10% frequency across all amplified loci were rechecked and confirmed. We calculated all summary statistics for genetic diversity as well as Hardy Weinberg equilibrium and linkage disequilibrium using program ARLEQUIN 3.1 [44].

Habitat connectivity in TAL

Assessing tiger connectivity directly (through radio-telemetry or camera trapping) at a landscape level is challenging due to their secretive behaviour, and logistical constraints resulting in infrequent information [45]. The TAL tiger habitat has 13 identified corridors [29] and we aimed to evaluate their current functionality. We took three independent indirect approaches to understand habitat connectivity in TAL. First, we generated individual level tiger genetic information across the entire tiger landscape and used this data to assess any spatial population structure (indicating loss of connectivity). Subsequently, we conducted a number of specific analyses to evaluate source-sink dynamics (using directional gene flow and migrant analyses as proxies of habitat connectivity) involving all the THBs within this landscape to corroborate the population structure results. Finally, we modelled the connectivity between PAs of TAL following Circuit Theory and identified critical corridors to maintain the functionality of this landscape.

For spatial clustering, we performed a Bayesian analysis using program TESS2.3.1 [46] with an admixture model with 60000 sweeps and 10000 burn in. We evaluated the most likely number of cluster (K), testing values between 2 to 10 [47], where clusters were identified through average deviance information criterion (DIC) value for each K. Further, we calculated genetic differentiation (F_{st} and G_{st}) among tiger genetic subpopulations identified through spatial clustering analyses in GenAlEx version 6.5 [48].

To assess tiger source-sink dynamics, we performed two different analyses focusing on 'detection of genetic migrants' and 'assessment of rate and direction of gene flow' within each tiger subpopulation. To detect tiger dispersals, we used two different approaches that use allele frequencies to detect migrant individuals. First, we used prior population information in the USEPOPINFO option implemented in STRUCTURE 2.3.2 [49] to detect first-generation migrants in our sampled populations. We detected the number of clusters (K between 1-10) based on 10 independent runs with 500,000 iterations and a burn-in of 50,000 assuming an admixture model. We considered the membership coefficient (q) above 0.9 as a realistic cut-off value to assign an individual to a population [24]. We assigned different migration rates (MIGPRIOR 0.01, 0.05, 0.1) as a sensitivity test. We ran the analysis with two separate dataset: a) individuals grouped as populations according to their sampling locations and b) individuals grouped as genetic clusters from our initial run. These different runs helped us to evaluate the consistency of the results across different genetic groups created.

Further, we used the 'Migrant detection' function in program GENECLASS 2.0 [50] to identify the first-generation migrant tigers. Here, we used a Bayesian approach as described by Rannala and Mountain (1997) [51] along with the resampling method of Paetkau *et al.* (2004) [52] for likelihood computation (L_home/L_max). The run parameters included 10000 simulated individuals with a threshold alpha value of 0.01 (Type 1 error) [52]. This method allows detection of migrants even when the overall differentiation between populations is low. Apart from first generation migrant detection, we did individual assignments in GENECLASS using Bayesian criterion of Rannala and Mountain (1997) [51] in combination without resampling with rest of the parameters same as described above. Subsequently, we measured the magnitude and direction of recent gene flow using the Bayesian MCMC based approach implemented in BAYESASS v.3.0.4 [53], with run parameters of 1000000 iterations and

100000 burn-in. This approach is generally used to detect recent gene flow (5-7 generations) between populations [54].

We developed habitat permeability layer to understand potential tiger connectivity in TAL, using the maximum entropy modelling approach in the program MaxEnt v 3.4.1 [55]. Habitat permeability layer and focal nodes (regions between which connectivity is to be modelled) are the primary input data for connectivity analyses, indicating the difficulty experienced by an individual in moving across a landscape [56]. We used tiger presence points and environmental variables in MaxEnt v 3.4.1 [55] to develop a habitat permeability layer, where each pixel is a proxy of the likelihood that individuals will move through that cell (i.e. conductance). After removing the spatial cluster between point locations, we used a total 465 presence points along with five environmental variables based on tiger's ecology and habitat requirements, viz. forest cover, distance to protected areas, distance from water, road, and settlements (Supplementary Table S2). For running the model, we split the presence points as training (70%) and testing (30%) during cross-validate run with 10 replicates and rest of the settings were kept as default. We used the average of all outputs (n=10 runs) as the habitat permeability layer for Circuitscape. Subsequently, we used export to Circuitscape tool (http://www.jennessent.com/arcgis/Circuitscape_Exp.htm) in ArcGIS 10.7 to export the focal nodes and habitat permeability layer to ASCII raster layers of equal extents, cell sizes, and spatial references. We used a total of 21 focal nodes (Supplementary Table S3) and habitat permeability layer in Circuitscape v.4.0 [56] to measure the corridor conductance across TAL. We provided weightage to each of the focal nodes based on tiger source-sink populations as identified in this study. We set the node weightages in four categories that ranged from 0 (nodes with no tiger presence), 0.25 (nodes that act as sink populations), 0.5-0.75 (nodes those are moderate-level source populations) and 1 (nodes those are primary source populations) (Supplementary Table S3) during analyses. Subsequently, based on conductance

value we classified the output corridors into three categories: low functioning (0-0.30), medium functioning (0.31-0.70) and high functioning (0.71-1.0).

To identify the corridor bottlenecks, we used 'Pinchpoint mapper' in Linkage mapper toolbox V2.0 in ArcGIS 10.7. We converted the habitat permeability layer into a resistance surface using SDM toolbox [57] in ArcGIS 10.7 (www.esri.com) for running the analysis. Finally, we modelled the least-cost pathways (LCPs) for tiger dispersal using Linkage mapper toolbox V2.0 in ArcGIS 10.7.

Results

Tiger data from TAL

Out of 1608 field-collected large carnivore faecal samples, we extracted DNA from 1524 samples (94.78%). Remaining 84 samples (5.22%) showed fungal growth across the top-layer and were not processed. Post tiger-specific assays we identified 743 tiger faeces (48.75%) (Supplementary Fig. S2) for downstream individual identification. Using 13 microsatellite panel [35,36] we subsequently identified 219 unique tiger individuals across TAL (Fig. 1b), covering approximately 35% of the estimated tiger population from this landscape (n=646 (567-726) [13]). Locus-wise mean success rate across all samples ranged between 53-97% (Table 1). We found no large-scale signatures of allele drop out or null alleles. The mean allelic dropout and false allele rates as 0.01 and 0.03, respectively (Table 1), indicating an overall low genotyping error rate across this marker panel. All 13 loci were polymorphic for TAL tigers with mean number of alleles, observed heterozygosity and allelic size range were found to be of 14.76±2.83, 0.35±0.17 and 45.38±10.96, respectively (Table 1). The cumulative $PID_{(sibs)}$ and $PID_{(unbiased)}$ values ($2.24*10^{-6}$ and $5.99*10^{-17}$, respectively) suggest a statistically strong result for unambiguous tiger individual identification (Table 1). We identified a total of 35 genetic recaptures across TAL. The details of area-wise genetic

recaptures are as follows: Rajaji TR- 10, Corbett TR- 11, Pilibhit TR- 2, Lansdowne FD- 5, Hardwar FD- 1, Ramnagar FD- 5 and Terai East FD- 1.

Of all the identified unique individuals (n=219) ~71% were from Uttarakhand (n=156, 35% of the state's population of 442 (393-491)), whereas ~20% were from Uttar Pradesh (n=44, 25% of the state's population of 173 (148-198)) and ~9% from Bihar (n=19, 61% of the state's population of 31 (26-37)) [13]. We confirmed tiger presence in 12 of the 13 earlier described THBs, representing 10 PAs, nine FDs and two SFDs (Supplementary Fig. S2, Supplementary Table S1). THB VII and part of THB IV did not show any tiger evidence (Supplementary Fig. S2). We achieved an 88% success rate in molecular sexing among the unique tigers (n=193, 89 males and 104 females, respectively). Interestingly, 54 of these males were found inside PAs and 35 individuals were from non-PAs (FDs and SFDs), whereas 59 females were from PAs and 45 from outside PAs, indicating that both sexes were using non-protected habitats across TAL.

Population structure and genetic differentiation of tigers across TAL

Our Bayesian clustering analyses with 219 individual tiger genetic data revealed four genetic lineages (K=4) across TAL. When examined closely, we found that Uttarakhand state holds two of these four genetic lineages as geographically mixed populations, whereas the remaining two lineages are roughly separated in states of Uttar Pradesh and Bihar, respectively, making three genetic subpopulations across TAL (Fig. 1b). Following the description of 'Tiger Habitat Blocks (THBs)' mentioned by Johnsingh *et al.* (2004) [29], we report these three genetic subpopulations as 'Tiger Genetic Blocks (TGBs)' while reporting the results.

Overall, these TGBs roughly correspond to the tiger population in the states of Uttarakhand (TGB I), Uttar Pradesh (covering mostly TGB II and small parts of TGB I and III) and Bihar (TGB III), respectively (Fig. 1b). The samples collected from THB I, II and III (n=171)

formed the first genetic subpopulation (TGB I) (Figs. 1a, 1b). The TGB I is spread between the Rajaji TR (western side) to Nandhaur Wildlife Sanctuary (WLS) (eastern side) in Uttarakhand, covered THB I, II and III and retains the highest tiger population in TAL[13] (Figs. 1a, 1b). This block represents six PAs, eight FDs, one SFD and nine earlier described corridors (Figs. 1a, 1b, Supplementary Table S1) [29]. The TGB II includes the samples collected from THB IV, V and VI (n=26) and spread between Pilibhit SFD (western side) to Katarniaghat WLS (eastern side) in Uttar Pradesh (Figs. 1a, 1b). This block retains two PAs, one FD, one SFD and three corridors (Figs. 1a, 1b, Supplementary Table S1) [29]. Finally, the samples collected from THB VIII and THB IX (n=22) together formed the TGB III and included only two PAs, Sohagibarwa WLS (western side) in eastern Uttar Pradesh and Valmiki TR (eastern side) in Bihar (Figs. 1a, 1b, Supplementary Table S1).

Our analyses revealed that the TGBs are genetically differentiated (Fst and Gst) at low, but significant levels. The Fst value ranged between 0.079-0.107, while the Gst value ranged between 0.067-0.087 (Table 2). Comparative summary statistic analyses among these TGBs showed that TGB I has the highest mean number of alleles (10.15±3.06) when compared to TGB II (7.00±2.11) and TGB III (9.08±2.24), respectively. However, TGB III showed higher observed heterozygosity and the allelic size range values (0.43±0.20 and 38.00±14.29, respectively) than TGB I (0.34±0.20 and 24.46±07.37, respectively) and TGB II (0.29±0.12 and 24.77±09.30, respectively) (Table 3).

Gene flow and tiger source-sink populations within TGBs

We analysed the genetic data to understand the tiger source-sink population dynamics (which also confirmed connectivity among populations) within each of the three already identified TGBs in TAL. Within the TGB I, 'genetic migrant detection' and gene flow analysis identified two major habitat complexes with genetic connectivity: The Rajaji-Lansdowne-Haridwar region and the Corbett-Ramnagar-Terai FDs-Haldwani region. In the Rajaji-

Lansdowne-Haridwar habitat complex we identified five first generation migrant tigers (Supplementary Table S4): two migrants each from Lansdowne FD to Rajaji TR and Haridwar FD and one from Rajaji TR to Lansdowne FD (Supplementary Table S4). Further Bayesian analyses show higher rates of gene flow between Lansdowne FD to Rajaji TR and Rajaji TR to Haridwar FD, whereas Najibabad SFD shows signatures of immigration from both Rajaji TR and Lansdowne FD (Fig. 2a). Combining this information, we interpret that both Rajaji TR and Lansdowne FD act as source tiger populations and Haridwar FD and Najibabad SFD are sink populations in this habitat complex, respectively (Fig. 2a).

Similarly, in the Corbett-Ramnagar-Terai FDs-Haldwani habitat complex we identified 12 first-generation migrant tigers (Supplementary Table S4), showing extensive genetic connectivity among the sampled areas within the habitat complex. We found three migrants from Ramnagar FD to Corbett TR and two migrants to Haldwani FD (Supplementary Table S4). We detected one migrant tiger from Terai West FD to Ramnagar FD (Supplementary Table S4). We found two migrants from Terai Central FD to Terai West FD, one migrant to Ramnagar FD and one to Champawat FD (Supplementary Table S4). We also found one migrant individual from Haldwani FD to Terai East FD (Supplementary Table S4). In addition, we detected one migrant individual from Najibabad SFD to Amangarh TR (Supplementary Table S4). Bayesian analyses show higher gene flow from Corbett TR to adjoining Najibabad SFD and Amangarh TR (Fig. 2a). Corbett TR also shows a high rate of bidirectional gene flow with Ramnagar FD and Terai West FD. Similarly, Terai West FD is highly interconnected with Amangarh TR, Terai Central FD and Ramnagar FD (Fig. 2a). The other areas such as Haldwani FD, Ramnagar FD, Terai Central FD and Terai East FD have lower rates of gene flow among them (Fig. 2a). Combined together, this information suggests that Corbett TR is the main source population for Ramnagar FD, Amangarh TR, Terai West FD and Najibabad SFD, whereas Ramnagar FD is the source tiger population for Haldwani,

Champawat and Terai East FDs (Fig. 2a). Within the two major tiger habitat complex in TGB I (Rajaji-Lansdowne-Haridwar and Corbett-Ramnagar-Terai FDs-Haldwani) connectivity is maintiained through the Najibabad SFD, which acts as a sink population for both Corbett TR and Lansdowne FD (Fig. 2a, Supplementary Table S4).

Within TGB II, the tiger habitats are comparatively more fragmented than TGB I and we identified 10 first-generation migrant tigers (Supplementary Table S4). We detected two migrants from Pilibhit TR to Dudhwa National Park (NP) (part of Dudhwa TR) and one migrant to Kishanpur WLS (part of Dudhwa TR) (Supplementary Table S4). We also found two migrants from Dudhwa NP to Pilibhit TR, one migrant to Kishanpur WLS (part of Dudhwa TR) and two migrants to South Kheri FD (buffer zone of Dudhwa TR) (Supplementary Table S4). Further, we detected two migrants from Kishanpur WLS to Pilibhit SFD (Supplementary Table S4). We measured higher gene flow from Pilibhit TR to Dudhwa NP, Kishanpur WLS, South Kheri FD and Pilibhit SFD (Fig. 2b). We also estimated higher rate of bidirectional gene flow among Dudhwa NP, Kishanpur WLS and Katarniaghat WLS, along with unidirectional gene flow to adjoining South Kheri FD (Fig. 2b). This information suggests Pilibhit TR and Dudhwa TR are the major source tiger populations, and South Kheri FD and Pilibhit SFD are the sink populations in the TGB II.

Finally, within TGB III we detected two first-generation migrants from west Sohagibarwa WLS to Valmiki TR (Supplementary Table S4). This result was surprising as within this habitat block Valmiki TR has the highest tiger population [13]. However, the Bayesian analysis results indicate higher gene flow from Valmiki TR and east Sohagibarwa WLS to west Sohagibarwa WLS (Fig. 2c). Combined together, we interpret that Valmiki TR-east Sohagibarwa WLS is the source population for west Sohagibarwa WLS in TGB III.

Corridor connectivity across TAL

Our habitat permeability analyses indicated certain habitat variables such as distance to forest cover and protected areas are the main governing factors of tiger dispersal (Supplementary Table S5). Out of the 13 earlier-described corridors [29], twelve showed conductance for tiger dispersal (Fig. 3a, Supplementary Table S6). Towards the western end of TAL Yamuna river corridor did not show any signatures of habitat conductance (Fig. 3a, Supplementary Table S6). The remaining corridors showed high to low conductances (Fig. 3a, Supplementary Table S6). The details of the least-cost pathways (LCP) are provided in Supplementary Table S6.

In addition, we identified seven new corridors in TAL. The first corridor (high conductance) connects Rajaji TR (eastern part) with Najibabad SFD through Lansdowne FD (Fig. 3a, Supplementary Table S6). The second corridor (high conductance) connects Jhilmil Jheel CR with Najibabad SFD through Haridwar FD (Fig. 3a, Supplementary Table S6). The third corridor (high conductance) connects Corbett TR with Najibabad SFD (Fig. 3a, Supplementary Table S6), making these areas very well connected with each other within TGB I. In TGB II, we identified two new corridors (high conductance), where the first one connected the northern and southern part of Pilibhit TR, and the second one connected Pilibhit TR to Kishanpur WLS through North Kheri FD, respectively (Fig. 3a, Supplementary Table S6). Finally, two new corridors were identified in TGB III, the first one (low conductance) connecting western and eastern part of Sohagibarwa WLS and the easternmost corridor (high conductance) connecting two parts of Valmiki TR, respectively (Fig. 3a, Supplementary Table S6). The corridor bottleneck analysis identified 10 critical tiger dispersal corridors in TAL (Fig. 3a, Supplementary Table S6).

Discussion

We used a multidisciplinary approach to evaluate all tiger corridor functionalities at a landscape level across the TAL. To the best of our knowledge, this is the first study using

424 genetic analyses and corridor modelling to understand tiger source-sink dynamics and
425 connectivity at a landscape level in TAL. We conducted extensive faecal sampling across all
426 tiger habitats and dispersal corridors and confirmed presence of tiger in most of the earlier
427 described THBs in TAL. These areas include already known TRs and FDs [13] along with new
428 areas in this landscape (Supplementary Fig. S2, Supplementary Table S1). Our field surveys
429 corroborated with early reports of tiger absence beyond western part of Rajaji TR, Suhelwa
430 WLS, and remaining areas between Dudhwa TR and Sohagibarwa WLS (Fig. 1b,
431 Supplementary Fig. S2) [13,29], possibly due to high anthropogenic pressure and habitat loss.
432 Further, we confirmed tiger presence from Jhilmil Jheel Conservation Reserve (CR)
433 (Uttarakhand), Pilibhit SFD and Najibabad SFD (Uttar Pradesh) where they were not
434 reported earlier (Supplementary Fig. S2) [13]. This expansion in tiger habitat occupancy is
435 possibly due to increasing tiger dispersals to newer areas driven by recent increase in their
436 population in this landscape [13]. Tigers are territorial and high resource-demanding (in terms
437 of both food and space) animals and such dispersal events are thus normal [58]. In the Indian
438 subcontinent such dispersal events have been reported from other landscapes also (Northwest
439 India [59], Central India [17,24,25], Nepal-TAL [27,28]) and ~35% of the country's tiger population is
440 reported to live outside the exisiting TRs [13]. Interestingly, we found that both male and
441 female tigers beyond protected areas including relatively marginal habitats of FDs and SFDs
442 indicating both-sex dispersal events in TAL, as found in other habitats (Central India [25],
443 Nepal-TAL [28]).
444 Our genetic analyses identified three genetic subpopulations (named as Tiger Genetic Blocks
445 or TGBs) in TAL. This was an unexpected result considering we found tiger presence in
446 majority of the protected and non-protected areas, except some parts of the central and the
447 eastern TAL (Fig. 1b). The easternmost subpopulation TGB III covering Sohagibarwa WLS
448 and Valmiki TR is physically separated from the central and western TAL without any forest

449 connectivity [29]. Tigers from Valmiki TR have also been reported earlier as genetically unique
450 [60]. Even in the Nepal part of TAL, three genetic subpopulations have been reported those are
451 connected with TGB II and TGB III [28], indicating genetic discontinuity across transboundary
452 sections of TAL in this region. However, the pattern of genetic discontinuity between TGB I
453 and TGB II within the seemingly continuous habitat of central and western TAL was
454 surprising. Similar patterns of tiger population structure has been earlier reported from other
455 tiger landscapes in the subcontinent (for example, Central Indian landscape [61,25], Western
456 TAL [62], Nepal-TAL [28]). Even in the same TAL landscape Bhatt *et al.* (2020) [63] has reported
457 two leopard genetic subpopulations, possibly driven by landscape features. When compared
458 with this study, it makes ecological sense that TAL leopards showed less population structure
459 than tigers (three TGBs) as they are more habitat generalist and even found in human-
460 dominated areas [64]. Unlike the other major tiger landscapes in India, both of these sympatric
461 species face one common problem related to this linear landscape where one-dimensional
462 space (resulting in restricted movement opportunities) combined with very high human
463 density makes the entire landscape extremely vulnerable to fragmentation at a relatively small
464 temporal scale [29]. We strongly feel that the genetic separation between TGB I and II is driven
465 by human-disturbance mediated loss of corridor functionality leading to population
466 differentiation at small, but significant levels (Table 2).
467 Within each of these TGBs the genetic data helped us to understand the source-sink dynamics
468 and fine-scale population connectivity patterns between the protected and non-protected
469 areas. Our analyses with tiger data from TGB I identified both migrant individuals (n=17)
470 and signatures of high gene flow among majority of the TRs and other surrounding areas
471 (Fig. 2a, Supplementary Table S4), indicating functional corridors within this region. We feel
472 that the complex, undulating forested Shivalik and Bhabhar habitat found in this region has
473 helped in tiger movements as hypothesized by Johnsingh *et al.* (2004) [29]. Similar pattern was

474 also identified in Western Ghats and central Indian landscapes where tigers were reported to
475 use rough terrain for dispersal and avoided human habitations [21,65]. In addition, this area also
476 retains the largest number of tigers, mostly within the PAs [13] and recent increase in tiger
477 population has probably resulted in migration of the surplus individuals to newer areas. Our
478 source-sink dynamics analyses results also corroborate this pattern, where we found that
479 majority of the sink populations in TGB I are in the flat, Terai region in the southern part
480 (Fig. 2a). The Terai habitat also supports very high human density and known to support poor
481 tiger populations in recent past [29]. Similarly, Terai habitat dominated TGB II (where majority
482 of the tiger populations are found within PAs) showed good connectivity (Fig. 2b,
483 Supplementary Table S4). Our analyses indicated that mostly the PAs are source population
484 and FD/SFDs are the sink populations (Fig. 2b), showing a contrasting pattern from TGB I
485 where even FDs were source populations (like Lansdowne and Ramnagar FDs) (Fig. 2a). In
486 TGB III, the largest tiger population Valmiki TR was the source whereas the western
487 Sohagibarwa WLS was the only available sink habitat (Fig. 2c).
488 The Circuitscape analyses provide strong support and possible explanations to the genetic
489 connectivity patterns we observed in this landscape. Overall, we identified a total of 10 high,
490 three medium and six low conductance tiger corridors across TAL (Fig. 3a, Supplementary
491 Table S6), all of them are outside PAs (Fig. 3a). First, the genetic discontinuity between TGB
492 I and II is possibly due to loss of functionality of the Kilpura-Khatima-Surai corridor (marked
493 as corridor J in Fig. 1a) [29]. This corridor structurally connects the Champawat-Haldwani-
494 Terai East FD complex of TGB I with Pilibhit-Dudhwa TR complex of TGB II but currently
495 dysfunctional (Fig. 1a). Earlier Jonsingh *et al.* (2004) [29] reported no tiger use in this corridor,
496 which has been further confirmed by Jhala *et al.* (2020) [13]. We believe that this loss of
497 connectivity between TGB I and II is possibly resulting from developmental activities (e.g.
498 highway, railway tracks, canal etc.) and habitat loss (from human settlements and

499 urbanization etc.) along the corridor (Fig. 3a). Some recent evidences suggest tiger dispersal
500 events in this corridor [66] indicating hope for this corridor but strong management
501 interventions are required to maintain its functionality and ensure one panmictic population
502 between Rajaji to Dudhwa TRs (Fig. 3a). Next, within the TGB I the southern part of Gola
503 river corridor (marked as corridor I in Fig. 1a) connecting Ramnagar-Terai Central FDs
504 complex with Champawat-Haldwani-Terai West FDs complex was identified as a weak
505 corridor (Fig. 3a). While the northern part of this corridor showed medium conductance, the
506 southern part is heavily affected by anthropogenic activities like human settlements,
507 urbanization, industrialization and large-scale boulder mining [29,67] leading to low genetic
508 exchange across it (Fig. 2a). Further, we identified that the Chilla-Motichur corridor (marked
509 as corridor C in Fig. 1a) that connects eastern and western parts of Rajaji TR as a weak
510 connecting link (Fig. 3a) [13,29]. Despite showing high conductance in Circuitscape analysis
511 there is no functional connectivity through this corridor, possibly due to intense
512 anthropogenic activities [29,68,69]. Further, one of the most important output of this study is the
513 identification of 10 critical bottlenecks distributed across the high, medium and low
514 conductivity corridors (Fig. 3a). Due to the linear geography of the TAL where only one-
515 directional movements are feasible, these corridor bottlenecks demand immediate
516 conservation attentions. Except these identified regions we could not find any other
517 alternative paths (high Himalayan area in north and human-dominated Terai flats in south)
518 and thus maintaining connectivity through these identified corridors are the only way these
519 tiger populations can survive in near future [67].
520 Finally, another important aspect of the results from this study is that despite having a large
521 tiger number and relatively connected populations the TAL tigers have relatively low genetic
522 variation compared to other tiger landscapes within India and Nepal-TAL. Different measures
523 of genetic diversity in TAL showed lower values when compared with central India (N_A-

524 12.43 ± 2.99 [61], 11.71 [24], 9.1 ± 2.2 [25]; H_O- 0.65 ± 0.09 [61], 0.54 [24], 0.70 ± 0.06 [25] and ASR-
525 28.8 ± 10.5 [25]) and Western Ghats (N_A- 3-12 [34] and H_O- 0.33-0.68 [34]) as well as with earlier
526 studies in TAL (N_A- 6.69 and H_O- 0.50 [62]) and Nepal-TAL (N_A- 3.0 ± 0.33-4.0 ± 0.32 and
527 H_O- 0.46 ± 0.08-0.58 ± 0.06 [28]). While such patterns can be possible due to different panel of
528 markers, the same markers have shown higher genetic variation in central India [24] and
529 Western Ghats [34,70]. Interestingly, similar pattern of lower genetic variation (compared to
530 other regions) has also been reported in leopards from TAL, which was attributed to severe
531 population decline in recent years [63]. TAL has a long history of extensive trophy and bounty
532 hunting of tigers and leopards since Mughal and British era [71], possibly leading to a small
533 founder population. Though the current population has increased in recent years [13] the small
534 founder population is probably the reason behind lower genetic variations. However, TGB I
535 showed higher genetic variations ($N_{A(TGBI)}$- 10.15 ± 3.06, $H_{O(TGBI)}$- 0.34 ± 0.2 and $ASR_{(TGBI)}$-
536 24.46 ± 7.37) than TGB II (Table 3), possibly because it hosts the largest tiger population
537 (~500 individuals) in this landscape [13]. Surprisingly, the small tiger population in TGB III
538 showed relatively higher genetic variation than in TGB II (Table 3). This is possibly due to
539 gene flow from larger tiger populations of Chitwan NP and Parsa Wildlife Reserve of Nepal
540 [27], which helped to retain high genetic diversity in the TGB III. The genetic varation of TGB
541 II can possibly be improved by ensuring tiger connectivity through Kilpura-Khatima-Surai
542 corridor from TGB I along with Shuklaphanta NP and Bardia NP of Nepal [27].

543 **Conservation implications**

544 Considering the linear shape of tiger habitats in TAL, fragmentation has always been a major
545 concern for conservation [29,72]. Maintaining the integrity of this landscape with very high
546 human density and associated linear developments will remain the most important challenge
547 for long-term persistence of tiger in this globally high priority tiger conservation landscape [29].
548 Our results on tiger population structure, connectivity and source-sink dynamics thus have

549 critical management implications specially in the backdrop of recent increase in tiger
550 numbers in TAL. One of the encouraging point is the revealing of only three TGBs compared
551 to the eight distinct THBs described by Johnsingh *et al.* (2004) [29], indicating considerable
552 amount genetic exchange within these THBs. However, within the TGBs we identified 10
553 critical corridors (Fig. 3a, Supplementary Table S6) that require urgent attention failing
554 which integrity of TAL will be severely compromised. Further analyses show that a total of
555 2707 sq. km. habitat (Fig. 3b, 3c, 3d, Table 4) needs appropriate conservation planning to
556 ensure panmictic tiger populations in TAL. Appropriate mitigation measures associated with
557 ongoing linear infrastructure developments, roads in particular, are a must in this highly
558 sensitive conservation landscape [69]. In addition, TGB II and III requires transboundary
559 cooperation between India and Nepal to ensure maintenance of genetic variation and source-
560 sink dynamics in this population.

561 We also anticipate another potentially important upcoming tiger conservation challenge in the
562 form of increasing tiger-human interactions. Our results show that significant proportion of
563 tigers in TAL are found outside PAs (Supplementary Fig. S2) (possibly driven by recent
564 increase in tiger population), co-existing with significant amount of human density and
565 associated livestock [13]. It is likely that the incidences of tiger-human conflict will increase
566 around the sink habitats and corridors in coming years and active management of such
567 conflict situations will be critical for these tigers living in marginalized habitats. Further,
568 adequate attention towards habitat and prey management in the FDs and SFDs where large
569 number of tigers are found (at least in TGB I and II) will play important role in maintaining
570 connectivity of the entire landscape.

571 India played a leading role in tiger conservation and has achieved a rare global success in
572 population recovery of a large-bodied, apex carnivore [13]. However, the future of these tiger
573 populations depend on appropriate management of the ever-shrinking habitats and

574 maintaining the existing populations as metapopulations. Our results from TAL showed the
575 functionality of the existing corridors and point out critical areas where immediate
576 conservation attention is needed. We believe that a focused approach to address such
577 concerns will improve the long-term sustainability of the tiger populations in TAL.

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593 manuscript.

594 **Author contribution**

595 S Mondol and B Pandav conceived the study idea, generated funds and supervised the study.
596 S Biswas and S Bhatt performed the sampling and data generation. Data was analysed by S
597 Biswas (molecular and GIS data), D Sarkar (GIS), G Talukdar (GIS) and S Mondol

598 (molecular data). S Mondol, S Biswas and B Pandav wrote the initial manuscript and all
599 authors approved the final draft.

600 **Competing interest**

601 The authors declare no competing interests.

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Figure legends

Figure 1: The tiger habiats within the Indian part of Terai-Arc landscape (TAL), encompassing both protected (National parks, Tiger Reserves, Conservation Reserves and Wildlife Sanctuaries) as well as non-protected areas (Forest Divisions and Social Forestry Divisions). The top plot (a) shows the entire landscape with marked 'Tiger Habitat Blocks' (THBs) and identified corridors (Johnsingh et al., 2004) [29]. The bottom plot (b) presents the 'Tiger Genetic Blocks' (TGBs) along with the genetic structure results from program TESS [45]. These TGBs roughly correspond to the western, central and eastern parts of the landscape.

Figure 2: Assessment of tiger source-sink dynamics within each TGB in the Indian part of TAL. The direction and magnitude of gene flow has been presented by different color allows among the protected and non-protected areas. The top plot (a), middlie plot (b) and the bottom plot (c) show the gene flow patterns in TGB I, II and III, respectively.

Figure 3: Results of the CIRCUITSCAPE analyses to identify the corridor conductances across TAL. Both 'Least Cost Pathways (LCPs)' as well as the critical corridors (pinchpoints) are shown here in (a). The critical corridor areas to maintain contiguous landscape and require urgent management attention are highlighted in (b), (c) and (d). Refer Table 4 for details of these critical areas.

Supplementary fig. 1: The locations of large carnivore faeces across TAL.

Supplementary fig. 2: The locations of genetically identified tiger faecal samples across TAL.

78°0'0"E 80°0'0"E 82°0'0"E 84°0'0"E

30°0'0"N 28°0'0"N 26°0'0"N

Himachal Pradesh
Uttarakhand
Haryana
Uttar Pradesh
Rajasthan
Bihar
NEPAL
INDIA

THB I
THB II
THB III
THB IV
THB V
THB VI
THB VII
THB VIII
THB IX

Khoh
Ramganga
Kosi
Boar
Gola
Ganga
Yamuna
Sharda
Ghagra
Gandak

a

TGB I
TGB II
TGB III

b

Protected Areas

1. Simbalbara NP
2. Kalesar WLS
3. Rajaji TR
4. Jhilmil Jheel CR
5. Corbett TR
   - 5a. Kalagarh TR
   - 5b. Corbett NP
6. Amangarh TR
7. Pawalgarh CR
8. Nandhaur WLS
9. Pilibhit TR
10. Dudhwa TR
    - 10a. Kishanpur WLS
    - 10b. Dudhwa NP
    - 10c. Katarniaghat WLS
11. Suhelwa WLS
12. Sohagibarwa WLS
    - 12a. West
    - 12b. East
13. Valmiki TR

Forest Divisions

i. Shivalik FD
ii. Dehradun FD
iii. Haridwar FD
iv. Lansdowne FD
v. Najibabad FD
vi. Terai West FD
vii. Ramnagar FD
viii. Terai Central FD
ix. Haldwani FD
x. Champawat FD
xi. Terai East FD
xii. Pilibhit Social FD
xiii. Shahjahanpur Social FD
xiv. South Kheri FD
xv. North Kheri FD

Corridors

A. Yamuna river
B. Kansrau-Barkot
C. Chilla-Motichur
D. Rajaji-Corbett
E. Kalagarh
F. Kosi river
G. Boar river
H. Nihal-Bhakra
I. Gola river
J. Kilpura-Khatima-Surai
K. Lagga bagga-Shuklaphanta-Tatarganj
L. Kishanpur-Dudhwa
M. Dudhwa-Katarniaghat

Service layer credits: Source: ESRI, Digitalglobe.

Tiger Habitat Blocks (THBs)
Rivers
International boundary
State boundary
Tiger Genetic Blocks (TGBs)
Individual Tigers (n=219)

0 50 100 Kms

78°0'0"E
79°0'0"E
80°0'0"E
30°0'0"N
29°0'0"N

TGB I

DFD- Dehradun FD
RTR- Rajaji TR
HFD- Haridwar FD
JJCR- Jhilmil Jheel CR
LFD- Lansdowne FD
CTR- Corbett TR
NJSFD- Najibabad SFD
ATR- Amangarh TR
TWFD- Terai West FD
TCFD- Terai Central FD
TEFD- Terai East FD
NWLS- Nandhaur WLS
HLFD- Haldwani FD
CFD- Champawat FD
PSFD- Pilibhit SFD
PTR- Pilibhit TR
NKFD- North Kheri FD

DFD, RTR, HFD, JJCR, LFD, CTR, NJSFD, ATR, TWFD, PCR, RFD, CFD, HLFD, NWLS, TCFD, TEFD, PSFD, PTR, NKFD
B, C, D, E, F, G, H, I, J, K
INDIA
NEPAL
N
a
0 50 100 Kms

80°0'0"E
81°0'0"E
28°0'0"N

TGB II

PSFD- Pilibhit SFD
PTR- Pilibhit TR
KWLS- Kishanpur WLS
DNP- Dudhwa NP
KGWLS- Katarniaghat WLS
NKFD- North Kheri FD
SKFD- South Kheri FD

PSFD, PTR, K, L, DNP, M, KWLS, KGWLS, NKFD, SKFD
INDIA
NEPAL
N
b
0 25 50 Kms

84°0'0"E
27°0'0"N

TGB III

W-SGLWS- Western Sohagibarwa WLS
E-SGLWS- Eastern Sohagibarwa WLS
VTR- Valmiki TR

VTR, W-SGLWS, E-SGLWS
INDIA
NEPAL
N
c
0 25 50 Kms

Legend

- Protected Areas
- Forest Divisions
- International Boundary

Gene Flow

- Unidirectional Lower Gene Flow
- Unidirectional Higher Gene Flow
- Bi-directional Higher Gene Flow

Service layer credit: Global Hillshade, ESRI

Corridors

A. Yamuna river
B. Kansrau-Barkot
C. Chilla-Motichur
D. Rajaji-Corbett
E. Kalagarh
F. Kosi river
G. Boar river
H. Nihal-Bhakra
I. Gola river
J. Kilpura-Khatima-Surai
K. Lagga bagga -Shuklaphanta-Tatarganj
L. Kishanpur-Dudhwa
M. Dudhwa-Katarniaghat

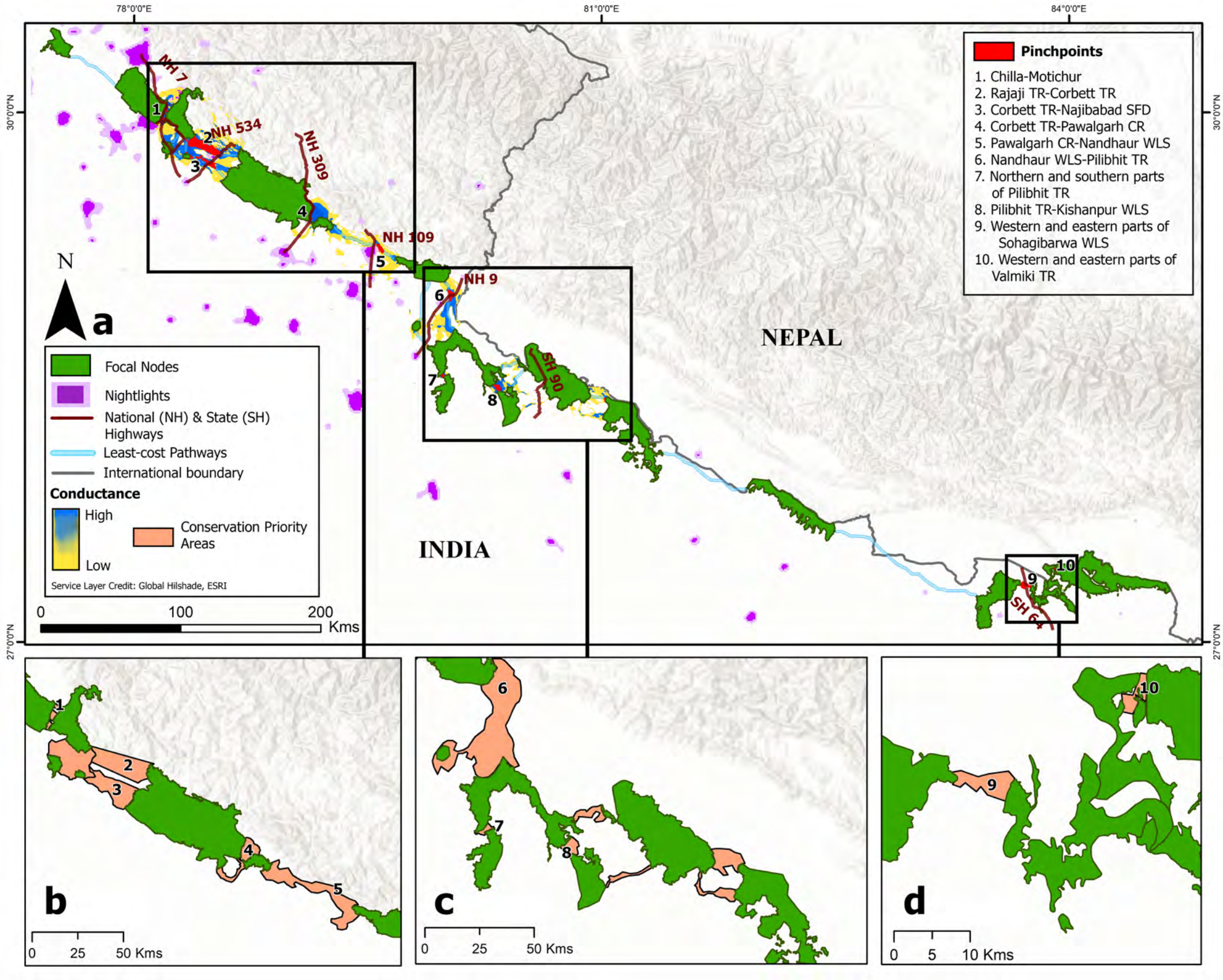

78°0'0"E
81°0'0"E
84°0'0"E
30°0'0"N
27°0'0"N
Pinchpoints
1. Chilla-Motichur
2. Rajaji TR-Corbett TR
3. Corbett TR-Najibabad SFD
4. Corbett TR-Pawalgarh CR
5. Pawalgarh CR-Nandhaur WLS
6. Nandhaur WLS-Pilibhit TR
7. Northern and southern parts of Pilibhit TR
8. Pilibhit TR-Kishanpur WLS
9. Western and eastern parts of Sohagibarwa WLS
10. Western and eastern parts of Valmiki TR
NH 7
NH 534
NH 309
NH 109
NH 9
SH 90
SH 64
NEPAL
INDIA
N
a
Focal Nodes
Nightlights
National (NH) & State (SH) Highways
Least-cost Pathways
International boundary
Conductance
High
Low
Conservation Priority Areas
Service Layer Credit: Global Hilshade, ESRI
0 100 200 Kms
b
0 25 50 Kms
c
0 25 50 Kms
d
0 5 10 Kms

Table 1: Genetic diversity and genotyping error details for the individual tigers (n=219) from TAL.

Locus	Repeat length	N_A	ASR	H_E	H_O	Cumulative $PID_{(unbiased)}$	Cumulative $PID_{(sibs)}$	ADO	FA	Success rate (%)	Reference
FCA090	2	19	38	0.88	0.31	2.266e-02	3.142e-01	0.011	0.030	77	[35]
msFCA506	2	12	38	0.85	0.26	7.991e-04	1.045e-01	0.003	0.050	59	[36]
FCA672	2	15	42	0.87	0.58	2.036e-05	3.330e-02	0.008	0.037	94	[35]
FCA304	2	14	42	0.73	0.17	2.305e-06	1.373e-02	0.003	0.024	92	[35]
FCA628	2	20	54	0.83	0.41	1.007e-07	4.721e-03	0.012	0.026	53	[35]
FCA232	2	16	32	0.70	0.27	1.340e-08	2.042e-03	0.005	0.028	97	[35]
FCA230	2	13	52	0.82	0.12	7.273e-10	7.226e-04	0.010	0.026	94	[35]
FCA279	2	16	36	0.87	0.51	1.761e-11	2.298e-04	0.000	0.031	91	[35]
FCA441	4	14	60	0.77	0.54	1.301e-12	8.834e-05	0.014	0.030	75	[35]
FCA069	2	16	40	0.79	0.30	7.176e-14	3.248e-05	0.014	0.014	79	[35]
msFCA453	4	12	72	0.71	0.62	8.955e-15	1.374e-05	0.010	0.010	94	[36]
msF115	4	09	48	0.60	0.08	1.869e-15	6.860e-06	0.019	0.019	56	[36]
msHDZ170	2	16	36	0.86	0.33	5.999e-17	2.245e-06	0.045	0.046	83	[36]
Mean		**14.76 (2.83)**	**45.38 (10.96)**	**0.79 (0.08)**	**0.35 (0.16)**			**0.012**	**0.028**	**80.3**	

N_A- Number of alleles, ASR- Allelic size range, H_E- Expected heterogygosity, H_O- Observed heterogygosity, PID- Probability of identity, ADO-Allelic drop out, FA- False allele

Table 2: Genetic differentiation (pairwise F_{st} and G_{st}) for three TGBs across TAL. The upper diagonal presents pairwise G_{st} values and lower diagonal presents the pairwise F_{st} values

	TGB I	**TGB II**	**TGB III**
TGB I	0	0.078*	0.067*
TGB II	0.089*	0	0.087*
TGB III	0.079*	0.107*	0

* p-value= 0.001

Table 3: Comparison of different genetic diversity indices among three identified TGBs in TAL.

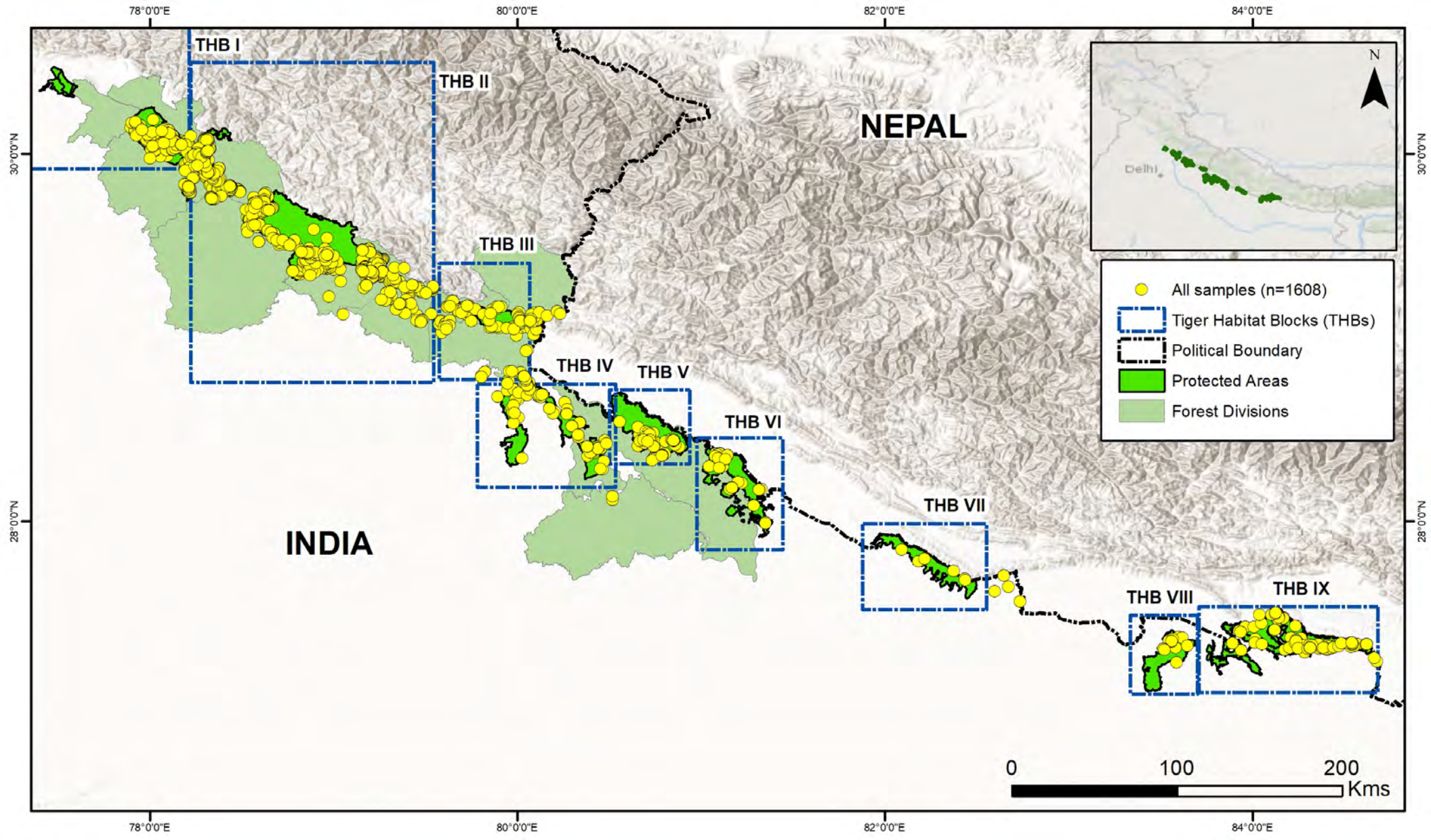
	TGB I (n=173)				TGB II (n=24)				TGB III (n=22)			
Locus	**N_A**	**ASR**	**H_E**	**H_O**	**N_A**	**ASR**	**H_E**	**H_O**	**N_A**	**ASR**	**H_E**	**H_O**
FCA090	12	28	0.85	0.31	08	18	0.70	0.29	09	28	0.78	0.37
msFCA506	11	24	0.83	0.21	08	16	0.72	0.42	08	34	0.84	0.38
FCA672	13	28	0.87	0.62	10	24	0.88	0.27	12	42	0.90	0.63
FCA304	11	28	0.73	0.14	05	20	0.62	0.30	05	10	0.73	0.32
FCA628	13	30	0.79	0.39	09	36	0.89	0.43	09	54	0.79	0.59
FCA232	11	22	0.68	0.20	07	20	0.53	0.43	11	32	0.81	0.71
FCA230	09	26	0.80	0.10	06	18	0.72	0.04	10	52	0.76	0.36
FCA279	13	24	0.86	0.53	09	22	0.59	0.20	11	32	0.77	0.73
FCA441	11	40	0.75	0.57	06	36	0.74	0.31	07	60	0.83	0.53
FCA069	03	26	0.71	0.33	10	28	0.89	0.30	09	32	0.84	0.15
msFCA453	04	08	0.65	0.70	05	20	0.69	0.38	10	60	0.82	0.33
msF115	10	16	0.44	0.03	05	48	0.74	0.35	05	24	0.67	0.06
msHDZ170	05	18	0.85	0.35	03	16	0.24	0.08	12	34	0.90	0.36
Mean	**10.15 (3.06)**	**24.46 (7.37)**	**0.75 (0.11)**	**0.34 (0.20)**	**07.00 (2.11)**	**24.77 (9.30)**	**0.69 (0.17)**	**0.29 (0.12)**	**9.08 (2.24)**	**38.00 (14.29)**	**0.80 (0.06)**	**0.43 (0.20)**

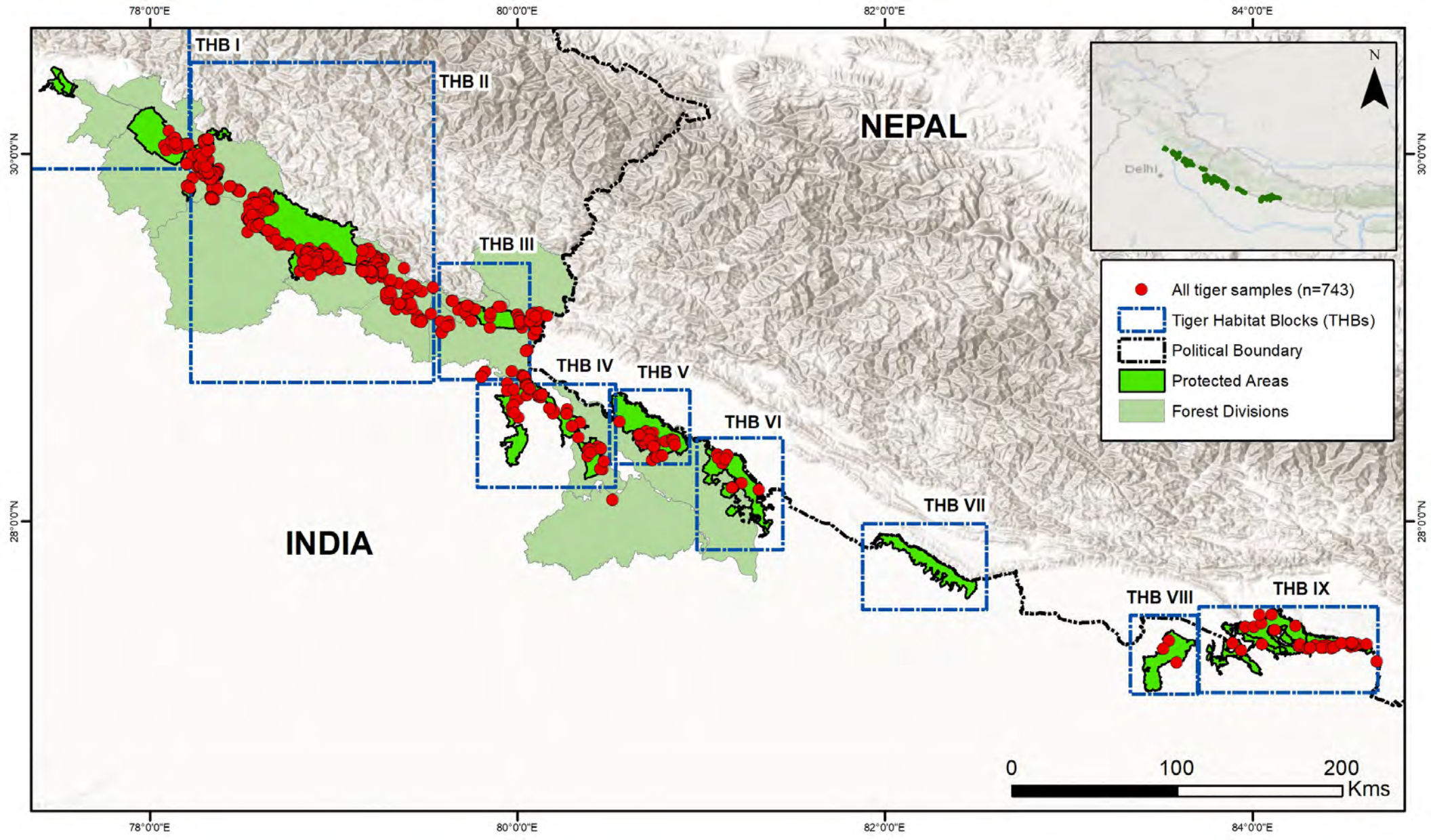
N_A- Number of alleles, ASR- Allelic size range, H_E- Expected heterozygosity, H_O- Observed heterozygosity

Table 4: Details of the critical corridor regions identified in this study.

SL	Geographic location	Area (sq.km.)	State
1	Connecting western and eastern parts of Rajaji TR (northern block)	9.73	Uttarakhand
2	Connecting western and eastern parts of Rajaji TR (central block)	14.12	Uttarakhand
3	Connecting western and eastern parts of Rajaji TR (southern block)	9.74	Uttarakhand
4	Connecting eastern Rajaji TR, Jhilmil Jheel CR and Najibabad SFD through Haridwar and Lansdowne FDs	345.07	Uttarakhand and Uttar Pradesh
5	Connecting Corbett TR and Najibabad SFD through Lansdowne FD and Najibabad SFD	267.16	Uttarakhand and Uttar Pradesh
6	Connecting Rajaji and Corbett TRs through Lansdowne FD	328.32	Uttarakhand
7	Connecting Corbett TR and Pawalgarh CR through Terai West FD	53.54	Uttarakhand
8	Connecting Corbett TR and Pawalgarh CR through Ramnagar FD	84.66	Uttarakhand
9	Connecting Pawalgarh CR and Nandhaur WLS through Ramnagar, Haldwani and Terai East FDs	405.37	Uttarakhand
10	Connecting Nandhaur WLS and Pilibhit TR through Haldwani and Terai East FDs and Pilibhit SFD	828.77	Uttarakhand and Uttar Pradesh
11	Connecting norther and southern parts of Pilibhit TR	17.4	Uttar Pradesh
12	Connecting Pilibhit TR and Dudhwa NP through North Kheri FD	56.76	Uttar Pradesh
13	Connecting Pilibhit TR and Kishanpur WLS through North Kheri FD	44.88	Uttar Pradesh
14	Connecting Kishanpur WLS and Dudhwa NP through North Kheri FD	34.83	Uttar Pradesh
15	Connecting Dudhwa NP and Katarniaghat WLS through North Kheri FD (northern part)	115.42	Uttar Pradesh
16	Connecting Dudhwa NP and Katarniaghat WLS through North Kheri FD (southern part)	64.46	Uttar Pradesh
17	Connecting western and eastern parts of Sohagibarwa WLS	18.41	Uttar Pradesh
18	Connecting western and eastern parts of Valmiki TR	8.9	Bihar
	Total	**2707.54**	

TR- Tiger Reserve, CR- Conservation Reserve, SFD- Social Forestry Division, FD- Forest Division, WLS- Wildlife Sanctuary, NP- National Park


78°0'0"E
80°0'0"E
82°0'0"E
84°0'0"E
30°0'0"N
28°0'0"N
THB I
THB II
THB III
THB IV
THB V
THB VI
THB VII
THB VIII
THB IX
NEPAL
INDIA
N
Delhi
All samples (n=1608)
Tiger Habitat Blocks (THBs)
Political Boundary
Protected Areas
Forest Divisions
0
100
200
Kms


78°0'0"E
80°0'0"E
82°0'0"E
84°0'0"E
30°0'0"N
28°0'0"N
THB I
THB II
THB III
THB IV
THB V
THB VI
THB VII
THB VIII
THB IX
NEPAL
INDIA
N
Delhi
All tiger samples (n=743)
Tiger Habitat Blocks (THBs)
Political Boundary
Protected Areas
Forest Divisions
0
100
200
Kms

Supplementary Table S1: Information of tiger populations, including habitat block details, state, protection status, corridors and identified genetic subpopulations (TGBs) across TAL.

SL No	THB	State	PA, FD, SFD and Corridors	Tiger presence (Unique tigers) (Reference)	TGB
1	THB I	HP	PA: Simbalbara NP	No (a)	
			Corridor: Yamuna river	No (b)	
		HR	PA: Kalesar WLS	No (a)	
		UP	FD: Shivalik FD	No (b)	
		UK	PA: Rajaji TR (western part)	Yes (2) (a)	TGB I
			FD: Dehradun FD	No (b)	
			Corridor: Kansrau-Barkot, Chilla-Motichur	No (b), No (b)	TGB I
2	THB II	UP	PA: Amangarh TR	Yes (20) (a)	TGB I
			SFD: Najibabad SFD	Yes (5) (e)	
		UK	PA: Rajaji TR (eastern part), Jhilmil Jheel CR, Corbett TR, Pawalgarh CR	Yes (37) (a), Yes (2) (e), Yes (231) (a), Yes (c)	
			FD: Haridwar FD, Lansdowne FD, Terai West FD, Ramnagar FD, Terai Central FD	No (b), Yes (34) (a), Yes (39) (a), Yes (37) (a), Yes (5) (a)	
			Corridors: Rajaji-Corbett, Kalagarh, Kosi river, Boar river, Nihal-Bhakra, Gola river	Yes (b), Yes (b), Yes (b), Yes (b), Yes (b), No (b)	
3	THB III	UK	PA: Nandhaur WLS	Yes (c)	
			FD: Haldwani FD, Champawat FD, Terai East FD	Yes (22) (a), Yes (9) (a), Yes (23) (a),	
			Corridor: Kilpura-Khatima-Surai	Yes (d)	
		UP	SFD: Pilibhit SFD	Yes (2) (e)	TGB II
4	THB IV	UK	FD: Terai East (eastern part)	Yes (c, e)	
		UP	PA: Pilibhit TR, Kishanpur WLS (part of Dudhwa TR)	Yes (57) (a), Yes (33) (a)	
			FD: North Kheri FD, South Kheri FD	No (a, e), Yes (2) (a)	
			SFD: Shahjahanpur SFD	No (b),	
			Corridor: Lagga Bagga-Shuklaphanta-Tatarganj, Kishanpur-Dudhwa	Yes (b), Yes (b),	
5	THB V	UP	PA: Dudhwa NP (part of Dudhwa TR)	Yes (20) (a)	
			Corridor: Dudhwa-Katerniaghat	No (b)	
6	THB VI	UP	PA: Katerniaghat WLS (part of Dudhwa TR)	Yes (29) (a)	
7	THB VII	UP	PA: Suhelwa WLS	No (a)	
8	THB VIII	UP	PA: Sohagibarwa WLS (western part)	Yes (a)	TGB III
9	THB XI	UP	PA: Sohagibarwa WLS (eastern part)	Yes (a)	
		BR	PA: Valmiki TR	Yes (33) (a)	

THB- Tiger habitat block, PA- Protected area, FD- Forest division, SFD- Social forestry division TGB - Tiger genetic block, NP- National park, WLS- Wildlife sanctuary, TR- Tiger reserve, CR- Conservation reserve, HP- Himachal Pradesh, HR- Haryana, UK- Uttarakhand, UP- Uttar Pradesh, BR- Bihar.

a= Jhala et al. 2020, b= Johnsingh et al. 2004, c= Jhala et al. 2015, d= Anwar and Borah 2020, e= This study

Supplementary Table S2: Details of habitat variables used to model tiger habitat permeability in TAL.

Source file	Covariates used	Source	Unit
Forest cover	Distance from open/dense forest	Forest survey of India	Meter
Protected area (PA)	Distance from PA	World database of protected areas	Meter
Road network	Distance from road	Diva-GIS	Meter
Settlement	Distance from settlement	Columbia-Village data	Meter
River network	Distance from river	Diva-GIS	Meter

Supplementary Table S3: Details of nodes and their weightages used in Circuitscape analysis.

SL	Name of the node	Protection status of nodes	Weightage (0-1)
1	Kalesar-Simbalbara complex	Protected area	0
2	Rajaji TR- western part	Protected area	0.50
3	Rajaji TR- eastern part	Protected area	1
4	Jhilmil Jheel CR	Protected area	0.25
5	Najibabad SFD	Non-protected area	0.25
6	Corbett TR	Protected area	1
7	Pawalgarh CR	Protected area	1
8	Nandhaur WLS	Protected area	0.75
9	Pilibhit SFD	Non-protected area	0.50
10	Pilibhit TR	Protected area	0.75
11	Pilibhit TR- southern part	Protected area	0.25
12	Kishanpur WLS	Protected area	0.75
13	Dudhwa NP	Protected area	0.75
14	Katarniaghat Wildlife Sanctuary	Protected area	0.25
15	Suhelwa Wildlife Sanctuary	Protected area	0
16	Sohagibarwa WLS- western part	Protected area	0.25
17	Sohagibarwa WLS- eastern part	Protected area	0.75
18	Sohagibarwa WLS- southern part	Protected area	0
19	Valmiki TR- western part	Protected area	0.75
20	Valmiki TR- central part	Protected area	0.75
21	Valmiki TR- eastern part	Protected area	0.75

TR= Tiger Reserve, CR= Conservation Reserve, SFD= Social Forestry Division, WLS= Wildlife Sanctuary, NP= National Park

Supplementary Table S4: Detail of the first-generation migrant tigers identified through GENECLASS and STRUCTURE in this study.

SL	Sampled location	Migrant from	GENECLASS likelihood computation (L home/L max)	*p* Resident	STRUCTURE USEPOPINFO (MIGPRIOR 0.05)
			TGB I		
1	Rajaji TR	Lansdowne FD	3.645	0.003	NA
2	Rajaji TR	Lansdowne FD	3.695	0.004	NA
3	Lansdowne FD	Rajaji TR	8.089	0.000	NA
4	Haridwar FD	Lansdowne FD	NA	NA	0.936
5	Haridwar FD	Lansdowne FD	NA	NA	0.937
6	Terai West FD	Terai Central FD	7.816	0.001	NA
7	Terai West FD	Terai Central FD	5.653	0.006	NA
8	**Ramnagar FD**	**Terai West FD**	**6.329**	**0.006**	**0.945**
9	Ramnagar FD	Terai Central FD	6.307	0.004	NA
10	**Haldwani FD**	**Ramnagar FD**	**2.347**	**0.006**	**0.960**
11	Champawat FD	Terai Central FD	4.993	0.000	NA
12	Terai East FD	Haldwani FD	2.374	0.000	NA
13	Corbett TR	Ramnagar FD	NA	NA	0.828
14	Corbett TR	Ramnagar FD	NA	NA	0.820
15	Corbett TR	Ramnagar FD	NA	NA	0.922
16	Amangarh TR	Najibabad SFD	NA	NA	0.995
17	Haldwani FD	Ramnagar FD	NA	NA	0.994
			TGB II		
18	Pilibhit TR	Dudhwa NP	4.293	0.008	NA
19	Pilibhit TR	Dudhwa NP	4.259	0.010	NA
20	Kishanpur WLS	Dudhwa NP	2.647	0.000	NA
21	**Katarniaghat WLS**	**Pilibhit TR**	**3.773**	**0.000**	**0.981**
22	Pilibhit SFD	Kishanpur WLS	NA	NA	0.985
23	Pilibhit SFD	Kishanpur WLS	NA	NA	0.986
24	Dudhwa NP	Pilibhit TR	NA	NA	0.942
25	Dudhwa NP	Pilibhit TR	NA	NA	0.985
26	South Kheri FD	Dudhwa NP	NA	NA	0.984
27	South Kheri FD	Dudhwa NP	NA	NA	0.989

TGB III					
28	Valmiki TR	Sohagibarwa WLS	5.204	0.001	NA
29	Valmiki TR	Sohagibarwa WLS	2.799	0.007	NA

TGB= Tiger Genetic Block, TR= Tiger Reserve, NP= National Park, WLS= Wildlife Sanctuary, FD= Forest Division, SFD= Social Forestry Division

Supplementary Table S5: Contribution of habitat variables in tiger dispersal across TAL.

Environmental variables	Contribution (%)
Distance from forest	66.8
Distance from protected areas	23.8
Distance from settlements	4.6
Distance from river	3.3
Distance from road	1.5

Supplementary Table S6: Details of least-cost pathways, corridor conductance and critical corridors of tiger dispersal identified across the TAL

SL	Corridors identified between	Length of the Least-cost pathways (km)	Conductance of corridors	Critical corridors
1	Simbalbara-Kaleser complex and Rajaji TR (western part)	41.5	NA	NA
2	Kansrau (western part of Rajaji TR) and Barkot (Dehradun FD)	No	Low	No
3	Chilla (eastern part) and Motichur (western part) ranges of Rajaji TR	3.9	High	Yes
4	Rajaji TR (eastern part) and Jhilmil Jheel CR	9.5	High	No
5	Rajaji TR (eastern part) and Najibabad SFD	9.9	High	No
6	Jhilmil Jheel CR and Najibabad SFD	9.5	High	No
7	Rajaji TR (eastern part) and Corbett TR	36.6	High	Yes
8	Corbett TR and Najibabad SFD	31.2	High	Yes
9	Corbett TR and Pawalgarh CR	1.9	High	Yes
10	Terai West and Terai Central FDs	NA	Low	No
11	Terai Central FD and Ramnagar FDs	NA	Low	No
12	Pawalgarh CR and Nandhaur WLS	53.8	Low	Yes
13	Nandhaur WLS and Pilibhit SFD	32.2	NA	NA
14	Nandhaur WLS and Pilibhit TR	38.6	Medium	Yes
15	Pilibhit SFD and Pilibhit TR	14	NA	NA
16	Northern and southern parts of Pilibhit TR	1.4	High	Yes
17	Pilibhit TR and Kishanpur WLS	5.6	High	Yes
18	Pilibhit TR and Dudhwa NP	14.8	Low	No
19	Kishanpur WLS and Dudhwa NP	16.2	Medium	No
20	Dudhwa NP and Katarniaghat WLS	11.5	Medium	No
21	Katarniaghat WLS and Suhelwa WLS	71.2	NA	NA
22	Suhelwa WLS and west Sohagibarwa WLS	120.3	NA	NA
23	Western and east Sohagibarwa WLS	7.4	Low	Yes
24	Western and eastern parts of Valmiki TR	0.5	High	Yes

TR- Tiger Reserve, CR- Conservation Reserve, SFD- Social Forestry Division, FD- Forest Division, WLS- Wildlife Sanctuary, NP- National Park