UNDERSTANDING SLOTH BEAR [*MELURSUS URSINUS*] GENETIC CONNECTIVITY ACROSS VIDARBHA LANDSCAPE, MAHARASHTRA, INDIA

THESIS
SUBMITTED TO THE
FOREST RESEARCH INSTITUTE (DEEMED TO BE) UNIVERSITY
DEHRADUN, UTTARAKHAND
FOR
THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN FORESTRY
(WILDLIFE SCIENCE)

BY
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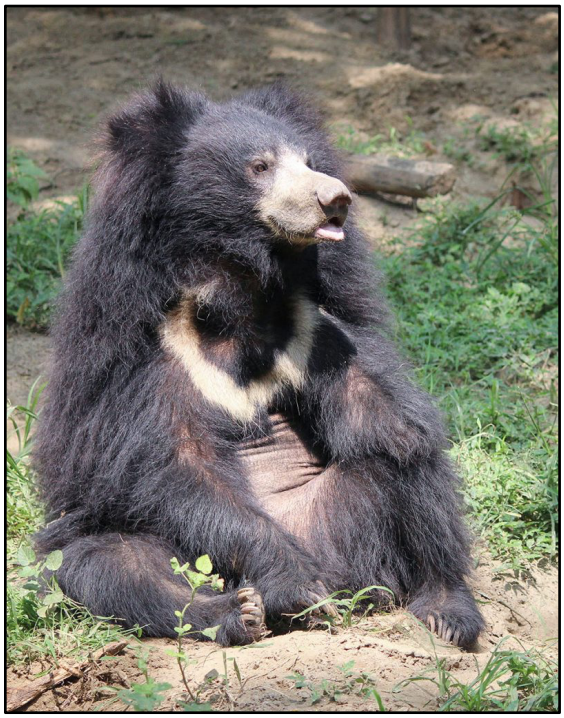
2024

Dedicated to….

ELVIS AND ROSE

- 

- 

- *Elvis lost his mother to illegal trading and Rose not only lost her mother but also a paw to illegal hunting. Both are currently housed at Wildlife SOS, Agra Sloth bear Rescue facility. Met them in 2016-2017 and started admiring sloth bears from day 1!*

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This is to certify that Ms. Lynette Gomes (Enrolment No. 17PHD460), carried out research work under Dr. Bilal Habib of Wildlife Institute of India. The topic of the research registered with Forest Research Institute (Deemed to be University) was " **Understanding sloth bear (*Melursus ursinus*) Genetic Connectivity across Vidarbha Landscape, Maharashtra, India**".The research scholar presented her work in the pre-thesis submission seminar held on 23rd February 2024, and the RAC found the work to be satisfactory and approved the work to be presented in the form of a thesis for evaluation by examiners for the "Award of Ph.D. Degree" by FRI, Dehradun.

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EXECUTIVE SUMMARY

In the face of the global Anthropocene range contraptions of different species both terrestrial and marine have increased. .Loss of habitat and fragmentation are thought to be the main causes of the decline in biodiversity. There is a longer-term increase in the likelihood of extinction when sprawling, connected forests are divided into isolated, human-inhabited areas. Loss of genetic diversity and an increase in random events are the key causes of the increased probability of extinction. Thus, the only feasible strategy for preserving population connectedness in the wild is to preserve the populations themselves. Strips of habitat that link fragments of habitat are called corridors. advancing the concepts of ecological integrity, ecological connectivity, wildlife corridors, and comprehensive landscape matrix conservation, large landscape conservation has emerged over the past three decades as a science-based response to increasing large-scale habitat fragmentation and degradation in the face of global threats

The goal of this thesis is to use non-invasive DNA sampling to study how sloth bears roam throughout the Vidarbha region of Maharashtra, India. Maharashtra's Vidarbha Landscape is a quickly changing area. The great biodiversity that lives there is under risk from the quick changes in the landscape. It's vital to note that there are about 200 instances of deadly human-sloth bear interactions in Maharashtra alone each year, and that number could increase in the years to come due to increased development activities in the area. Wide ranging or long dispersing species who have extensive movements across a landscape are the ones most affected by range contraptions and fragmentation of the habitat. The broad objectives of this thesis were- 1. Understand Genetic Structure, Variation and relatedness

amongst sloth bear populations in Vidharbha Landscape and 2. Evaluate genetic relatedness with respect to permeability of the landscape and connectivity.

For my study I employed microsatellite markers keeping in mind the funding and the laboratory facilities available with me. Sloth bear specific markers are not available as of now hence I used markers that have been standardized on other bear species both for microstaellites and sex markers. Softwares such as CERVUS, GIMLET have been used calculate probability of identity, expected, and observed hetrozygosity, number of alleles, false alleles, and allelic dropout. The probability of identity (siblings) in this study is 1.0e-05 which is sufficient to conduct a large-scale spatial study.The standardized sexing markers gave us a three-band pattern in males (100bp, 191bp, 245bp) and 1 band in females (245 bp) and was used for sexing data generation in the forthcoming chapters.

The standardized markers were used for population level study in the landscape. 565 samples had been collected from of the study areas from 2016-2019. For data analyses for metapopulation dynamics the genetic softwares used were CERVUS, MICROCHECKER, GIMLET, STRUCTURE, TESS,GENPOP ,ARLEQUIN, BAYESASS, R- software with packages- DiveRsity, adegenet and DAPC.The outputs showed a clustering of k=5 based on TESS output showing admixture between PTR, NNTR and UKWLS . Signatures of MTR was seen in STR which has been discussed and has been attributed to the role of probable stepping stone forests. The G'st value ranged from 0.14- 0.40. the highest differentiation was found to be between MTR and PTR (0.40) while the lowest was found to be between NNTR and MTR. Similarly, Jost'D varied between 0.005-0.178. The rate of migration was represented by the circus plot by the proportion of individuals migrating was found to be the highest from the NNTR to PTR (0.214) and from TATR to UKWLS (0.177).

I further tried to undertstand whether or not bears showed sex biased dispersal. I had hypothesized that like other bears and owing to the maternal role in caregiving for the

offspring, sloth bears may show a male-biased dispersal. To understand that I used Fis, Fst, Relatedness , Assisgnment and Spatial autocorrelation indices. My results supported my hypothesis. I obtained a high Fis and vAIC but a lower Fst, mAIC and relatedness for the dispersing males than the more philopatric females.

Next for my landscape, I wanted to understand the factors that affect or influence the habitat use of sloth bears is important when one to looks at the movement of sloth bears. Camera trapping has been conducted in different protected areas with 2406 stations. Presence absence data from this was taken along with covariates at three spatial scale 0m, 250m and 500m.R packages MuMIN with dredging was done to obtain the best models and covariates that explained the occupancy of sloth bears. Across all three spatial scales, the top contributing models were selected and included disturbance covariates (population, livestock, nightlight, and distance from road) and environmental factors (temperature, elevation, forest type and NDVI).

Using genetic data and the covariable generated I ran connectivity using CIRCUITSCAPE in JULIA interface. The surfaces were optimized in single and composite surfaces. It was established through the modelling that distance from roads NDVI and population had the most impact on sloth bear movement. Therefore, these features are strong influencers of geneflow across the landscape. Using the top models generated after optimization of the surfaces with the genetic distance data, connectivity map and pinch point maps were generated and presented. The heat map generated along with the pinchpoint map shows that the landscape is highly permeable for movement but also has greater number of pinchpoints owing to which the access to the greater landscape might be hindered.

The functional corridor obtained for sloth bears is the main result of this thesis. This can be used in a number of ways by managers, planning bodies, and stakeholders. First, the occupancy and connection would assist indicate key movement regions and areas of concern when evaluated in conjunction with the documented incidents of sloth bear attacks. The pinchpoints could be focussed upon and conserved as intact corridors. Secondly, the movement areas could be modelled with documented bear attacks to highlight the probable corridors or movement areas that need awareness to avoid bear attacks.

In spite of being the most dreaded animals in the landscape with the highest number of attacks, sloth bears are overlooked in most management plans. It is imperative to produce more plans and management options keeping the species in mind.

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CHAPTER 1

Habitat Fragmentation and Connectivity Modelling

1.1 Introduction

Habitat fragmentation stands as one of the most pressing challenges in today's world, exerting profound impacts on ecosystems, biodiversity, and human societies. As landscapes continue to be altered by urbanization, agriculture, and infrastructure development, natural habitats become increasingly fragmented, leading to a plethora of ecological consequences that ripple through the intricate web of life.

1.1.a. Understanding Habitat Fragmentation

Habitat fragmentation refers to the process by which large, contiguous habitats are divided into smaller, isolated patches. These isolated patches are often connected to each other through a matrix of different habitats which differ from each other (Wilcove, McLellan and Dobson,1986). This division is often a result of human activities, such as the construction of roads, cities, and agricultural fields. It transforms once-cohesive ecosystems into a mosaic of disconnected patches, altering the balance between flora and fauna and disrupting the intricate interactions that have evolved over millennia. Researchers often confuse between habitat loss and habitat fragmentation and these two are very different, even though very much associated, according to one school of thought. Independent of habitat loss, habitat fragmentation should only be used to describe configuration changes in habitat that come from habitat breaking apart (Fahrig, 2003).

Biodiversity, the cornerstone of healthy ecosystems, suffers immensely due to habitat fragmentation. Numerous factors cause animals to migrate, including the pursuit of food, protection from predators and other agents of death, avoidance of competition (such as natal dispersal), and the desire to be close to conspecifics for mating and other social interactions

conspecific attraction (Fahrig, 2007). It is obvious that the many purposes of movement are connected to survival and procreation, and as a result, natural selection affects the factors that control movement. Species that were once able to move freely across their native habitats now find themselves confined to smaller areas. This restricted movement can lead to reduced access to resources like food, water, and mates, ultimately hampering reproductive success and genetic diversity.

Small and isolated populations are particularly vulnerable. They face a higher risk of inbreeding and genetic drift, which can lead to decreased fitness and an increased susceptibility to diseases and has already been seen in several species (Furlan, et al. 2012; Neaves, et al. 2015). Over time, this may result in a "genetic bottleneck," where the genetic diversity necessary for adaptation and survival is severely limited, putting species at greater risk of extinction.

1.1.b. Fragmentation and its effects on wide ranging species

Among the species most significantly affected by this phenomenon are wide-ranging species. These remarkable creatures, often characterized by their extensive movements across large areas, face dire challenges as their habitats become increasingly isolated and fragmented (Rabinowitz and Zeller, 2010).

Wide-ranging species, including large mammals like elephants, big cats, and certain bird species, require extensive territories to fulfil their biological needs (Brodies, et al. 2014). These needs encompass finding food, mates, and suitable areas for reproduction. Habitat fragmentation constrains their movements and can isolate populations, causing them to lose access to crucial resources. For instance, a tiger's range can span hundreds of square kilometres, and when these areas are divided by roads, urban sprawl, or agricultural expanses, the ability to

find sustenance and establish territories is severely curtailed (Joshi, et al. 2013; Karanth, et al. 2011). As a result, wide-ranging species often resort to using suboptimal habitats, leading to reduced reproductive success and overall fitness (Smith,1993).

Conserving wide-ranging species in the face of habitat fragmentation poses complex challenges. The preservation of large, contiguous habitats is vital, but often challenging due to competing human needs for land. Creating wildlife corridors—connective pathways between fragmented habitats—has emerged as a promising solution. These corridors allow animals to traverse human-altered landscapes, maintaining their movements and facilitating gene flow between populations.

1.1.c. Connectivity and Corridors

Connectivity plays a pivotal role in maintaining the delicate balance of ecosystems. As habitat fragmentation continues to carve up landscapes, conservationists turn to an ingenious solution: corridors. These ecological bridges, threading through the mosaic of isolated habitats, offer a lifeline for species, ensuring genetic diversity, movement, and resilience in the face of mounting challenges. The concept of “connectivity”, first surfaced in the field of landscape ecology in the 1984 and introduced by Dr.G.Merriam (Merriam, 1984). Down the years, with the onset of rapid urbanization and developmental activities the concept has found an important place to aid in counter-balancing the negative effects of developmental activities which cause habitat fragmentation. A particular landscape is perceived differently by different species (Taylor, et al. 1993). A landscape is considered to be highly permeable in terms of connectivity for a particular species, when individuals of that particular species can move freely from one habitat patch to another (Merriam, 1988). Therefore, any landscape may have low connectivity for one species while high connectivity for another species as shown in figure below. For example wide ranging canids may find it easier to move in the landscape over snakes or snails.

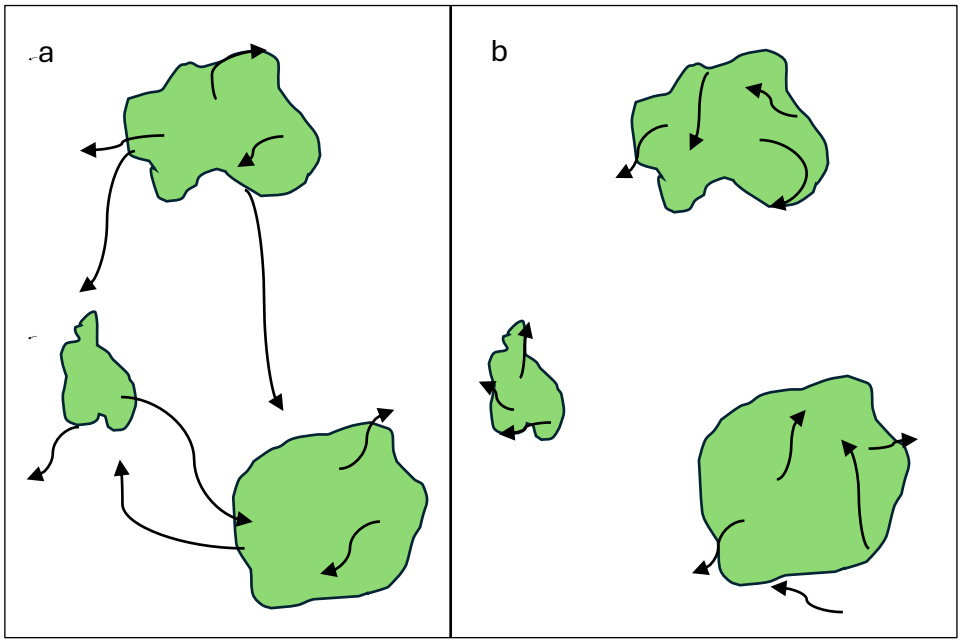

Figure 1a: A habitat may show higher connectivity for one species as depicted in a, but lower connectivity for another species, as shown in b.

Such functionality of connectivity is influenced by two very important factors for each species- (i) structure and (ii) behaviour of the species (Bennett,1990). The structural factor specifically refers to the spatial arrangement of various habitat types in a landscape Factors affecting it include the availability of adequate suitable habitat, gaps between patches, distance travelled by the species, and alternate pathways/networks that may be used by the travelling species (Forman,1983; Forman and Godron,1981).

The behavioural component that is also a major component of connectivity, refers to how individuals of a species respond to the physical structures that are present in the landscape. Factors influencing a species' behaviour include its perception and movement scale, habitat requirements and specialization, tolerance to disturbances, life stage and timing of dispersal, and response to predators and competitors in the landscape. Species with different behavioural reactions (e.g., to habitat disturbance) may perceive and behave differently in terms of connectivity in the focal landscape (Logan, et al. 2020; Gaston, 2003).

1.1.d. Modelling and Understanding Connectivity

In the quest to mitigate the impacts of habitat fragmentation and preserve biodiversity, conservationists have turned to a powerful tool: connectivity modelling. This innovative approach harnesses the power of technology, ecological data, and spatial analysis to map and predict how species move through landscapes, guiding the creation of effective corridors and connectivity networks. However, this entails understanding the how species disperse, the many requisites and barriers to dispersal of a species (Vasudev, et al. 2015).

Connectivity modelling is a computational technique that simulates the movement of species across landscapes. It involves integrating various types of data, such as habitat maps, species distribution information, topography, and human activities. Using simulations, conservationists can use to predict how different areas of habitat are connected, how species might move between them, and where potential barriers or bottlenecks exist. In fact, the study of the effect of connectivity is of immense scientific interests since managing and conserving sustainable landscapes depends on our ability to comprehend the variables that sustain or modify the local dynamics of organisms (Rudnick, et al. 2012). A well-connected environment results from successful dispersal of individuals, a life-history feature essential for reproduction and survival of many species (Baguette, et al. 2013).

1.1.e. Types of Connectivity Modelling

Several types of connectivity modelling techniques exist, each suited to different conservation goals and scales:

Circuit Theory: This approach is rooted in electrical circuit theory and conceptualizes landscapes as conductive surfaces. It considers how species might flow through habitats based on factors like resistance (difficulty of movement) and voltage (species movement motivation). Circuit theory can identify optimal pathways for connectivity.

Least-Cost Path Analysis: Least-cost (LC) modelling was first established in the context of geographic transport and consists of determining the path with the shortest cost distance among all potential paths connecting predefined sources and destinations. This technique calculates the path of least resistance for species movement between two points. It takes into account factors such as slope, distance, and habitat suitability, providing insights into potential corridors that offer minimal challenges to species.

Graph Theory: Graphs represent landscapes as networks of nodes (habitat patches) connected by edges (potential pathways). By analysing the structure of these networks, researchers can identify key patches and corridors crucial for maintaining connectivity.

1.1.f. Connectivity in Central Indian landscape

The connectivity of species is vital for maintaining ecological balance and biodiversity. It enables gene flow, promoting genetic diversity and resilience to environmental changes. Central India landscape is a vital landscape especially for the conservation of tiger. Several studies , in the landscape, have focussed upon understanding the connectivity with respect to tigers.

However it has also been established that this landscape is also an excellent habitat for a number of other mammals (Jhala, Qureshi and Yadav, 2021) and astonishingly only a handful of studies have tried to understand the connectivity of these mammals in the landscape. Three significant Central Indian corridor complexes are located in Maharashtra- Complex A (Melghat-Bor-Pench), Complex B (Umred Karandhla, NNTR, and TATR) and Complex C (Tipeshwar, Painganga, Chaprala, and Pranhita). However, these corridors have been designated based on tiger telemetry alone and highlights the specific ecological needs for movement of the big cat . Such traditional approaches of establishing corridors for conservation planning was based on the assumption the increase in number of corridors for bigger iconic species would automatically provide habitat connectivity for other species as well (Wang, et al. 2018). However, sympatric species requires varying configurations and scales of connectivity and therefore corridors based on a single species approach would not serve the purpose of other species (Breckheimer, et al. 2014). No such studies have been done for sloth bears and it is not sensible to plan management strategies based on corridor modelling conducted based on a single-species approach in spite of the landscape being shared by other long-dispersing sympatric species such as leopards, dholes and sloth bears

1.2 Study Area

A significant portion of the Central Indian landscape is made up of the western Indian state of Maharashtra. It has a tropical monsoon climate with dry summers and hot, chilly, and rainy seasons. 16.5 % of the state's total land area is covered by forests which is about 61,907.08 sq km. There are four different types of forests: Very Dense Forest (VDF), Moderately Dense Forest (MDF), Open Forest (OF), and Scrub Forests (SF).Maharashtra network of protected areas, which includes six tiger reserves, fifty wildlife sanctuaries, and fifteen conservation

reserves, accounts for 3.03 percent of the state's total land area. The three primary types of forests are moist teak forest, dry teak forest, and southern moist mixed deciduous forest.

1.2.a. Tadoba Andhari Tiger Reserve (TATR)

Tadoba Andhari Tiger Reserve (henceforth known as TATR in the thesis), the second officially recognized tiger reserve, was created in 1995 and is in the Chandrapur district of Maharashtra, Central India. Located in the Central Plateau Province of the Deccan Peninsula biogeographic zone, it is a mega-biodiversity hotspot. The park is situated at an elevation of 188 meters between 79° 11' 50" E and 20° 29' 44" N. It is distinguished by a southern tropical dry-deciduous forest that is home to most of the Maharashtra's tiger population. It has a diverse range of flora and animals, such as little Indian civets, tigers, leopards, sloth bears, gaurs, dholes, nilgai, teak, ain, bija, arjun, and bamboo. The reserve has a total area of 1727.59 square kilometres (625.82 square kilometres in the core; 1101.77 square kilometres in the buffer).

1.2.b. Nawegaon- Nagzira Tiger Reserve (NNTR)

The Gondia and Bhandara districts of Maharashtra are home to the Nawegaon-Nagzira Tiger Reserve (henceforth referred to as NNTR), which has an area of 1894.94 sq. km. (core: 653.67 sq. km.; outer: 1241.27 sq. km.) (NTCA 2020). In the Vidarbha region, which is the easternmost section of Maharashtra, the reserve is known as a "Green Oasis" because of its wet deciduous forest. In 2012, this area was designated as a tiger reserve. With a relatively low population of tigers, large dhole and sloth bear population, the reserve is rich in biodiversity. The highest point of the uneven terrain is approximately 702 meters above sea level.

1.2.c. Pench Tiger Reserve (PTR)

Pench Tiger Reserve (henceforth known as PTR) is situated in the Satpuda-Maikal hills of the Nagpur district, bordering Madhya Pradesh on one side and the district on the other. (NTCA 2020) Its entire area is 741.22 sq. km. (buffer: 483.96 sq. km.; core: 257.26 sq. km.). The Pench River, which flows 74 kilometres through the reserve from north to south, is the source of the reserve's name. The Maharashtra state proclaimed it a tiger reserve in 1999. The reserve's predominant forest type is southern tropical dry-deciduous forest, which is home to a diverse array of plant and animal life. Animals such as sambar, chital, barking deer, leopard, tiger, dhole, sloth bear, and other animals are among the main wildlife. Conversely, the predominant flora consists of teak, ain, haldu.

1.2.d. Melghat Tiger Reserve (MTR)

Melghat Tiger Reserve (henceforth known as MTR), is in the Vidarbha area of Central India, bordering Madhya Pradesh to the north and east, in the southern offshoot of the Satpura hill ranges. Geographically speaking, it is situated between 21°26′45″N and 77°11′50″E, 312 M to 1178 M above mean sea level. With a total area of 2768.52 sq. km. (core: 1500.49 sq. km.; buffer: 1268 sq. km.), it was established as a tiger reserve in 1974. It was the first in Maharashtra and among the largest in the world. (NTCA 2020) (NTCA 2020). Melghat, which derives its name from the convergence of several "ghats," or valleys, is distinguished by a tropical dry-deciduous forest type that is primarily dominated by *Tectona grandis*. MTR is inhabited by a number of different species of animals including tigers, leopards, sloth bears, gaur, chital, sambar, dhole, flying squirred, forest owlet to name a few.

1.2.e. Sahyadri Tiger Reserve (STR)

The Sahyadri Tiger Reserve (henceforth known as STR), is a crucial biodiversity hotspot located in the Western Ghats of India. Spread across the states of Maharashtra and Karnataka, this reserve spans over 1,600 square kilometres and is recognized for its rich flora and fauna. Established in 2008, STR aims to protect the endangered species inhabiting the region, with a primary focus on the conservation of tiger. The biodiversity within the reserve includes a variety of plant species, mammals, reptiles, and birds, making it a haven for nature enthusiasts and researchers alike. Apart from tigers, the reserve is home to leopards, gaur, chital, and numerous other wildlife species.

1.2.f. Bor Tiger Reserve (BTR)

The Bor Tiger Reserve (henceforth known as BTR), located in the Indian state of Maharashtra, spans over 138.12 square kilometres and is a crucial wildlife habitat. Established in 1970, it gained the status of a tiger reserve in 2014. The reserve is renowned for its diverse flora and fauna, housing not only the majestic tigers but also leopards, sloth bears and deer species. Bor is situated centrally and is surrounded by other important protected habitats such as Pench Tiger Reserve and Navegaon Nagzira Tiger Reserve to the north-eastern side, Umred Karhandla Wildlife Sanctuary and Tadoba Andhari Tiger Reserve to the south-eastern side and Melghat Tiger Reserve towards the North-Western side.

1.2.g. Umred -Karhandla Wildlife Sanctuary (UKWLS)

Umred Karhandla Wildlife Sanctuary (Henceforth referred to as UKWLS) , located around 58 kilometres from the city of Nagpur and about 60 kilometres from Bhandara. UKWLS is connected to Tadoba Andhari Tiger Reserve via the forest along the Wainganga River. The

sanctuary is home to a number of different species including resident breeding tigers, leopards, gaur, wild dogs, sloth bears and other cervids.

1.2.1 Rationale for Selecting the Study sites

Selecting the study area for understanding the connectivity of sloth bears among the specified protected areas is crucial for effective conservation efforts. These areas are geographically proximate, creating a network of habitats that sloth bears can traverse, which facilitates movement and gene flow essential for maintaining genetic diversity. Each area offers suitable habitats, including forests and grasslands, providing insights into their movement patterns and habitat preferences (Refer figure 1 b and 1c). Identifying connectivity can help establish conservation corridors, enabling safe movement between habitats and reducing the risks of inbreeding. The study will also address the impacts of human activities, such as agriculture and urbanization, informing management strategies to mitigate human-wildlife conflict. The diverse ecosystems within these protected areas support varied wildlife, enhancing our understanding of sloth bear interactions with other species. Many of these regions are already the focus of conservation research, allowing for a more comprehensive understanding of sloth bear ecology. Additionally, evaluating connectivity will help assess threats like habitat fragmentation and poaching, guiding targeted conservation actions. The findings will inform local and regional wildlife management policies, contributing to effective conservation frameworks that benefit both sloth bears and surrounding biodiversity. Overall, this study in the selected sites, will significantly enhance our understanding of sloth bear ecology and support their conservation efforts.

Figure 1b: Picture shows monsoon in the landscape at Melghat Tiger Reserve

Figure 1c: A glimpse of the landscape showing dry and sparse vegetation at Tadoba Andhari Tiger Reserve

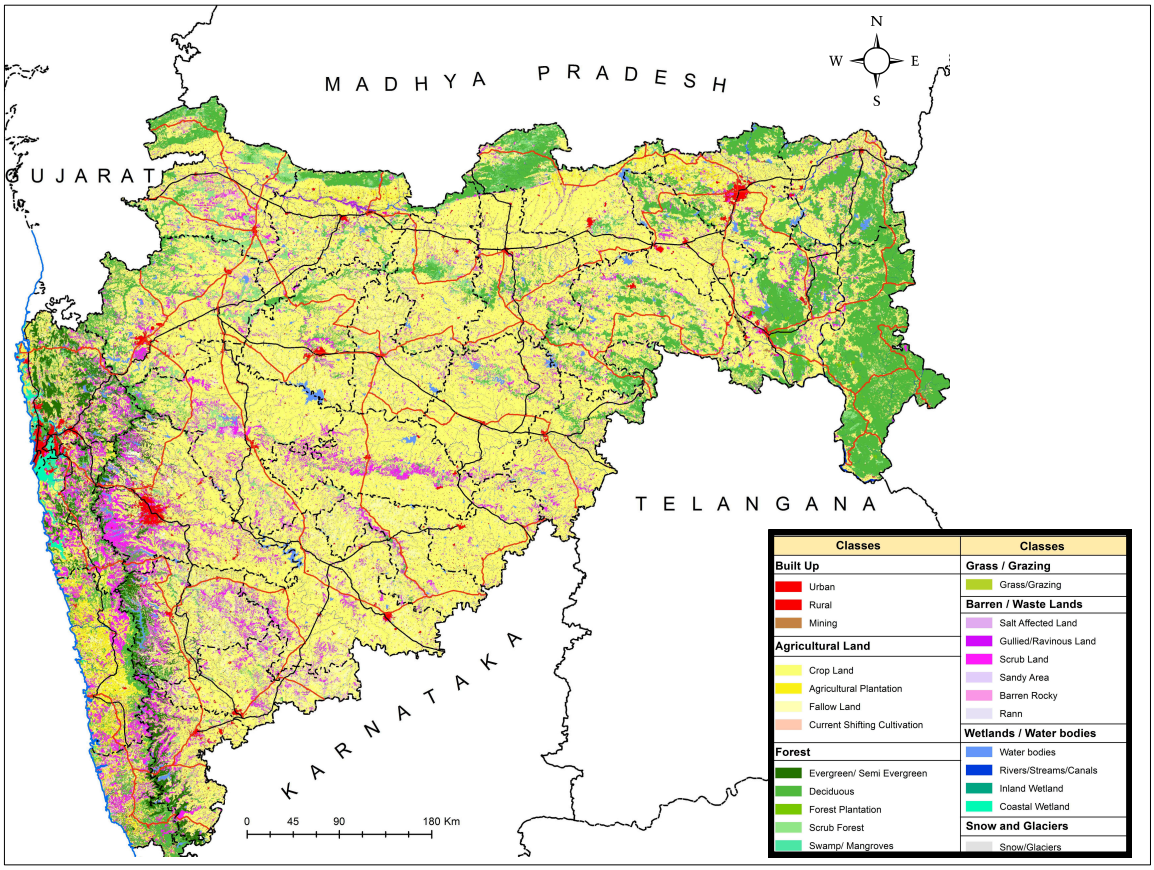

Figure 1d: Land Use map (2016-2017 data) for the state of Maharashtra featuring different land use classes

Protected Area	Area	TATR	PTR	STR	NNTR	BTR	UKWLS	MTR
TATR	1727	0	151	440	157	64	44	371
PTR	758	151	0	378	226	138	116	487
STR	1620	440	378	0	473	445	388	264
NNTR	1894	157	226	473	0	134	98	530
BTR	139	64	138	445	134	0	64	448
UKWLS	189	44	116	388	98	64	0	425
MTR	1,600	371	487	264	530	448	425	0

Table 1a: Matrix depicting all seven protected areas along with their respective areas in sq km and the approximate distance (shortest aerial) between each of them

1.3 Objectives of the Thesis

The goal of this doctoral research is to gauge and understand the effect of habitat fragmentations and other disturbances on the genetic connectivity of sloth bears and study the connectivity across the landscape. Using the data acquired after analysing samples from field, I was not only able to compare the genetic structures and describe gene flow of sloth bears in the study landscape, but also incorporate effects of variables (both environmental and man-made) and understand the differences in functional connectivity between different study areas.

There two broad objectives for the study:

1. Understand the genetic structure, variation, connectivity amongst sloth bear populations in the Vidarbha landscape.
2. Evaluate the permeability of the landscape and develop landscape connectivity maps.

Each objective was then fulfilled by posing more specific question.

For objective 1, the questions posed were:

a. Investigate the amount of genetic variation in sloth bears across the Vidarbha landscape.
b. Estimate genetic structure across the existing sloth bear populations using molecular tools.
c. Determine the extent and direction of gene flow across populations, if any.
d. Estimate sex ratio and inbreeding status within local populations of sloth bears.

For objective 2, the questions posed included the following:

a. Develop landscape connectivity maps for sloth bear incorporating species-specific traits.

b. Estimate relatedness and evaluate relatedness patterns.

1.4. Chapter organization

To ensure easy flow of understanding and collation of the data and outcomes, I have organized this thesis into 6 Chapters.

Chapter 1 is introduction to the core idea of the thesis along with description of each of the study area that had been sampled for scats of sloth bears. It also talks about the study objectives of the thesis and the questions that had been posed.

Chapter 2 focusses on our focal species i.e. sloth bears and outlines the behavioural and ecological traits of the species. It also describes how these traits affect movement and dispersal of the species across a particular landscape. The chapter also outlines studies other relevant studies conducted on sloth bears till date.

Chapter 3 is the first technical chapter. It describes the methods for selection and standardization of markers used to study sloth bears- both microsatellites and sex identification markers.

Chapter 4 is the second technical chapter. It describes the genetic diversity, differentiation and dispersal of sloth bears in the fragmented landscape based on data generated using the markers described in chapter 3.

Chapter 5 is another technical chapter for which camera trapping and field data was used along with modelling for occupancy. It describes the various covariates that affect the occupancy of sloth bears across the landscape and describes the same at three different spatial scales. The

covariates which showed high effect on sloth bear occupancy was used as resistance surfaces to model connectivity.

Chapter 6 describes the final objective. The chapter determines the effect of fragmentation on sloth bears. It describes the different habitat and landscape requirements for sloth bears to movement across a landscape. The variables obtained in chapter 5 are optimized and used to develop a map based on circuit theory.

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CHAPTER 2

Ecological traits and its Role in Genetics in Sloth Bears

2.1. Introduction- Role of movement in genetic exchange

Movement is a fundamental and indispensable aspect of an animal's existence, serving a myriad of crucial functions essential for survival, adaptation, and overall well-being within their respective ecosystems. The evolutionary imperative for mobility is deeply rooted in the biological, ecological, and behavioural requirements of diverse species. Firstly, movement is intricately tied to the procurement of sustenance. Animals must actively traverse their habitats in search of food and water sources. Predators, employing swift and agile movements, pursue prey, while herbivores engage in persistent foraging behaviours to ensure an adequate supply of nutrition. The efficiency of this movement directly influences an animal's ability to secure sustenance, impacting its energy balance, health, and reproductive capabilities.

Additionally, movement plays a pivotal role in reproductive strategies and behaviours. Animals often embark on journeys to find suitable mates, participating in elaborate courtship rituals or displays to attract potential partners (Fijin, Heimstra, Philips, & Van Der Winden 2013). This necessitates not only physical mobility but also behavioural adaptations that facilitate successful reproduction. Migration, a form of purposeful movement, is particularly noteworthy in this context, as many species undertake long-distance journeys to breeding grounds or areas conducive to raising offspring (Bauer & Farnsworth 2021; Alerstam & Backman 2018). The synchronization of these movements with reproductive cycles is critical for the perpetuation of species and the maintenance of genetic diversity.

Secondly, movement is a paramount mechanism for defence against predators and environmental threats. The ability to swiftly evade danger through running, flying, or swimming enhances an animal's chances of survival. This evolutionary adaptation is evident in the agility of prey species and the specialized locomotion methods of predators. Furthermore, animals exhibit territorial behaviours, as can be seen in canids, necessitating movement for patrolling and defending their territories (Mastrantonio, 2020). These territories often contain

essential resources such as food, water, and shelter, and the strategic use of movement is vital for maintaining access to these resources and ensuring successful reproduction (Kannowski & Johnson, 1969).

Moreover, movement facilitates adaptation to changing environmental conditions. Animals must navigate through their surroundings to respond to fluctuations in temperature, weather patterns, and resource availability. Migration, a remarkable manifestation of this adaptive movement, allows species to cope with seasonal variations, optimize access to food, and exploit favourable breeding conditions. Movement also enables animals to explore and discover new habitats, promoting biodiversity and enhancing their resilience to environmental changes. It is a multifaceted and dynamic phenomenon that underpins essential biological functions, from procuring sustenance and reproducing to avoiding predators and adapting to environmental shifts. The intricate relationship between movement and survival reflects the evolutionary wisdom encoded in the behaviours of diverse animal species, highlighting the indispensable role that mobility plays in shaping the ecological balance of our planet.

2.2. Ecology of Sloth Bears

Originally from the Indian subcontinent, the sloth bear (*Melursus ursinus*), commonly referred to as the labiated bear, is a nocturnal insectivorous bear species. Through convergent evolution, the sloth bear shared traits with other insect-eating animals after evolving from ancient brown bears throughout the Pleistocene (Sacco & Valkenburgh, 2006) . Sloth bears are lankier in build than brown or black bears, with long, shaggy coats that form a mane around the face, long, sickle-shaped claws, and a lower lip and palate that are uniquely specialized for swallowing insects .

Figure 2a: Picture above shows a sloth bear using sickle shaped claws to dig out insects (above). A close up of the claws specialized for digging (below)

Sloth bears are highly adaptable in nature especially when it comes to their diet which can vary both seasonally and differ across the geographical range specifically based on the type of tress, availability of insect mounds etc (Gokula, Sivaganesan, & Varadarajan, 1995; Baskaran, 1990).

2.3. Evolutionary History of Sloth Bears

One of the largest terrestrial carnivores are Ursid. Bears are a one of the most eminent case where contradictory gene trees and an uncertain fossil record make it difficult to evaluate their evolutionary history (Wagner, 2010). According to the IUCN , the range of extant bear species expands over thousands of kilometers and across several countries.

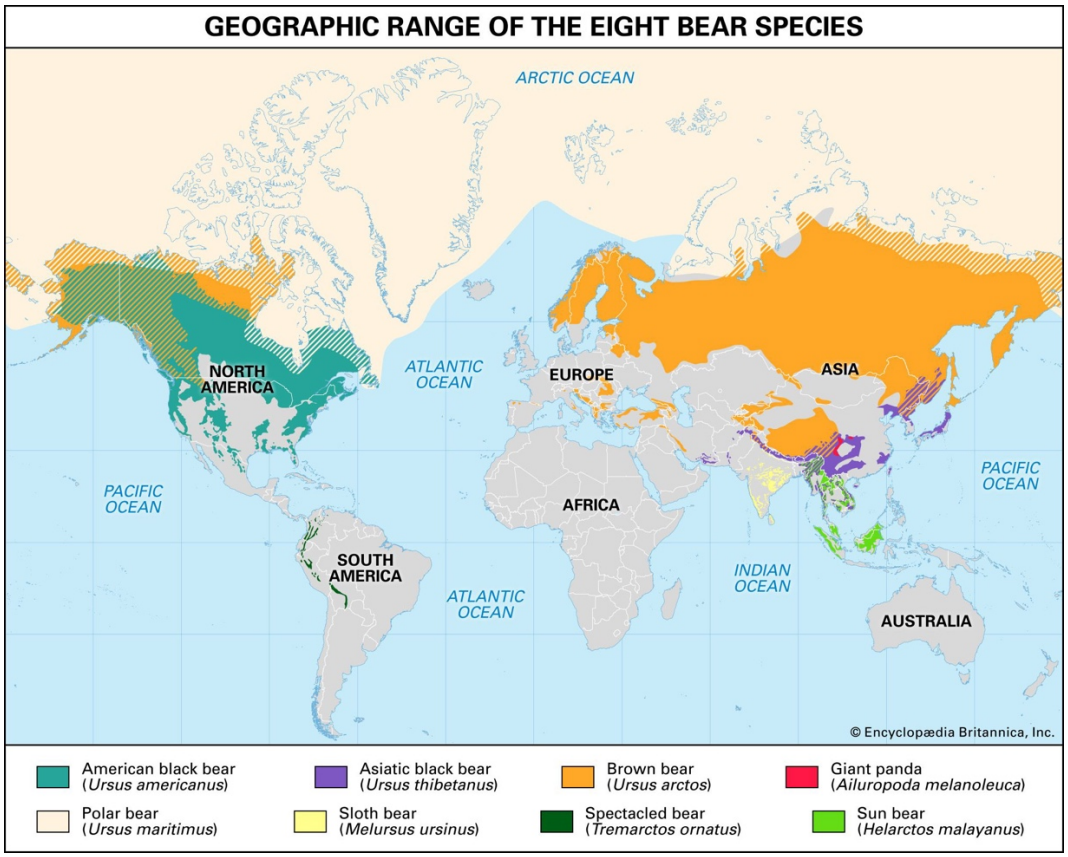

Figure 2b : Th map depicts the geographic ranges of all eight bear species found in the world

Previously, most classifications and groupings were based purely on morphological indications alone. There existed a conflict between studies done using mitogenomics, autosomal sequencing and morphological studies of the sloth bears (Kutschera, Hailer, Rodi, Fain, & Janke, 2014; Krause.J., et al., 2008).Recently, a paper by Kumar et al (2017) based on phylogenomic analyses using representation from all of the bear species was able to obtain a conducive coalescent species tree.

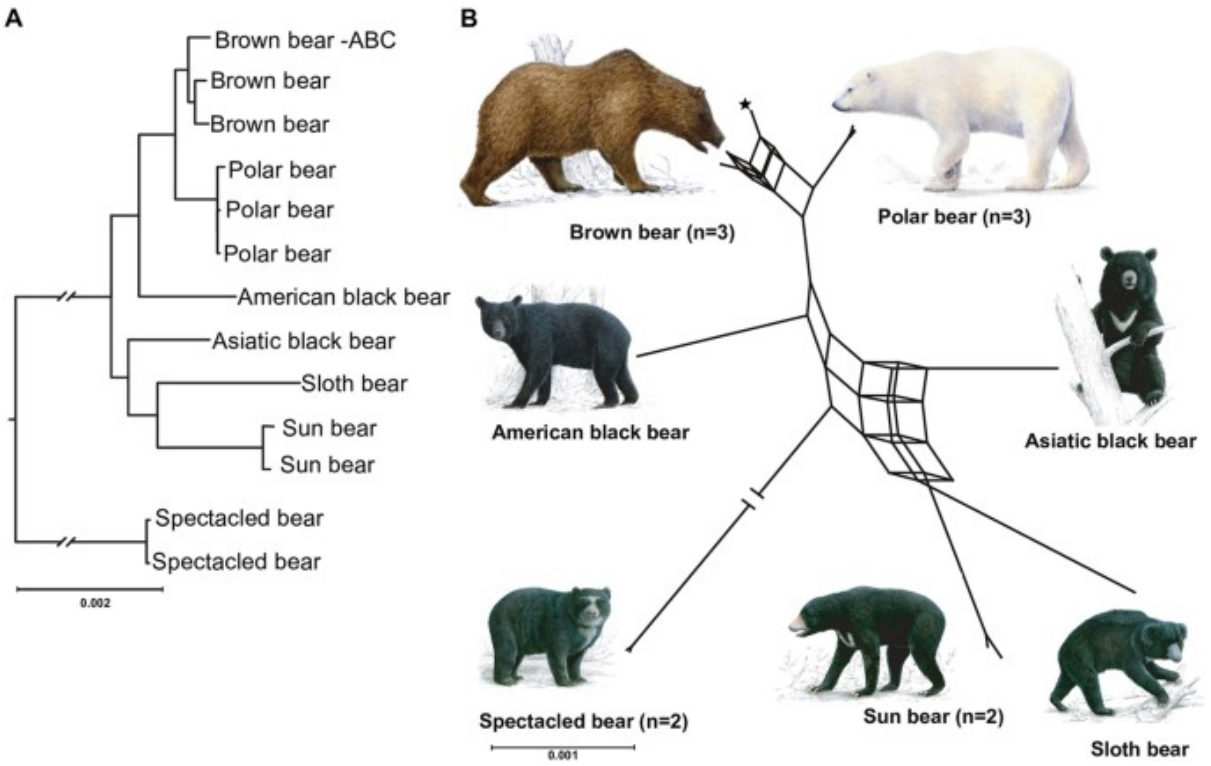

Figure 2c: Shows a coalescent species tree (A) and split network analysis with 100% bootstrap support (B) (Kumar, et al., 2017)

2.4. Genetic Differentiation and Diversity

There have been very few studies on sloth bears that explored their home range or dispersal. Having a very limited data about movement patterns in mind, I hypothesized that sloth bears, having relatively low dispersal range will show a high level of genetic differentiation. Additionally, I also investigated genetic differentiation and demographics using a few indexes of the sloth bear population of Eastern Vidarbha Landscape.

These questions have been addressed using a microsatellite panel that has been standardized and described in details in the later Chapter 3 using 256 scat samples collected from seven protected areas which were successfully amplified and used for all the analyses.

2.5. Sex-biased Dispersal

Kin competition, inbreeding avoidance, and spatiotemporal heterogeneity in resource or habitat suitability are all factors that favor dispersal along the tree of life (Pusey, 1987). These factors differ in how strongly they favor male and female dispersal, and selection on dispersal of one sex typically depends on how much the other disperses (Greenwood, 1990). For example, one sex dispersing away from the birth site may be sufficient to avoid inbreeding. Many bird and mammal species are devoted to their birth and breeding grounds or groups. The majority of them have one sex that is more philopatric than the other. Females often scatter more than males in birds; males typically disperse more than females in mammals (Greenwood, 1990).

In long-ranging animals such as bears dispersal is mostly male-biased (Swenson, Sandegren, & Soderberg, 1998; Rogers, 1987) . In several studies conducted on different bear species the results of sex-biased dispersal have been incompatible. For example, black and brown bear dispersal studied for both stable and declining populations have shown that the dispersal characteristics of both the sexes differ (Rogers, 1987).

Males frequently disperse far, however females are philopatric and establish their home ranges in or near their mothers' home ranges. Female black bears, for example, have been found to occupy the nearest suitable region if they disperse at all. Females can spread far, according to research on spatially expanding bear populations in Scandinavia and Finland, and their proportion among emigrants is relatively substantial (Kojola et al, 2003). Based on the variations in female dispersal between Scandinavian and other bear populations, it was predicted that long-distance female dispersal occurs more regularly in geographically growing populations but is minimal in stable and declining populations.

Sex-biased dispersal studies in sloth bears have not been conducted and hence no information about dispersal especially sex-based dispersal is available.

2.6. Concept of Landscape Genetics

For a long time researchers and ecologists had aimed to combine the fields of ecology and population genetics more robust. However, it was a difficult task at hand given the dissimilarities in ecological approach between the scientific communities. However, through time each field evolved and made way for landscape genetics. Over several years, landscape genetics has helped in understanding two very basic concepts- gene flow and adaptation of a species (Manel & Holdregger, 2013).

Our ability to study the effects of landscape variables, such as altitude, topography, and ground cover, on genetic variation and structure has greatly advanced as a result of technological advancements in spatial analyses, increased availability of spatial data, and hypervariable genetic markers. Gaining knowledge about how the terrain affects genetic connectedness can help one better understand basic biological processes like speciation, metapopulation dynamics, and eventually the creation of species' ranges (Schoville, et al., 2012). In addition to being highly valuable for applied science, landscape genetic analyses can also be used to identify specific anthropogenic barriers that hinder gene flow or genetic diversity, forecast how proposed management alternatives will affect population connectivity and genetic variation, and find potential biological corridors that could help reserve designers.

Landscape genetics offers a set of methods for correlating the Landscape heterogeneity and gene estimates flow has inspired various methodological and conceptual breakthroughs. Landscape-genetic studies typically use Mantel tests to compare genetic distances between individuals or populations to geographical distances, such as those based on single (Cushman, 2006) or multiple least-cost paths (circuit theory), to analyze the impact of landscape on gene flow. Mantel tests, however, has been criticized, as have associated techniques like multiple regression on distance (Raufaste & Rousset, 2001) . The real degrees of freedom are unknown due to the non-independence of the response and predictor variables, thus popular techniques like R^2 and the Akaike information criterion (AIC) to choose the best model from a group of

alternative models are not valid . Using mixed effect models that take into account the covariance structure of allele frequencies is one way to address the nonindependence issue (Goldber & Waits, 2009). Therefore, most studies dealing with landscape genetics now, measure gene flow directly from the data generated and the allele frequencies calculated and then induct the usage of multiple regression analysis.

2.7. Review of Studies on Sloth Bears

The extant population of sloth bears currently inhabit India, Nepal and Sri Lanka with almost 80% of the population inhabiting our country. However, research studies conducted on sloth bears in India is extremely limited skewed when compared to other iconic species such as the tiger, elephant, or leopard. This is in spite of the fact that in India, the reported number attacks by sloth bears is much more than any other large carnivore (Bombieri, et al., 2023).

In lieu of this, the present number of studies is very disproportionate. A large number of studies primarily deal with sloth bear ecology and seasonal feeding variation which describe the adaptable nature of sloth bears depending on the availability of resources (Rather, Tajdar, Kumar, & Khan, 2020; Palei, Debata, & Sahu, 2019; Palei,Mohapatra & Sahu 2013; Sukhadiya, Joshi, & Dharaiya, 2013;Bargali, Akhter, & Chauhan, 2004; Joshi, Garshelis, & Smith, 1997).

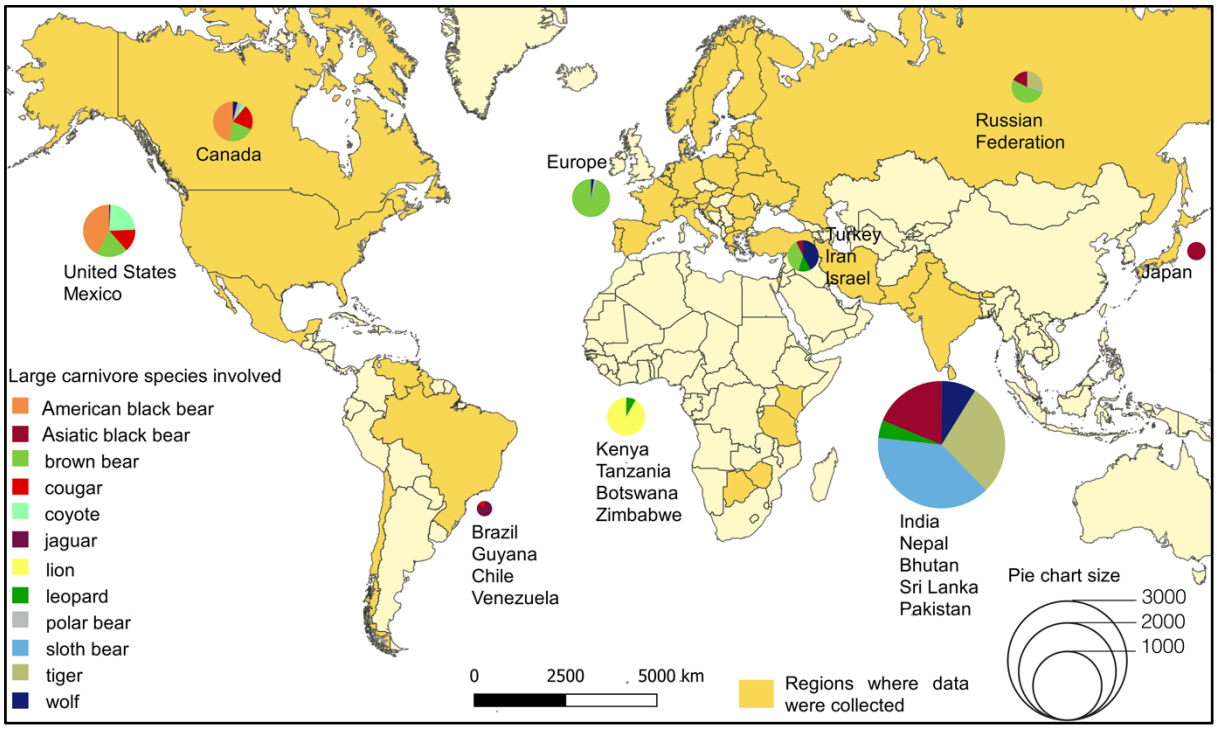

Figure 2d: Map depicts spatial distribution of attacks by large carnivores between 1950-2019 (Bombieri, et al., 2023)

A few studies describe the various factors affecting occupancy and habitat use of sloth bears (Sultana, Khan, & Nabi, 2015; Puri, et al., 2015; Das, et al., 2014 ; Ramesh, et al., 2012). However, only two studies have established the effect of fragmentation and loss of habitat on the genetic diversity of sloth bears (Thatte, et al., 2020; Dutta, Sharma, Maldondo, & Panwar, 2015). Till date, no studies have integrated genetic studies with mapping of corridors for management purposes. This thesis, is also an attempt to integrate molecular monitoring with connectivity modelling and contributing in development of wildlife corridors that are inclusive of lesser studied species as well ensuring a safe passage for wildlife and mitigation of conflicts.

Figure 2e: The pictures above show real life vitims of sloth bear attack and the picture below shows a sloth bear killed by retaliation

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CHAPTER 3

STANDARDIZATION OF MOLECULAR MARKERS FOR INDIVIDUAL IDENTIFICATION AND SEXING IN SLOTH BEARS

3.1. Conservation Genetics as a tool- an Introduction

The field of conservation genetics plays a crucial role in species protection by providing insights into population health, diversity, and adaptation. Through genetic analysis, researchers can identify unique traits, assess inbreeding, and understand migration patterns (Andrew, et al., 2013). Such data guides effective breeding programs, prevents genetic bottlenecks, and enhances species resilience (Forcina & Leonard, 2020). It aids in the identification of distinct populations and their vulnerabilities, enabling targeted conservation strategies. Monitoring of illegal trade and assessing the impact of habitat loss gains accuracy and momentum when supported by genetic analyses.

3.2. Concept of Genetic Monitoring and Individual identification

Stochasticity in the ever-changing habitat and its demographics has pronounced effect on population status of any species (Ouborg, 1993). Historically, sloth bears were widely distributed throughout the Indian subcontinent, but their population has declined significantly over the years due to habitat loss and fragmentation caused by human activities such as deforestation, agricultural expansion, and urbanization (Shankar & Murthy, 1995). Human-induced alterations create disruption in population as well as in genetic connectivity subsequently lowering genetic diversity and creating a risk of local extinction (Isagi, et al., 2020). Such an exigency entails implementation of non-invasive, cost effective and reliable molecular methods to understand and study populations of sloth bears.

Genetic monitoring methods are used extensively in other bear species around the world to study demographics, genetic variation and inbreeding status (Tallmon, Bellemain, Swenson, & Taberlet, 2004; Kruckenhauser, Rauer, Daubl, & Haring, 2009) and serve as key tools for biodiveristy monitroing approaches (Carroll, et al., 2018) however, studying sloth bear populations using genetic methods is sporadic in India. Till date, there has been just a couple of

studies that standardized ursid microsatellite primers in sloth bears for individual identification (Sharma et al, 2013;Thatte, et al, 2020) while no studies standardizing sexing primers to identify sex from non-invasive samples, have been conducted . Sharma et al (2013) used 7 micrsoatellite primers yielding a value of 2.15e-3 but Wang et al (2021) showed that genotyping with less than 11 or 12 markers leads to significant changes in population stucture and genetic variation within metapolutaions (Wang, Yang, Wang, & Xiao, 2021). In 2020, Thatte et al, tested 13 microsatellite markers with a slighly better value of probability identity between siblings at 1.4e-3. In this study, we optimized and used the same number of markers to obtain an even better value of 1.0e-5 PID_{sibs} cumulative. This study is also the very first to accurately demonstrate usage of a multiplex of 2 sexing primers using scat samples from sloth bears for sex detemination. Previously, there was just a single study ((Bidon, et al., 2013) that used three sexing primers on blood and tissue samples of sloth bears without testing their efficacy and reproducibility in scat samples. Our study demonstrates the use of a gel-based two-primer panel assay in tissues and scats for non-ambiguous and cost effective sexing approach. The study landscape was not the habitat of any other ursid species, and since the scat samples collected were accurately identifiable, no species identification was required prior to generation of data.

3.3. Methods

3.3.a. Study Area

This study for standardization of the markers was conducted in tiger reserves of the Eastern Vidarbha landscape (EVL) of Maharashtra, India. The landscape has a forest cover of 22,508 sq km accounting for areas both inside and outside of protected areas (Indian State of Forest Report, 2021). Bordered by three states- Madhya Pradesh in the North, Telangana to the South and Chhattisgarh to the East, the protected areas of EVL, is well connected to forest areas of its neighboring states. The region is semi-arid and has mixed dry deciduous forests. The major tiger protected areas of EVL are Tadoba Andhari National park , Bor Tiger Reserve, Navegaon-

Nagzira Tiger Reserve, Pench Tiger Reserve, Sahyadri Tiger Reserve and Umred-Karhandla Wildlife Sanctuary .

3.3.b. Sampling

For standardization, we used both positive tissue and scat samples. A total of 138 samples was used for the purpose of standardization. These 138 samples consisted of confirmed tissue samples (hair, skin, meat, bone, n=14) and scat samples (n=124). The tissue samples had been provided by Nagpur Zoo (2 blood samples) and Forensic laboratory facility of Wildlife institute of India (4 hair, 5 skin, 3 meat, 2 bone). The scat samples were collected during intensive sampling in the study sites was conducted from 2016-2019 for sample collection. Out of the total number of scat samples collected i.e. 565 samples, 131 scat samples were randomly selected from all the samples we had collected during our survey to represent the landscape and used for standardization purpose. We made sure to use at least 1/4th of the total samples for standardization purpose to test the efficacy of the selected microsatellites and their reproducibility. Little is known about preferred latrine sites in the case of sloth bears, which made sampling both labor and time intensive. We conducted our search on foot and used four wheeled vehicles only when feasible. We focused our search mainly on dry riverbeds, rocky surfaces, near fruiting trees or trees with beehives, trees with scratch markings and areas near bear dug-outs or termite mounds each of which indicated presence of bears. Sloth bear scats are remarkably different than scats of other large carnivores, lesser cats or langurs based on their size, shape, appearance and presence of seeds, ants and termite remnants (Laurie & Seidensticker, 1977). Once a scat was located, a bolus of the scat sample was collected and kept directly on a butter paper. Each butter paper containing a sample was then stored in individual zip lock bags. Details such as location, date, state of scat and locale associated details such as substrata or terrain type were recorded. Upon reaching the field station all ziplocked bags were stored in a box containing silica beads to minimize chances of fungal growth till it was brought to the laboratory for further processing.

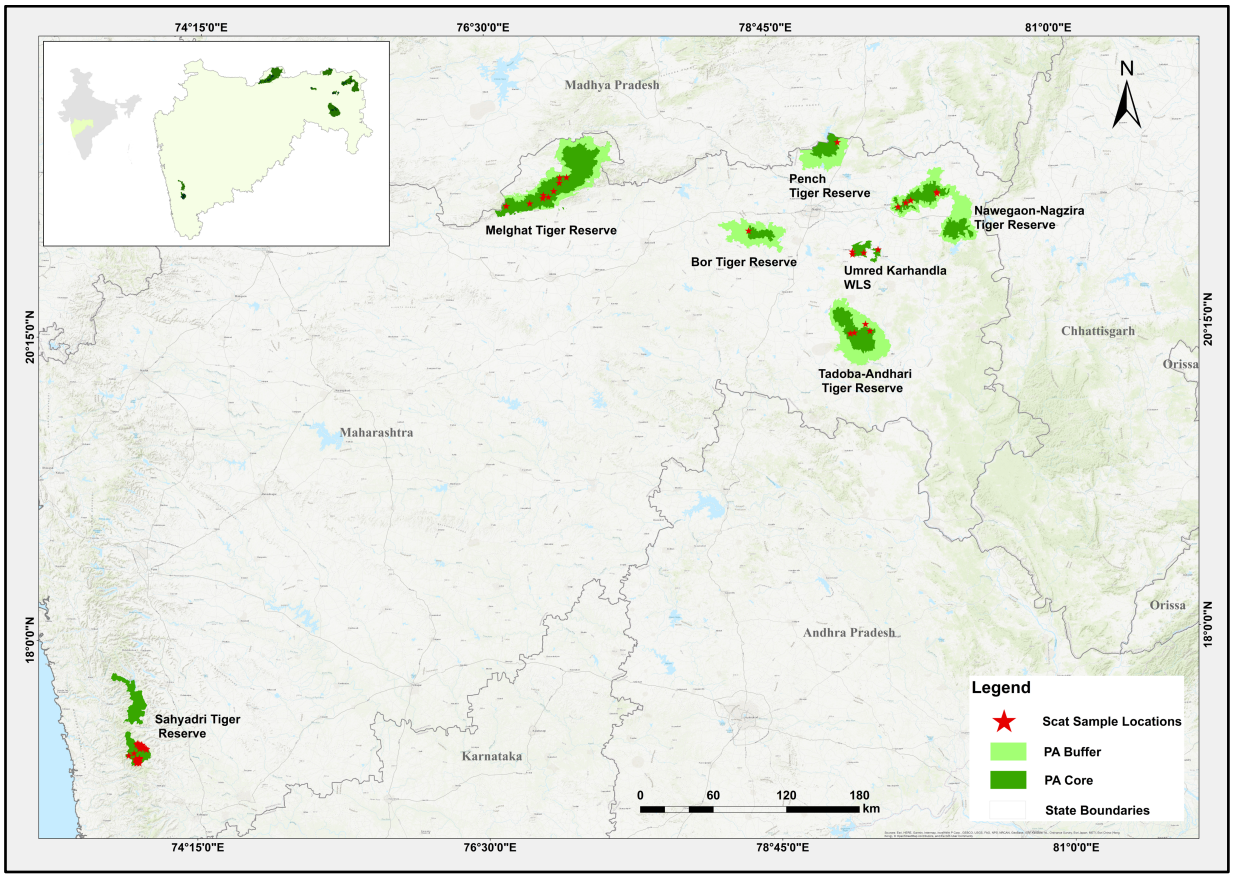

Figure 3a: Map depicting sampling sites from where scats were sampled for standardization of the primers (n=124; STR=38 , MTR=41, BTR=1, PTR=12, NNTR=12, TATR=15, UKWLS=5)

3.3.c. Primer Selection

Since there are no sloth bear-specific microsatellite markers published for sloth bears, we selected and screened a panel of 25 cross-species markers developed on different species of bears and canids . These markers were selected based on their polymorphic information content, heterozygosity and the number of alleles per locus and rates of allelic dropouts.

For molecular sexing a multiplex combination of two markers were used conforming to a double Y male specific amplification approach. SMCY (Y specific) developed in bears (Bidon, et al., 2013) and SE 47/48 developed in bovids (Ennis & Gallagher, 1994) and tested positively on black bears (Yamamoto, et al., 2002) was used

3.3.d. DNA Extraction

The tissue and the scat samples were extracted following different standardized protocols. Each scat sample brought to the laboratory was swabbed twice using sterile swabs (HiMedia) dipped in PBS buffer (Biswas, et al., 2019) . The swabs were then placed in 2ml eppendorf tubes until extraction. Extraction of the swabbed samples was done using DNeasy kit (QIAGEN) following the instructions stated in the kit's instruction manual with a few standardized modifications. An overnight lysis was performed with 330ul InhibitEx buffer (QUIGEN) and 20 µl Proteinase K and DNA was extracted the following day following the steps provided by the manufacturer. In the final step the DNA was eluted twice with 100ul of 1X TE buffer and stored in -30°C for long term storage. Care was taken to include negative controls during each set of 12 extractions to monitor chances of possible contamination during handling and extraction steps. Standard manufacturer's protocols were followed for DNA extraction from tissue (DNeasy kit , Quigen) and bone (GeneiPureID DNA Isolation Kit-Bone, Merck) samples used in the study.

3.3.e. PCR Standardization and Data Validation

The selected primers were first individually standardized using the confirmed tissue samples and using gradient temperature PCR method to obtain optimum annealing for each primer. PCR reaction for each eluted sample was performed using 3.5ul hotstart taq mix (Qiagen Inc., Valencia, CA, USA), 3 ul of BSA 1 uM of primer mix (florescent tagged forward primer and a reverse primer) and 2 ul of DNA extract under conditions that included initial denaturation (95°C for 15 minutes); 55 cycles of denaturation (94°C for 30 seconds), annealing (temperature as calculated for each primer for 30 seconds) and extension (72°C for 30 seconds); final extension (72°C for 10 minutes). The negative controls were also included in the PCR process to ensure quality of the process and monitor chances of any cross-species amplification. The

PCR products post amplification were then prepared using GeneScan 500 LIZ for genotyping and the plates were run on ABI 3500xl Genetic Analyzer. The results of the alleles were scored using GeneMarker v3.0.1 (Softgenetics Inc., State College, PA, USA). Allelic bins for each locus was created using the data generated from the tissues and scat samples.

For data validation of the scat samples a multi tube approach was used (Mondol, et al., 2009). Each sample was amplified and genotyped thrice for each locus and a consensus final data was used for quality index analysis as described in Miquel et al, 2006. In this protocol, the alleles are scored. If the repeats are identical to the first allele call then they are assigned '1'"and if they are different due to non-amplification, allelic dropout of allelic slippage then '0'is given. The quality index for each locus is calculated by adding the scores of each loci and dividing each with the number of repeats for that loci. If this value is equal to or above 0.75, the sample was then used for further downstream analyses (Miquel, et al., 2006). Monomorphic markers and those not conforming to the cut-off were not used further. The average rate of amplification success was calculated as the percent of positive PCR amplifications for each locus.

The allelic dropout and false allele rates were calculated manually as well as through MICROCHECKER v 2.2.3 (Van Oosterhout et al., 2004). To calculate allelic dropout and FA , the number of drop outs or FAs over the total number of amplifications was calculated (Broquet et al., 2004). The FA frequency for each locus, in case of both homozygous and heterozygous genotypes, was calculated as the ratio of amplifications with one of more FAs to the total number of amplifications. The allelic dropout was calculated for heterozygotes, as the ratio between the number of amplifications showing loss of one allele to the total number of positive amplifications. Null alleles (NAs) was calculated using the program FreeNA (Cahpuis & Estoup, 2006) based on maximum likelihood from incomplete data using EM algorithm proposed by Dempster et al in 1977 (Dempster, Laird, & Rubin, 1977)

For molecular sexing, PCR reactions were prepared using multiplex buffer (Qiagen), 4ul BSA buffer, 0.5 mM primer mix and 2 ul of extracted DNA. Accordingly. the protocol included an initial denaturation (95°C for 15 min) followed by 55 cycles of denaturation, annealing and extension (94°C for 30 s, 59°C for 30 s, 72°C for 30 seconds) and a final extension (72°C for 10 minutes). 3.5 ul of the amplified products was then electrophoresed in 3% agarose gel using Firefly dye, DNA visualization stain.

3.4. Data Analyses

CERVUS 3.0.7 (Kalinowski, Taper, & Marshall, 2007) module of identity analysis was used to identify genetic recaptures (identical genotypes) by comparing the consensus data generated from all loci for each sample. The recaptures were removed from the analysis. GIMLET (Valiere, 2002) was used to calculate PID values and genetic variations in the loci such as expected heterozygosity, observed heterozygosity, number of alleles, false alleles and allelic dropout. Hardy Weinberg equilibrium and linkage disequilibrium for each locus was calculated using ARLEQUIN (Excoffier, Laval, & Schneider, 2005).

The results from sex identification was obtained by running the amplified products in a gel and visualizing the bands. Each sample was amplified three times and the products run three times in the gel and the results were analysed visually to reach a consensus before assigning a sex to a sample.

3.5. Results

3.5.a. Individual identification

During trial experiments, a total of 25 primers had been used firstly on the confirmed tissue samples. 2 out of the original 25 primers did not amplify at all even with tissue samples and were removed from subsequent analyses. The remaining 22 markers were amplified with all 138 samples out of which 13 loci generated data that were consistent (Refer 3a). No large allelic dropouts were seen in these 13 microsatellites. Genotyping results of the tagged primers showed clean peaks and less stutters. Only those samples that generated data for at least 7 loci was taken in to consideration. This cut-off of at least 7 loci was decided based on the statistical support that produced a food PID_{sibs} of 1 in 10,000 produced by these loci. Given that the abundance of sloth bears in India is ballparked somewhere between 10,000 and 20,000 (Sathyakumar et al , 2012; Yoganand, Rice, Johnsingh, & Seidensticker, 2006), it was concluded that this PID value is sufficient for individual identification at even local scales. Considering this and the aim to make the study cost effective, the cut-off was finalized.

79 of the 138 samples amplified positively and yielded data for at least 7 loci and were considered for further analysis. It was seen that most of the samples that amplified successfully were the ones that were collected fresh from the field. Dried and degraded samples either failed to amplify at all or amplified very inconsistently. An amplification success rate of 57.4% for scat samples was obtained for the scat samples obtained from field. None of the final 13 loci showed any signatures of large-scale allelic dropouts. The average allelic dropout was 0.042 while average FA across all 13 loci was 0.03. The frequency of NA was 0.13 indicating that genotyping errors for 13 loci panel was minimal. The summary statistics for each of the 13 microsatellites are given in the table below.

Overall, data was generated for 79 samples (13 tissues samples and 66 scat samples). Cumulative PID sibs value of 1.48×10^{-5} was found suggesting good statistical support for

unambiguous individual identification. We were able to identify 75 individuals from this pool while matches using the exact match criteria were removed.

Locus	Primer Sequence	Source
UarT838	5'-3' TCTCTACATCCTTGCCAGC CGCAAATCAAAACCACAATG	(Kleven, et al., 2012)
UT1	5'-3' AGCAACTCTTCTCAGATGTTCACAAA CCCAGGTCAGCACTTGGCATAC	(Shih, Huang, Li, Hwang, & Lee, 2009)
UT38	5'-3' ATTATTGATGAGCAGGGACAG CTAAAGCAACAACATGTGAATG	(Shih, Huang, Li, Hwang, & Lee, 2009)
UarT259	5'-3' CTCTGGACTTCTGGCTCAGG TGAAGCCATCAACATTGCTC	(Kleven, et al., 2012)
Umar2	5'-3' TCACGGGTTTGTAGTAAACA CACAAAGTGGATGCTAAGAA	(Poissant & Davis, 2011)
UT4	5'-3' GAGTTATTGGCACTAAAATCTAATG CTGCAAATCCCTGCTCAACTTTC	(Shih, Huang, Li, Hwang, & Lee, 2009)
UamD112	5'-3' GAATCCTCTCCAAGACCTATG GTTTTCCTTATCCCTGAACTG	(Meredith, Rodzen, Banks, & Jones, 2009)
G1D	5'-3' GATCTGTGGGTTTATAGGTTACA CTACTCTTCCTACTCTTTAAGAG	(Paetkau, Calvert, Stirling, & Strobeck, 1995)
G10L	5'-3' GTACTGATTTTATTCACATTTCCC GAAGATACAGAAACCTACCCATGC	(Paetkau, Calvert, Stirling, & Strobeck, 1995)
G10B	5'-3' AAGCCTTTTAATGTTCTGTTG AGGACAAATCACAGAAACCT	(Paetkau, Calvert, Stirling, & Strobeck, 1995)
UT29	5'-3' GACATTGCCTTTTACAGAGCAG GGGCAGATCTCAACCACCATAAGC	(Shih, Huang, Li, Hwang, & Lee, 2009)
CXX203	5'-3' TTGATCTGAATAGTCCTCTGCG AGCAACCCCTCCCATTTACT	(Binns, Holmes, Marti, & Bowen, 1995)
Mu23	5'-3' GCCTGTGTGCTATTTTATCC TTGCTTGCCTAGACCACC	(Taberlet, et al., 1997)

Table 3a : 13 microsatellites developed on different bear species selected and used for population level study of sloth bears across the landscape

3.5.b. Sexing

The sexing multiplex gave us a sex discrimination rate of 83%. Each sexing experiment for each sample was conducted three times for statistical accuracy and allow sex discrimination in degraded samples which failed to amplify in a single experiment. This touchdown approach gave us a three-band pattern in males (100bp, 191bp, 245bp) and 1 band in females (245 bp). We identified 39 males and 26 females.

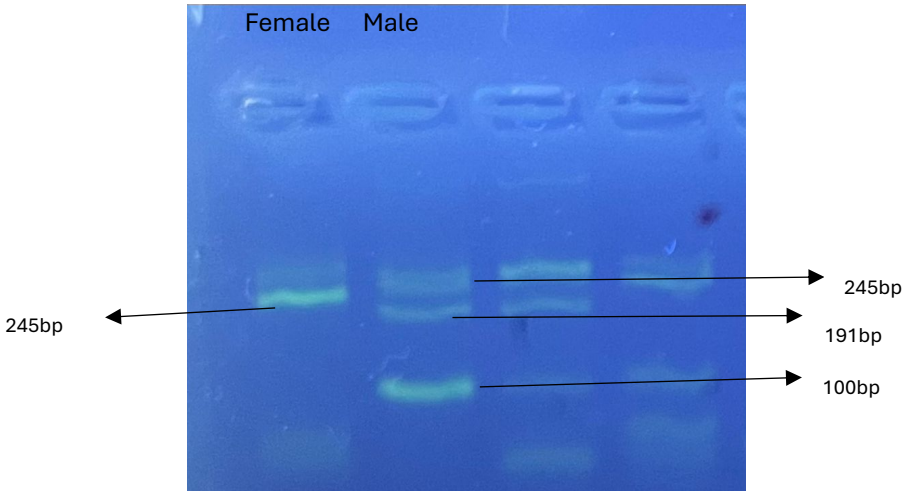

Figure 3b: Representative gel of PCR amplified products of sexing primers. Lane 1 shows amplification from known female sloth bear and Lane 2 shows amplification from known male sloth bears.

3.6. Discussion

In this paper we have tested and standardized a panel of 13 microsatellite tagged primers to be used for individual identification and sexing which may further finds its use in genetic monitoring. Using a larger number of primers at the initial stage (25 markers in this case) allowed us to test for appropriate panels to identify individuals with robust statistical bolstering. The final panel of 13 microsatellites were further standardized in to 5 multiplex reactions to make amplification costs effective and time efficient. We tried to keep a balance while selecting microsatellites and the final panel had a ratio of 2:1 of tetra against di nucleotide markers. Care was taken to opt for more tetranucleotide markers in the beginning that have low stutter peaks and sharper allele characteristics during calling while dinucleotide markers are known for their

efficiency in higher amplification success even from degraded samples (Walsh, Fildes, & Reynolds, 1996; Broquet, Menard, & Petit, 2006). One of the core motivations to conduct and develop upon this method is that since the sloth bear is unmarked, any kind of population study using field methods alone is next to impossible. For genetic estimation of population size in each area, it is recommended that the PID_{sibs} value be double than the expected population size (Waits, Luikart, & Taberlet, 2001). Using 13 microsatellites the PID_{sibs} value obtained in this study 1.48×10^{-5} should be sufficient to conduct a large spatial scale study. A success rate of 57.4% was obtained in this study and even though it might not be very high, similar studies conducted on sloth bear have shown a similar trend. For example. a study conducted by Dutta et al yielded a success rate of around 30% using a 7 panel marker. (Dutta, Sharma, Maldonado, Panwar, & Seidensticker, 2015).Thus it was concluded that the success rate may be dependent on the sample quality i.e. the freshness or age of the sample along with its condition.

The molecular sexing method tested here is advantageous because of its reproducibility, efficiency, and cost effectiveness. Moreover, since it employs the double Y test, the probability of misidentification is reduced.

In a much broader context, in order to understand sloth bear dynamics amongst sub-populations at a larger scales such as EVL, genetic sampling is the only way especially for a monomorphic species.

These standardized markers 13 microsatellite (for individual identity) and 2 sexing markers (for sexing) were then used in the following studies in the thesis to understand various genetic parameters

Locus	T_a	Allelic size	Number of alleles	H_o	H_E	P_{ID}[a]	$P_{ID(sibs)}$[b]	$P_{ID(cum)}$[c]	$P_{ID\ (Sibs-cum)}$[d]	ADO	FA
UarT838	57°C	97-145	4	0.66	0.80	1.777e-01	4.649e-01	1.777e-01	4.649e-01	0.183	0.024
UT1	57°C	176-192	5	0.63	0.36	1.786e-01	4.825e-01	3.172e-02	2.243e-01	0	0.028
UT38	53°C	196-232	12	0.68	0.24	1.118e-01	4.396e-01	3.548e-03	9.860e-02	0	0.038
UarT259	53°C	153-177	10	0.46	0.18	2.923e-01	5.975e-01	1.037e-03	5.891e-02	0	0.038
Umar2	55°C	185-227	10	0.65	0.31	1.472e-01	4.632e-01	1.527e-04	2.729e-02	0	0.056
UT4	55°C	157-182	8	0.75	0.55	9.237e-02	3.984e-01	1.410e-05	1.087e-02	0.225	0.054
UamD112	55°C	142-210	12	0.84	0.29	3.785e-02	3.411e-01	5.339e-07	3.708e-03	0	0.042
G1D	59°C	176	5	0.60	0.47	2.031e-01	5.048e-01	1.084e-07	1.872e-03	0.072	0.035
G10L	59°C	165	7	0.73	0.84	1.063e-01	4.149e-01	1.153e-08	7.766e-04	0.026	0.012
G10B	56°C	133-143	14	0.89	0.45	1.910e-02	3.123e-01	2.203e-10	2.425e-04	0.063	0.051
UT29	58°C	168-192	10	0.85	0.53	3.598e-02	3.353e-01	7.927e-12	8.131e-05	0.105	0.025
CXX203	58°C	122-146	7	0.69	0.46	1.366e-01	4.404e-01	1.083e-12	3.581e-05	0.288	0.024
Mu23	56°C	164-180	11	0.73	0.25	9.926e-02	4.093e-01	1.075e-13	1.466e-05	0.015	0.024
Mean	**-**	**-**	**8.**	**0.71**	**0.45.**	**-**	**-**	**-**	**-**	**0.042**	**0.03**

[a] Probability of identity
[b] Probability of identity between siblings
[c] Cumulative probability of identity
[d] Cumulative probability of identity between siblings
ADO- allelic drop out

Table 3b: Summary statistics of the 13 microsatellites standardized in the chapter to study the various genetic parameters across the study landscape

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CHAPTER 4

Metapopulation Dynamics of Sloth Bears across Vidarbha Landscape

4.1. Genetic Differentiation and gene Flow

4.1.1. Introduction

Habitat fragmentation, a consequence of human activities such as urbanization, agriculture, and infrastructure development, has profound implications for the gene flow of different species (Ledig 1992; Fahrig 2003). Gene flow, the transfer of genetic material between populations, is a critical factor influencing the genetic diversity and adaptability of species (Ouborg, Verheer and Mix 2006). In addition to implying habitat loss, this process also suggests a change in habitat design, which suggests a variation in the number of pieces or the relative isolation between them (Fahrig, 2003). When habitats become fragmented, populations face challenges in maintaining connectivity, and this can lead to various impacts on gene flow. A crucial component of populations' adaptive evolutionary potential is their quantitative genetic variety, which serves as the foundation for phenotypic development. Genetic variation for ecologically significant features may improve an organism's capacity to adapt to changing environmental conditions, which may have an impact on population persistence and fitness (Jump, Marchant and Penuelas 2009).

A number of studies have been conducted in the central Indian landscape on other large carnivores such as tigers, leopards and dholes. The works done by Sharma et al (2013), Joshi et al (2013),Yumnam et al. (2014), Dutta et al (2015), Thatte et al (2020) and Modi et al (2022) have used non-invasive methods to understand genetic connectivity across the landscape. All of these studies indicated that genetic distance supplemented by fragmentation and loss of habitat connectivity has profound influence on the genetic structure and flow of genes between sub-populations. The limited works of sloth bear genetic connectivity have shown that loss of habitat affects movement of sloth bears (Thatte et al, 2020).

I hypothesised that sloth bears would show less genetic differentiation, due to their adaptability in terms of habitat. Since no study has yet been published on their dispersal and home range data is limited too, I based my hypothesis purely on their adaptive nature. Using non-invasive genetic sampling and genetic tools, I derived the (a) genetic diversity of sloth bears in the sampled protected areas; (b) Derived population structure and compared them. These questions were answered using 13 microsatellites loci in 5 panels standardized in the last chapter.

4.1.2. Methodology

4.1.2.a. Sampling

To generate landscape data, intensive sampling was conducted and a total of 565 scats were collected during field surveys. This study was conducted in the Vidarbha landscape (VL) of Maharashtra, India. The landscape has a forest cover of 22,508 sq km, accounting for areas both inside and outside of protected areas. The major protected areas of the VL in which sampling was conducted include the Melghat Tiger Reserve (MTR, 2768.52 sq km), Sahyadri Tiger Reserve (STR, 1166 sq km), Tadoba Andhari Tiger Reserve (TATR, 1727.59 sq km), Bor Tiger Reserve (BTR, 816.27 sq km), Navegaon-Nagzira Tiger Reserve (NNTR, 1894.94 sq km), Pench Tiger Reserve (PTR, 741.22 sq km) and Umred-Karhandla Wildlife Sanctuary (UKWLS, 189 sq km). Field sampling was done from 2016-2019.

4.1.2.b. Individual Identification

The microsatellites (n=13) standardized in the previous chapter was used generating data on individual identification for all samples collected from the landscape (N=565).Each sample was genotyped three times independently to ensure a good consensus and data quality.

4.1.2.c. Data Analyses

Consensus data was obtained for the genotypes for each locus, for all of the 565 samples using the multiple tube technique in conjunction with the quality index protocol to produce the highest quality data possible for analysis as described in previous chapter during standardization procedure. Only the genotypes, which yielded information for a minimum of seven of the consensus's twelve loci was taken in to account. In order for the samples to be accepted for further analyses, a quality index threshold of 0.66 per locus and an average quality index of 0.75 across loci were established. The total genotyping error rates (allelic dropout and false alleles) using the genotyping error estimation module of GIMLET (Broquet and Petit 2004) and used MICROCHECKER v 2.2.3 (Van Oosterhout, et al. 2004) to identify substantial allele dropouts were calculated. Using the identity analyses module of CERVUS (Kalinowski et al., 2007), all genetic recaptures were eliminated, permitting up to two mismatches. To evaluate the panel's differentiation power, the cumulative PID(unbiased) (probability of identity) and PID(sibs) value (Waits et al., 2001) was calculated using GIMLET (Valière, 2002). Using HP-RARE, the allelic richness was also calculated by applying the rarefaction method while taking into account the different population sample sizes. Checks for deviations from linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were done using GENPOP and ARLEQUIN (Excoffier et al., 2005).

4.1.2.d. Sloth Bear Metapopulation Structure

Multivariate analysis and Bayesian clustering was used to infer any potential genetic structure among the sampled areas for sloth bears. These studies were limited to populations for which data were available from 10 or more different individuals.Using program STRUCTURE v.2.3.4 (Pritchard, Stephens and Donnelly 2000), I put the Bayesian clustering strategy into practice. Ten independent runs with 100,000 burnin and 500,000 iterations were carried out for a range of population values (K = 1 to 10). Admixture models that took into

account correlated allele frequency and ancestry from one or more of K genetically different sources were used to run the models. Using the deltaK technique in STRUCTURE HARVESTER (Evanno, Regnaut and Goudet 2005), the ideal number of clusters was ascertained. With CLUMPAK, the average of the admixture proportion of individuals across ten replicates was calculated (Kopelman, et al. 2015).

TESS (Caye, et al. 2015) was also used for population clustering in addition to STRUCTURE. This is because some studies have reported that TESS performs better than STRUCTURE in the detection of the number of genetic populations and also works well in cases where moderate admixture may be present (Vergara, et al. 2015). Ancestry estimation algorithms are implemented by TESS for geographical population genetic investigations. The tool can be used to run genome scans for selection, as well as individual geographic assignment and admixture analysis. TESS is especially useful for assessing regionally changing individual mixing proportions and searching for genetic discontinuities in continuous populations. TESS provides textual output of the admixture Q matrix along with graphical displays of geographical cluster allocations or admixture proportions (based on the model chosen).

The program Discriminant Analysis of Principal Component (DAPC) (Jombart et al., 2010) was used to identify the presence or absence of genetic structuring across the sampled sites. This multivariate analytical technique does not require the population to be in Hardy-Weinberg equilibrium, nor does it require geographical information (Jombart, 2008a; Jombart et al., 2010). Using the discriminant function, the genetic data is first converted into principle components, which are then used to cluster the individuals into groups with the least within-group variation and the most between-group variation. The results of the studies were obtained by using the Bayesian Information Criterion (Jombart et al., 2010) in R studio 1.1.453 (R Development Core Team, 2018) with the adegenet package 2.1.1 and dapc cluster finding function.

4.1.2.e. Genetic differentiation and migration between Sloth Bear Population

Using the R package DiveRsity 1.9 (Keenan, et al. 2013)in R studio 3.1, I evaluated genetic differentiation by various indices (G'st and Jost D) (Hedrick 2007; Jost 2008). Previously it had been argued that popularly used indices such as Nei's G(ST) and Wier and Cockerham's θ used to understand genetic differentiation are 'bound' to not equal one in some scenarios that appear to reflect maximal differentiation and hence is often supplemented by G'st and Jost D . To clarify the asymmetric migration (Sundqvist et al., 2016) and differentiation among the sub-populations (Ryman et al., 2009; Meirmans et al., 2011), I employed both these differentiation indices.

The study landscape's migration rates in between the protected areas were estimated using the BayesAss software version 3.0.3, which makes use of genotype information from multi-locus data (Wilson & Rannala 2003). The software was configured with 5 × 106 iterations and 105 burn-in, with sampling occurring every 2000 iterations. The "circlize" package, version 0.34, in R version 1.2.5 was used to show the BayesAss results (Gu, et al. 2014).

4.1.3. Results

4.1.3.a. Genetic Diversity

A total of 565 samples in total was collected from entire landscape 2016– 2019. Sloth bear scats are characteristic in nature and the landscape is not shared by any other bears; hence, species identification was not done. Using the panel of standardized markers and deleting genetic recaptures all genetic parameters were calculated. The PIDsibs value was low (1.55×10^{-5}), suggesting that microsatellites had a strong ability to distinguish related genotypes from individual genotypes and providing a statistically reliable result for individual identification.

Table 4a provides the summary statistics for each of the protected regions. There was no indication of substantial linkage disequilibrium between any pair of loci in the panel. In our

analyses, we obtained a low error rate of 0.06 which proved that our panel was robust to conduct a landscape level population study (Bonin, et al. 2004).

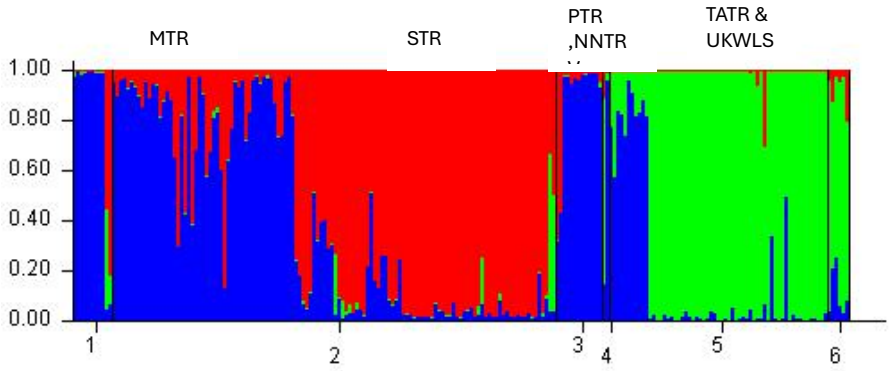
Parameters		Sloth Bears
Error Rate		0.06
PID (sibs)		1.55×10^{-5}
Ho/He	STR	0.46 /0.78
	MTR	0.62/0.88
	PTR	0.59/0.59
	NNTR	0.52/0.55
	UKWLS	0.31/0.59
	TATR	0.44/0.63
	BTR	NA

Table 4a: Shows summary statistics of 565 samples

4.1.3.b. Population Structure of Sloth Bears

Sampling strategy for collecting of scat samples was aimed at ensuring maximum coverage of the sampling sites across the landscape to enhance the probability of assessing any possible population structure within the sampled areas.

The output from structure gave a cluster of k=3 (Cluster 1= MTR+PTR+NNTR , Cluster 2= STR, Cluster 3 = TATR+UKWLS).



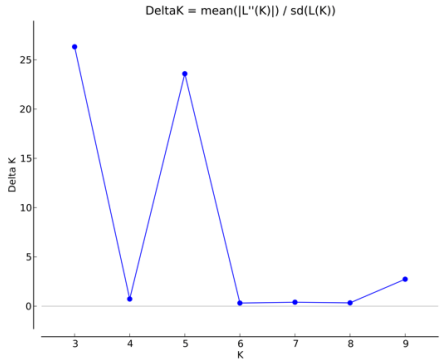


Figure 4a: STRUCTURE output showing clusters of k=3.Each colour signifies a cluster. X axis is the population and Y axis is the assignment to a population.

The results from TESS clustering analysis showed k=5 clusters for sloth bears (cluster 1= NNTR+PTR+UKWLS, cluster 2 and cluster 3= STR, cluster 4= MTR, cluster 5= TATR).

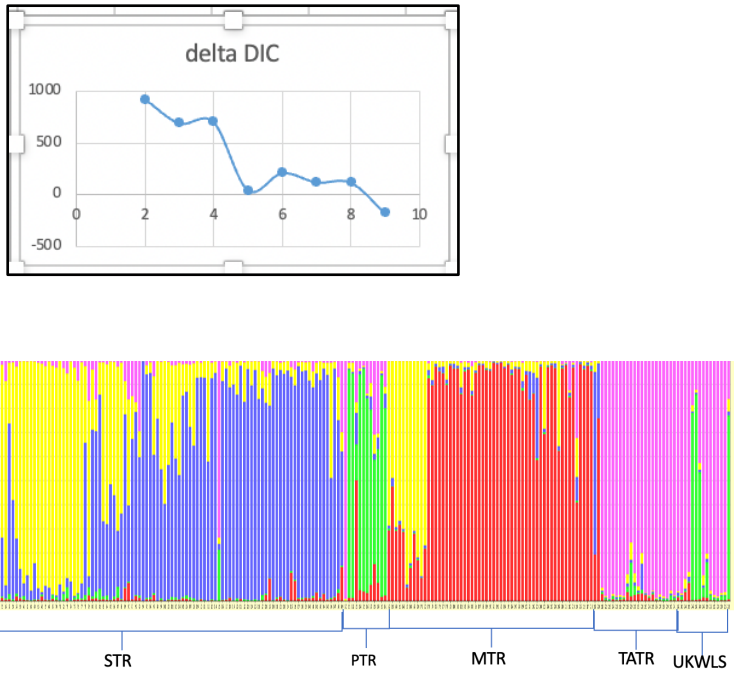


Figure 4b: TESS output showing clusters of k=5.Each colour signifies a cluster. X axis is the population and Y axis is the assignment to a population.

DAPC however, showed a clustering of populations to 4 different clusters. Two of the clusters were showed very little or no admixture while the other 2 clusters (TATR, UKWLS, NNTR, PTR) showed some admixture.

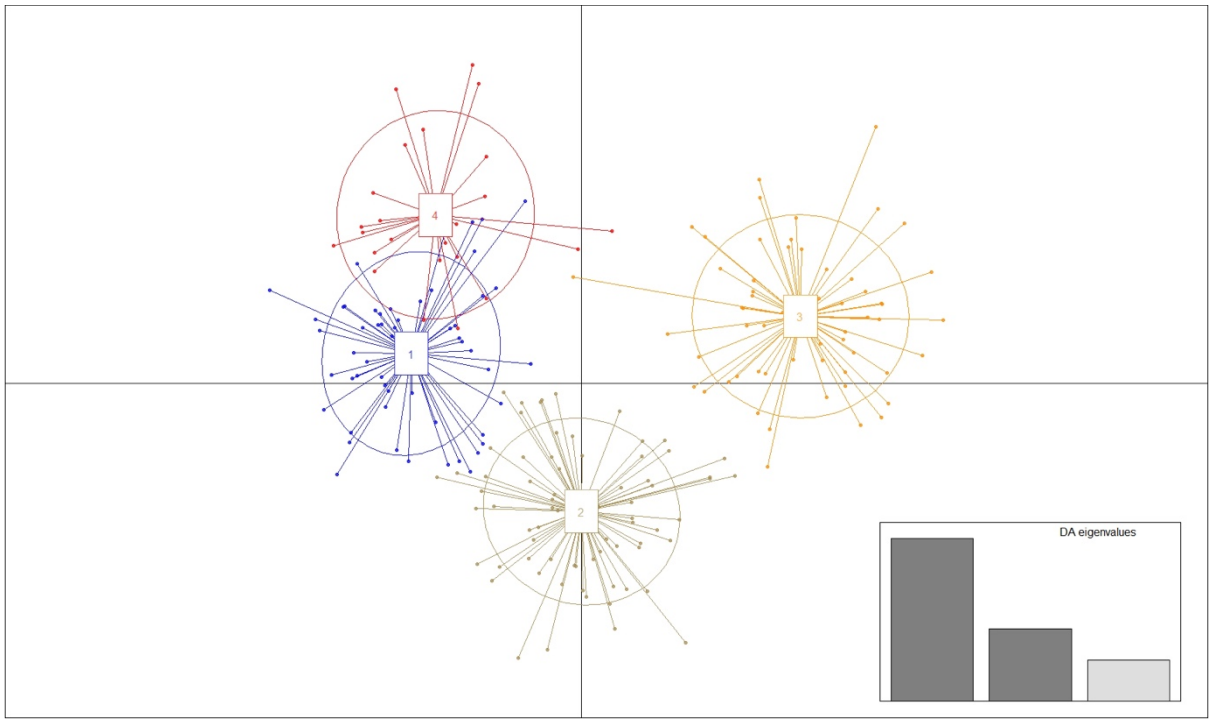


Figure 4c: DAPC output showing clustering of populations in to k=4. Where, Population 1 =TATR+UKWLS, Population 2 = MTR, Population 3= STR, Population 4= NNTR+PTR

4.1.3.c Genetic differentiation and gene flow

The G'st value ranged from 0.14- 0.40. the highest differentiation was found to be between MTR and PTR (0.40) while the lowest was found to be between NNTR and MTR. Similarly, Jost'D varied between 0.005-0.178. The lowest was again found to be between NNTR and MTR while NNTR , PTR and STR showed differentiation on the higher side (Table 4b).

G'_{ST}/JOST'D	Melghat Tiger Reserve	Navegaon-Nagzira Tiger Reserve	Pench Tiger Reserve	Tadoba Andhari Tiger Reserve	Umred Karhandla Tiger Reserve	Sahyadri Tiger Reserve
Melghat Tiger Reserve		0.005 (-0.16-0.22)	0.112 (-0.10-0.30)	0.120 (-0.19-0.15)	0.037 (-0.12-0.25)	0.120 (-0.002-0.28)
Navegaon-Nagzira Tiger Reserve	0.144 (-0.01-0.49)		0.178 (0.01-0.36)	0.048 (-0.08-0.14)	0.073 (-0.03-0.19)	0.174 (0.09-0.27)
Pench Tiger Reserve	0.406 (0.14-0.52)	0.266 (0.13-0.48)		0.042 (-0.10-0.27)	0.038 (-0.03-0.30)	0.162 (0.04-0.31)
Tadoba Andhari Tiger Reserve	0.166 (0.12-0.39) (	0.207 (0.08-0.34)	0.330 (0.13-0.52)		0.085 (-0.35-0.25)	0.039 (-0.04-0.14)
Umred Karhandla Tiger Reserve	0.165 (-0.03-0.44)	0.203 (0.03-0.44)	0.332 (0.16-0.51)	0.237 (0.06-0.39)		0.094 (-0.01-0.27)
Sahyadri Tiger Reserve	0.265 (0.11-0.46)	0.258 (0.17-0.36)	0.283 (0.13-0.39)	0.116 (0.03-0.23)	0.234 (0.08-0.42)	

Table 4b : G'st (lower diagnonal) and Jost'D values across all study areas

Results of the BayesAss program estimated the average contemporary gene flow rate. The rate of migration represented by the proportion of individuals migrating was found to be the highest from the NNTR to PTR (0.214) and from TATR to UKWLS (0.177) (Table below).

A(horizontal) B (Vertical)	NNTR	STR	PTR	MTR	TATR	UKWLS
NNTR	0.9145	0.0362	0.0097	0.01	0.0098	0.0098
STR	0.0026	0.9815	0.0026	0.0029	0.005	0.0027
PTR	0.2145	0.0198	0.6863	0.0195	0.0201	0.02
MTR	0.005	0.0698	0.005	0.905	0.0051	0.005
TATR	0.0105	0.0116	0.0104	0.0167	0.93	0.0105
UKWLS	0.0442	0.0221	0.0222	0.0223	0.1777	0.689

Table 4c: Results of migration of sloth bears from BayesAss output in EVL. The direction of gene flow is from A to B.

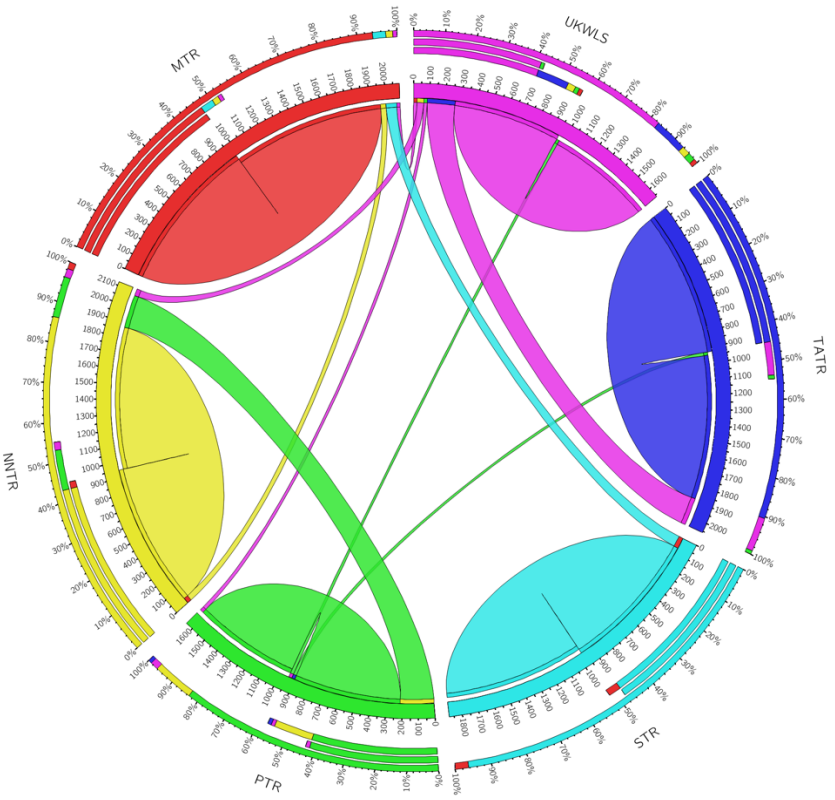


Figure 4d : BayesAss output visualization using circus plot to represent gene flow amongst the different population.

4.1.4 Discussion

It is imperative for the conservation of sloth bears, to generate comprehensive data on the demographic aspects of these animals in today's fragmented and anthropogenically altered ecosystems in order to maintain and restore their genetic diversity and connectedness. The main aim of this study was to understand genetic diversity and other parameters for sloth bears across the Vidarbha landscape and create a baseline data for bears.

I used a multidisciplinary strategy to address the spatial genetic patterns and structure of the species in the protected areas of Maharashtra. This technique included field sampling, genetic information, and multivariate and Bayesian analytical frameworks. Rapid urbanization, growing agricultural, infrastructural development, mineral acquisition, and economic growth are all causing substantial changes in the land-use patterns in this region (Reddy et al., 2013; Dutta et al., 2020).

Using landscape-scale microsatellite data, several genetic analyses first showed moderate to high levels of genetic differentiation in sloth bears as can be seen in Table 4b. Out of the six study areas that had been sampled- STR, MTR, TATR, NNTR, PTR and UKWLS, clustering showed the existence of 5 clusters based on TESS clustering. I considered the results for TESS to understand the structuring in the landscape because compared to the other analyses it not only considered the spatial locations but also gave a better and nuanced clarity on the clustering of populations. In the output admixture of bears of NNTR, PTR and UKWLS is clearly seen. UKWLS also shows some genetic signages of TATR. This is given the fact that UKWLS is placed geographically between NNTR and TATR and could be the sink for sloth bears moving across the landscape through UKWLS. In STR we clearly see two different population signatures. STR geographically has two components with Koyna wildlife sanctuary forming the northern part and Chandoli National park in the South. The genetic cluster of MTR and STR was clearly separated from rest of the study areas. Interestingly, MTR showed some genetic signatures of STR. This could be attributed to two reasons which may be sued to understand this. Firstly the employment of microsatellites used may be affecting the signatures. Secondly, it is plausible that before the landscape was divided, populations of the STR and MTR may have migrated through other forest sections, including the modern-day Gautala Autramghat Sanctuary and Yawal Wildlife Sanctuary. Investigations employing mtDNA could shed light on this. However no connection of STR to other study areas in the landscape was seen indicating complete loss of connectivity .

To ensure the survival understanding the genetic structure, gene flow, and demographic patterns is imperative to create effective conservation plans. The baseline data from the genetic database and the comparative variations in the genetic diversity and differentiation between different protected areas is presented in this study. In addition to helping to understand the effects in a similar landscape across the distribution range, my hope is that the findings and

recommendations from this study will generate important information on the genetics of the species from Maharashtra where conflicts between bears and humans are on the rise.

4.2. Sex-Biased Dispersal

4.2.1 Introduction

Dispersal occurs everywhere in the tree of life. Kin competition, avoiding inbreeding, and spatiotemporal variation in resources or habitat suitability are some of the mechanisms that select for dispersal. Dispersion is a major factor in many fields of ecology and evolutionary biology and influenced by species behaviour. Dispersal appears to be a dangerous behaviour since it frequently leaves behind suitable habitat and requires dispersers to travel through unfavourable habitat. However, successful dispersal and successful mating lay the groundwork for gene flow, which lessens the degree of genetic heterogeneity of populations that are semi-isolated.

The term "sex-biased dispersal" describes how males and females disperse differently within a community. Dispersal behaviour between the sexes has been documented to differ in several animals, including bears and it is essential to population dynamics and gene flow. The resource competition hypothesis put forth by Greenwood, (Greenwood 1980) the local mate competition hypothesis, and the inbreeding avoidance hypothesis are some of the theoretical theories that have been put forth to explain the evolution of this bias. According to such theories, the direction of this bias should primarily be explained by the mating system.

Given that no previous study had been conducted on the dispersal of sloth bears, I predicted that the dispersal will be male -biased based on studies done on other bear species where females do not disperse much and offspring care is solely done by the females.

4.2.2. Methodology

4.2.2a. Study area Sampling and Sexing

The study was conducted on scat samples from NNTR, PTR and UKWLS since the structure analysis showed admixture of these populations in to one cluster and hence these three study areas were considered for this analysis. This was primarily done because the it was assumed that since there was admixing in genes, there must be an stablished gene flow and hence dispersal. The molecular sexing was conducted on scat samples from each of these study areas. Sexual identification was conducted three times for each sample, independent of each other. The consensus of each sample was then used for analysis. A total of 110 scat samples had been used from the three study sites (UKWLS=27, PTR=35 , NNTR=48) for sexual identification.

4.2.2.b. Data Analyses

Genetic Structure in Males and Females

I employed the Bayesian clustering method implemented in STRUCTURE (Pritchard et al., 2000) to gain a broad picture of the population structure at the protected area level. To ascertain their place in the population, the analysis of males and females was done independently. It is expected that genetic structuring would be visible in individuals with philopatry (Pakanen, et al. 2021). An admixture model with 100,000 burn-in steps and 1000000 Markov Chain Monte Carlo (MCMC) repetitions was used for ten independent runs for k=1 to 10. This was done to make sure the chain stabilized. Additionally, in the event when the population's structure is weak, I improved the genetic assignment using the non-loc prior model followed by the loc prior as well. Using STRUCTURE HARVESTER v6.8, the ad hoc ΔK

(Evanno's approach) and the greatest estimated log-likelihood helped establish the optimal k (number of clusters) (Earl and vonHoldt, 2012; Evanno et al 2005).

Sex-Biased Dispersal

Fis Values

To find out how well the genotype frequencies within the population fit in the H-W equilibrium, I computed the inbreeding coefficient (Fis) by sex for each subpopulation. It is expected that with the presence of a sex-bised dispersal, the dispersing sex would have a positive Fis i.e. the dispersing sex's Fis value ought to be greater than the philopatric sex's as a result of Wahlund effect since the disperser's genotype originated in a different subpopulation (Wahlund 1928). Using R version 1.1.453 (R Development Core Team 2018), the evaluation was conducted using the demerelate function for Fis calculation included in the r package 'Demerelate' (Kraemer & Gerlach, 2017).

Fst

Fst estimators, which are computed for both males and females among sub-populations, can also be used to infer a bias in the migration rates between genders. This parameter measures the genetic differentiation between populations. It is expected that the sex dispersing will show a lower between-sub-populations Fst value compared to the philopatric sex (Weir and Cockerham 1984). GenAIEx 6.5 program was used to calculate pairwise Fst for the population (Peakall and Smouse, 2012) .

Intra-subpopulation Relatedness

The relatedness between the males and females of the three study areas with all the panels of microsatellite markers was conducted using Mueller and Goodnight Index (Queller

and Goodnight 1989) which eliminated biases related to inequal and small number of samples. This index calculates the relatedness between two samples by pair-wise comparison of the alleles shared between them and with the other samples. The R value i.e. the coefficient of relatedness has a range between -1 to 1. Therefore, a lower index is expected in case of sex-biased dispersal than the more philopatric sex i.e. individuals of the philopatric sex will have higher relatedness than those dispersing.

Assignment Index

The assignment indexes of males and females in a population were compared using Program FSTAT 2.9.4. the corrected assignment index, mAIC and vAIC values was calculated. In case of sex -biased dispersal the mean AIC (mAIC) would be lower than the variance of AIC (vAIC) when compared to the more philopatric sex (Goudet, Perrin and Waser 2002) . These indexes were selected because several studies have shown their efficacy in detecting intense biases in dispersal (Goudet, Perrin and Waser 2002; Mossman and Waser 1999).

Spatial Autocorrelation

Spatial genetic autocorrelation, often known as the spatial genetic structure between the sexes, was compared using Genalex 6.5 (Paekall et al., 2012). The pairwise genetic and geographic distance matrices are used to calculate the spatial autocorrelation coefficient (r) . For each individual whether male or female, I first determined the linear genetic distance and pairwise codominant genetic distance separately. The GPS coordinates of the samples were entered into the Universal Transversal Mercator (UTM) coordinate system, and GenAlEx program was used to determine the distances between locations.

4.2.3. Results

4.2.3a. Genotyping and Sex Identification

The total number of samples taken for the analysis was 110 samples, out of which 92 samples yielded data and could be assigned a gender i.e. either Male of Female. The rest of the samples for which sexual identification could not be done was not used further. The samples from each study was UKWLS=29 PTR= 20 and NNTR= 43 that could be assigned a gender. It was seen that the proportion of males was slightly more than that of females in all of the samples.

	UKWLS n=29	PTR n=20	NNTR n=43
Males	18	13	26
Females	11	7	17
PID sibs	5.048 e-06	5.34 e-07	4.404 e-07
PID	3.101 e-06	4.10 e-03	6.15 e-04

Table 4d: the tables shows the number of males, females , PID and PID sibs for the samples from NNTR, PTR and UKWLS

4.2.3c. Sex-Biased Dispersal

By running structure for all the three study areas we a fine scale genetic structuring for males and females at all the three study sites. The log-likelihood values for PTR M was k=2 while that for PTR F was k=3. Similarly, for NNTR M, cluster was k=2 and for NNTR F ,k=3 and finally a similar pattern was seen in UKWLS M where k=2 and for UKWLS F, k=3. This gives a slight idea that females appeared to be slightly more structured than male suggesting females may be philopatric in nature.

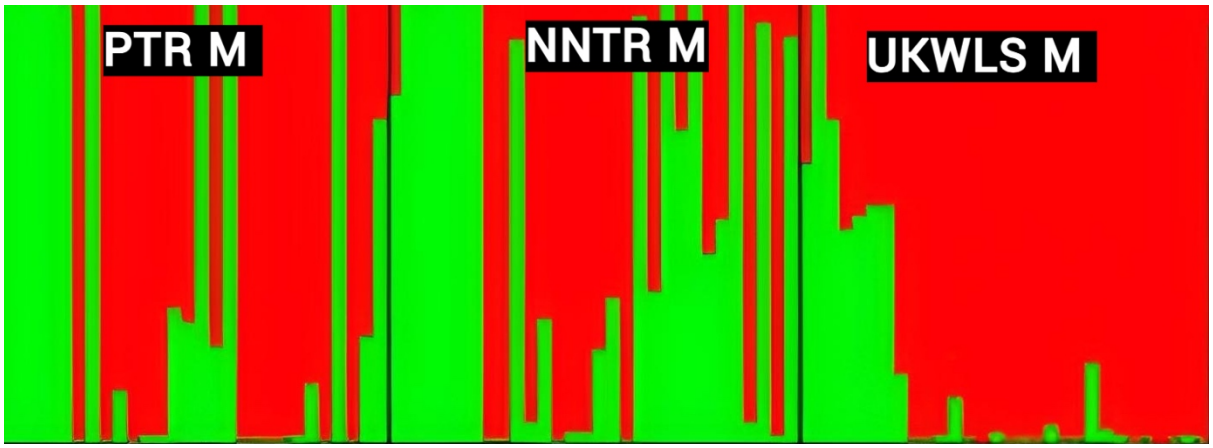


Figure 4e: Structure output for Males. The log-likelihood confirms number of clusters PTR M=2, NNTR M=2 and UKWLS M= 2.

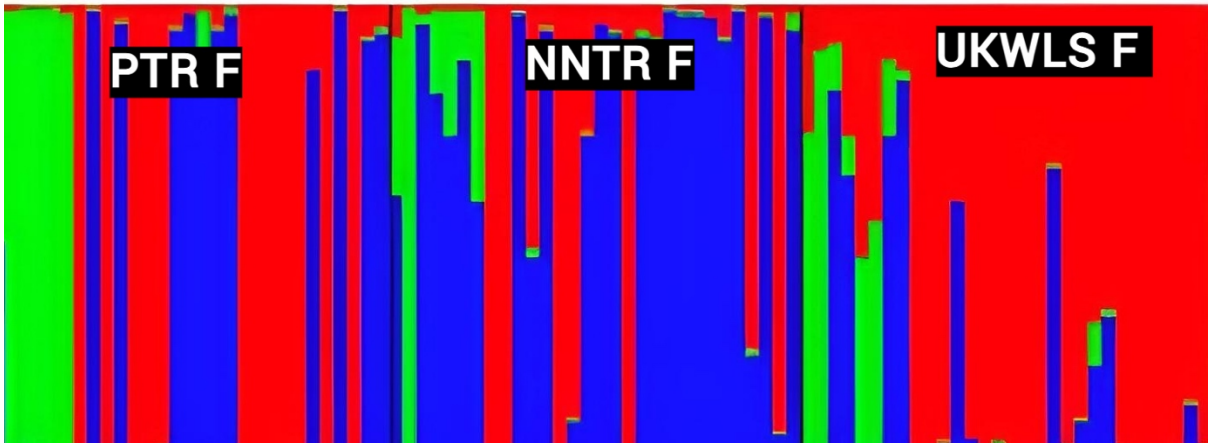


Figure 4f: Structure output for females . The log-likelihood confirms number of clusters PTR F=3, NNTR F=3 and UKWLS F= 3.

The Fis values of males is more than that of females as can be seen in the table below. This may indicate that females are more philopatric than males. This is because individuals of the dispersing sex display a higher Fis Value (heterozygote deficit) owing to Wahlund effect.

Fis Value (p-Value)	
PTR_male	0.187
PTR_female	0.045
NNTR_male	0.212
NNTR female	0.098
UKWLS male	0.29
UKWLS female	0.137

Table 4e: Fis Values for males and females across PTR, NNTR and UKWLS

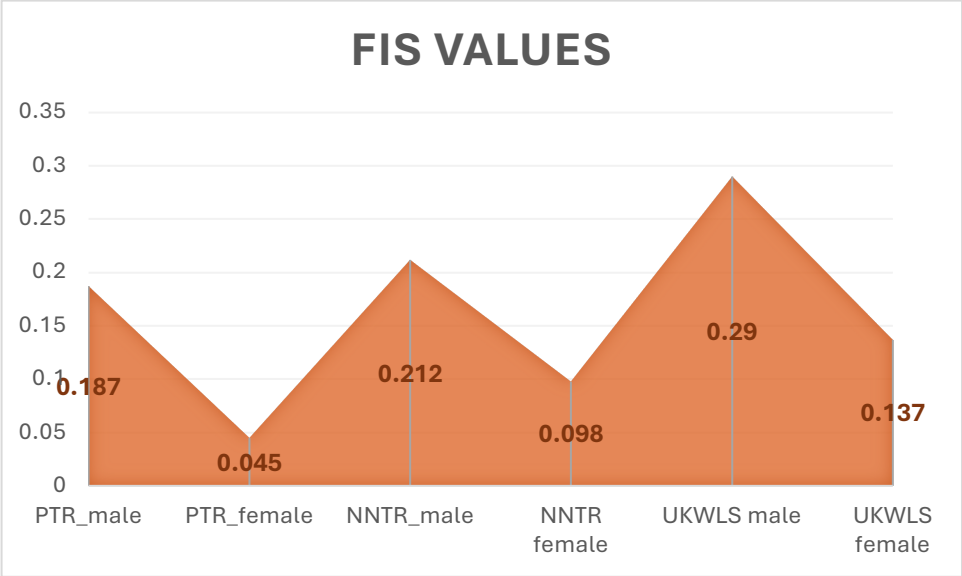


Figure 4g: Depicts Fis values of males and females. The peaks are of males while the dips in the chart are of females.

Subpopulation	PTR	NNTR	UKWLS
PTR	0	0.167	0.156
NNTR	0.051	0	0.061
UKWLS	0.0138	0.054	0

Table 4f: Fst values for females (above diagonal) and males obtained from. All F_{ST} values are significant at $P < 0.01$.

The relatedness coefficient r as derived from the Quellar-Goodnight Index showed that overall the males showed lower value than the females indicating that possibility that the females within a population were more related than the males and that males may be dispersing bolstering the hypothesis that dispersal is male-biased (refer Table 5g).

STUDY AREA	F-F	M-M	F-M
PTR	-0.020	-0.023	-0.003
NNTR	-0.045	-0.021	-0.020
UKWLS	-0.042	0.010	-0.019

Table 4g : Mean relatedness using Quellar-Goodnight Index between females-females, male-males and female-males

Assignment test was conducted on all samples from the study areas. The males across all the study area samples showed a lower mAIC value than females (Table 4h). Further, for males versus females in each of the study areas the value of the mean AIC was less than that variance. In case of PTR the p values were not every significant however, for the other two populations the p values were significant hence, it can be said that overall the mean AIC was less than v AIC significantly indicating a male biased sex dispersal.

	mAIC	vAIC
PTR Males	-1.69	1.22
PTR Females	1.154	0.68
NNTR males	-1.03	1.88
NNTR females	1.11	1.23
UKWLS males	0.6	0.56
UKWLS females	1.23	0.012
P value	0.037	0.777

Table 4h: mAIC and vAIC values for males and females with their P Values.

t-test	**PTR_M -PTR_F**	**NNTR_M -NNTR_F**	**UKWLS_M -UKWLS_F**
mAIC	-0.716	-0.89	0.616
p-value	0.44	0.02	0.01
vAIC	0.775	1.45	0.313
p-value	0.78	0.03	0.03

Table 4i: mAIC and vAIC values in assignment test with the significant values across PTR, NNTR and UKWLS

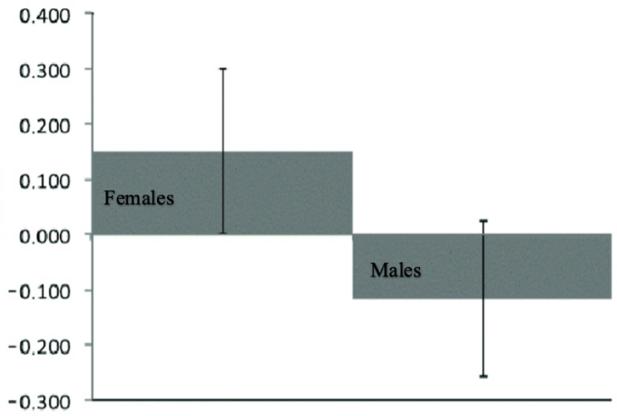


Figure: 4h The graph shows mAIC of males and females.

The data for females and male sloth bears had been combined to obtain the spatial autocorrelation for the individuals. The females showed significant decreasing spatial autocorrelation (r) up to 45 km (P value 0.00). The males, did not show any decreasing spatial autocorrelation (p value 0.028) lending support to a male-biased dispersal.

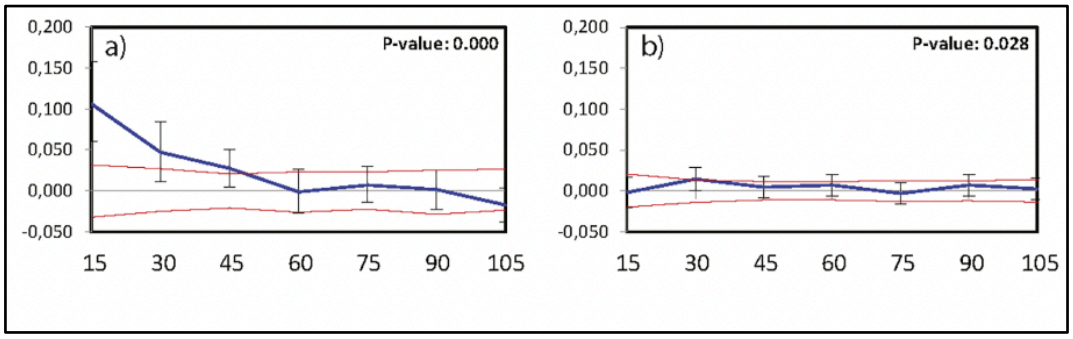


Figure 4i: Shows spatial autocorrelation of females (a) and males (b) combined of all three study sites.

4.3. Discussion

A number of factors both known and unknown determine the decision of a species to disperse. Sloth bears shares its landscape with tiger, leopards and dholes however no resource competition exists between these four species. Thus the drivers of dispersal in case of sloth bears purely depends on its own behaviour and intra-species factors.

The different tests conducted on the samples of males and females to determine their dispersal patterns indicates that sloth bears show male-biased dispersal. The Fis values between males and females showed that differentiation in males were higher than that of females indicating that in sloth bears the females were more philopatric. Similarly the fst and relatedness was lower for the dispersing sex (male) than the philopatric sex (females).

In case of sloth bears, which is mostly solitary in nature information about territory defence and competition for resources and mates is extremely limited. According to studies

(Gour et al., 2013; Biek et al., 2006; Janecka et al., 2007), the majority of solitary carnivores have a male-biased dispersal pattern and are typically characterized by uniparental care. Females Sloth bears are widely known for their parental care role. Cubs between 1.5-2 years stay with their mothers before dispersal. Hence, it may be said and as supported by the results of this study that, in sloth bears the males disperse more than the females.

Understanding the role of sex biased dispersal is important to understand behaviour of a species and also answer several genetics based questions such as inbreeding depression, bottlenecking etc. This study is the first to highlight the sex-biased dispersal in sloth bears. This may further be supported by data from other landscapes as well.

Developing a more comprehensive framework for the long-term persistence of populations in this anthropogenic period requires an understanding of the distribution pattern of this vulnerable monomorphic species. This study will aid in the development of targeted conservation plans for the species with limited ranges and poorly understood ecosystems. Dispersal patterns and a thorough understanding of the same will help in tailor-making of conservation and management plans.

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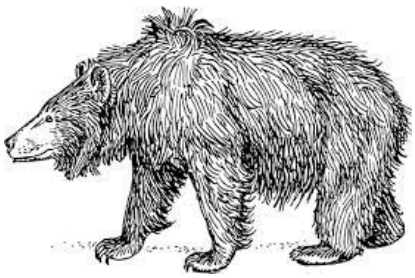
CHAPTER 5

Occupancy Modelling of Sloth Bears Across Eastern Vidarbha Landscape



5.1. Introduction

Species occupancy and distribution are fundamental concepts in ecology that provide insights into the geographic range and ecological role of a particular species. Distribution refers to the geographic area in which a species is found. It delineates the extent of the species' presence and includes all the locations where individuals of that species can be observed. Distribution can vary widely among species, ranging from local to global scales. Factors such as habitat suitability, climate, and ecological interactions influence a species' distribution. Occupancy, on the other hand, pertains to the presence of a species within a specific habitat or area. It focuses on whether a species is present or absent in a given location. Occupancy data can be used to assess the habitat preferences and ecological requirements of a species, helping researchers understand its interactions with its environment. Studying species occupancy and distribution is crucial for conservation efforts. It aids in identifying critical habitats, assessing the impact of human activities, and monitoring changes in populations over time. With habitat loss and climate change affecting ecosystems worldwide, understanding how species occupy and distribute themselves provides valuable information for making informed conservation decisions to protect biodiversity and ensure the long-term survival of species in their natural habitats.

5.2 Occupancy of Sloth Bears

5.2.a. Occupancy Modelling

Occupancy modelling is a statistical approach used in ecology to estimate the presence or occupancy of a species in a particular area while accounting for the imperfect detection of that species. It's a powerful tool for understanding species distribution patterns and the factors influencing their presence in each habitat. The basic idea behind occupancy modelling is to differentiate between two processes: the true presence or absence of a species in a site (its

ecological occupancy) and the probability of detecting that species if it's present (detection probability). This approach acknowledges that species might be present even if they are not detected due to factors like survey methods, weather conditions, or the species' behaviour. Occupancy models use data collected from repeated surveys of sites to estimate the probabilities of occupancy and detection. These models can incorporate various environmental variables such as habitat type, vegetation cover, and human disturbance, allowing researchers to analyse how these factors influence a species' distribution. Occupancy modelling finds applications in conservation planning, habitat management, and assessing the effectiveness of conservation efforts. It helps researchers make more informed decisions by accounting for detection biases and providing insights into the ecological requirements of species. Overall, occupancy modelling is an invaluable tool for understanding the complex dynamics of species presence, especially in the face of habitat changes and conservation challenges.

The Anthropocene has seen humans as major participants in shaping environmental and biological factors around them. The population explosion followed by the per capita resource exploitation corroborate role of humans as pivotal forces of environmental modification ((Crutzen & Stoermer, 2000). Such modifications often lead to ecosystem loss and fragmentation, both of which, have been acknowledged as primary causes of species extinction (Wilcox & Murphy, 1985; Rosenberg & Raphael, 1986). The accruing effects of human activities over the years has caused population reduction of several species and almost 1 million more stand threatened of extinction in the coming decade (IPBES, 2019). One such species, whose numbers have plummeted over the last few decades, is the sloth bear. The Sloth bear (Melursus ursinus) is an important but poorly understood species of the Indian subcontinent. Being endemic to the region and recently extirpated from some of its habitat mostly due to factors such as hunting and loss of habitat (Islam, et al., 2013) it is evident that the species continues to be under threat, even to this day. The IUCN has conferred the ‘Vulnerable’status

to the species considering the trend in deterioration of its habitat and a gradual decline in its numbers (Dharaiya et al, 2016).

Historically, the species was found in several places of the Indian subcontinent including India, Sri Lanka, Nepal, Bhutan and Bangladesh. However, in 2014 it was declared as extinct in Bangladesh (Islam, et al., 2013)after an extensive survey. Even though it continues to exist in the other regions, their distribution has become highly fragmented. In India, central India and the Western Ghats remain the prime strongholds of sloth bear distribution (Yoganand, Rice, Seidensticker, & Johnsingh, 2006). These areas have also emerged as prime areas for developmental activities such as laying of roads, railways etc, all of which comes at a cost to nature (Congo, 1956). As development continuous to advance in various forms, it throws several challenges to the natural environment around it.

In landscapes that are dominated by humans, sloth bears are seen to be nocturnal and avoid interactions with them (Seidensticker, Yoganand, & Johnsingh, 2011) . However, human activities such as logging, mahua collection, defecation, cattle grazing often lay the foundations for human-bear conflicts. Therefore, in a continuously modifying landscape, it becomes indispensable to understand what factors influence their occupancy in any given area.

Habitat usage and occupancy have been described before using techniques such as radio-collaring, indirect evidence and direct field observations. However, in the recent years camera trapping has been acclaimed because of being – (i) non-invasive in nature, (ii) Efficient and continuous (both day and night) sampling (iii) Sampling in inaccessible areas (Trolliet, Huynen, Vermeulen, & Hambuckers, 2014). Sloth bears are elusive and shy and hence, and camera traps are an ideal tool to study them. Only two studies have been conducted so far using camera trapping technique to understand habitat use by sloth bears (Rather, Kumar, & Khan, 2021;Chaudhuri et al 2022). This thesis chapter aims to add to the existing knowledge about environmental, ecological and anthropogenic factors influencing space usage by sloth bears

5.3. Study Area

The study was carried out across protected areas in the Vidharbha Landscape, Maharashtra, India. It covers seven districts of the state- Amravati, Bhandara, Chandrapur, Gadchiroli, Gondia, Nagpur and Wardha. The area has diverse fauna and is home to large carnivorous mammals such as the tiger, leopard, dhole and the sloth bear. The protected areas in which sampling was conducted include- Melghat tiger reserve, Pench Tiger Reserve, Nawegaon-Nagzira Tiger Reserve, Umred-Karhandla Wildlife Sanctuary, Tadoba Andhari Tiger Reserve, Bor tiger reserve , Tippeshwar wildlife sanctuary and Brahmapuri. Among the study sites, Melghat, Pench and Tippeshwar are not connected directly through contiguous forests to the rest of the sites. The remaining seven sites share corridors which aid in movement of animals from one protected area to another and to other areas as well.

The Waiganga, a tributary of Godavari is the largest flowing river in Vidarbha. The main forest types include tropical dry deciduous forests, moist deciduous forests and sub-tropical hill forests (Champion & Seth, 1968). The region experiences dry conditions during the summers with temperature soaring to 40-48 degrees Celsius. The winters temperatures are moderate and range from 12- 25 degrees Celsius (Tiple, Andrew, Subramanian, & Talmale, 2013) .

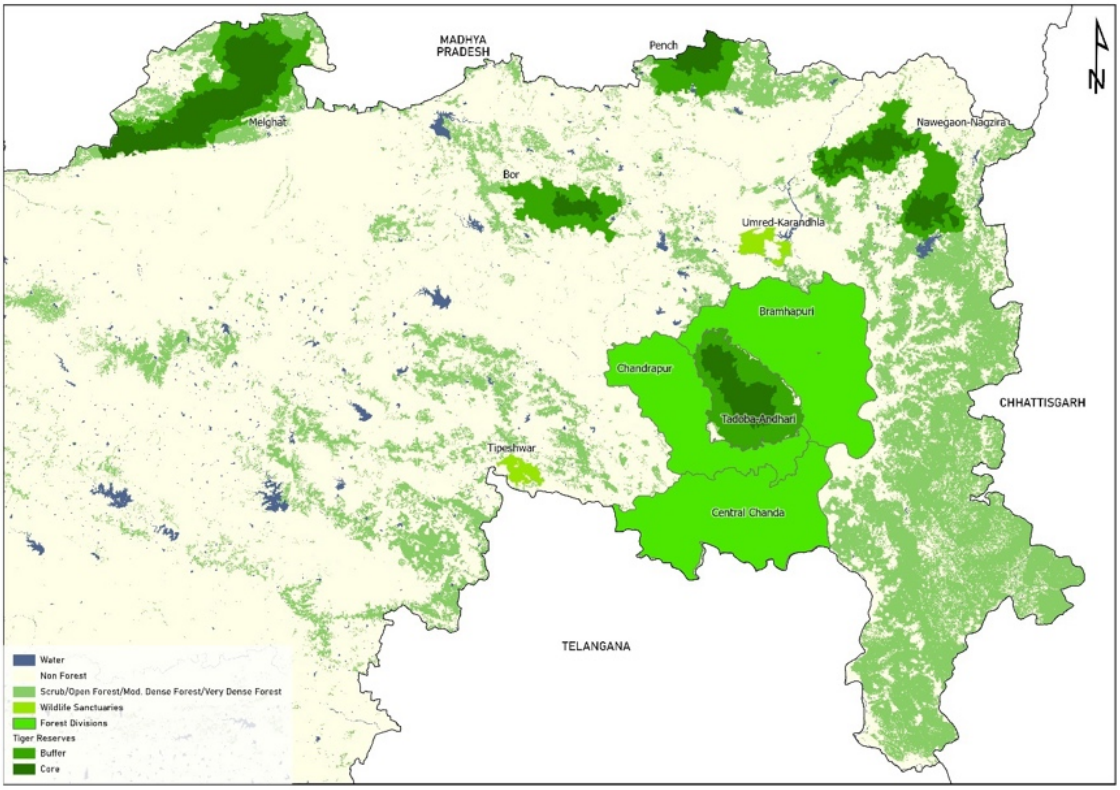


Figure 5a: Map depicting all of the study sites for camera trapping exercise namely Melghat Tiger Reserve, Tadoba Andhari Tiger Reserve, Tippeshwar, Bor Tiger Reserve, Pench Tiger Reserve, Brahmapuri, Central Chanda and Chandrapur districts

5.4. Methodology

5.4.a. Field Method

Camera traps were deployed across the landscape in selected protected and a few adjoining areas. Prior to deployment, a survey of the areas was conducted along forest paths, animal trails, stream beds and dirt tracks to record signs of animal presence. This was done in accordance with the protocol prescribed by Karanth and Nichols (1998). Using ArcGIS, camera sites were selected by laying out grids measuring 2 sq km over the study areas. This was done to ensure maximum coverage for sampling without leaving any large gaps. Motion triggered camera traps were placed during the trapping exercise and were either tied to poles or on tree trucks at a height of about 25-30 cm above ground. The cameras were set on multi-shot mode with a delay of 5s between each shot. The trapping exercises were conducted between the

months of February-May, for a period of 25-30 days. Since the traps were expected to be functional throughout the entire sampling period (day and night), regular monitoring of the traps and battery replacement was done to ensure maximum functional efficiency.

5.4.b. Sloth Bear Occupancy

At each of the camera site, occupancy rates of sloth bear (ψ) was estimated following a likelihood-based methodology using 'Unmarked' extension package in R (Mackenzie & Royle, 2005).Package "CamTrap" in R was used to generate species detection history (Niedballa, Courtiol, Wilting, & Sollmann, 2016). Detection histories for sloth bear was constructed at each camera trap site over a period of 30 days. For each site, a binary indicator of sloth bear detection or non-detection was recorded in a matrix. Thus, each detection (photo capture) of a sloth bear was indicated by '1' while each non-detection was '0'.Each sampling unit i.e. camera trap is regarded as a 'site' as described in MacKenzie et al (MacKenzie, et al., 2002). It is assumed that detection of sloth bears at each site is independent and that the state of occupancy across all sites is constant (i.e. close to changes) (Mackenzie & Royle, 2005). Detection histories from all the sites across the landscape was pooled together and used for single species single-season occupancy modelling in R.

5.4.c. Covariate Modelling

Previous studies on sloth bears have revealed that sloth bears share several ecological relationships with the surroundings they thrive in. Therefore, in order to incorporate these known relationships in our occupancy modelling, we included site based and GIS extracted variables as predictors of occupancy and detection.

The *a priori* factors shaping sloth bear occupancy, that we predicted, were primarily based on previous studies conducted on the species .Forest cover and presence of large boulders that could be used for denning have an effect on sloth bear occupancy (Akhtar, Bargali, &

Chauhan, 2007; Yoganand et al, 2005), hence we derived factors such as elevation, rugedness and forest type.Even though sloth bears are considered to be fairly shy in nature, there have been several reports about sloth bear-man conflicts (Dhamorikar, Mehta, Bargali, & Gore, 2017) (Singh, Sonone, & Dharaiya, 2018). Dharaiya reported (2009) that bears were often seen to visit villages that added to the chances of interactions between humans and the bears. In their study, Dhamorikar et al (2017) found that maximum interactions occurred within forests while some also occurred along forest edges. Therefore, we predicted that human factors such as settlement, population, distance from road and night light would have a negative influence on sloth bear occupancy. Sloth bears are found in a wide range of habiatas, mostly forests (Dharaiya, 2020)Since, dry and moist broadleaf forests are considered to be essential sloth bear habitats (Seidensticker, Yoganand, & Johnsingh, 2011) along with scrublands and grasslands (Joshi, Garshelis, & Smith, 1995) (Ratnayeke, Manen, Van pieris, & Pragash, 2007), we predicted that dry forests would have a positive impact on sloth bear occupancy.Therefore, we used LULC along with Forest Type to understand their impact.

Further, it is also known that space usage by animals often depends on the spatial scale (Mayor, Schneider, Schaefer, & Mahoney, 2009).There is a dearth of information as to how landscape cues affect the habitat use of sloth bears. Therefore, to examine the effect of different covariates (landscape and ecological), we aggregated GIS based landscape features at 3 scales. The smallest scale was the point scale where the immediate covariate readings of the deployed camera trap was taken. The intermediate scale was the 250m scale, where covariates were taken at a 250m radius around the camera trap point. The largest scale was the 500m scale, where using a radius of 500m around the site location was set up and covariates were derived. The roads, water bodies and settlement data were converted into distance rasters using the conversion tool in the ‘Distance’ toolset (ESRI Inc, 2020). The distances were calculated using ArcGIS Pro v. 2.7.26828 (ESRI Inc, 2020) proximity toolset called “Near”. This toolset

calculates the distance and other additional proximity features between the input feature and the closest feature in another raster layer.

The variables to be used were first arranged and then correlated to detect any correlation to one another. We used package 'Unmarked'in R to procure candidate models, assessing the covariates at three different sptial scales. The best model was selected based on AIC values (Burnham & Anderson, 2004)

5.4.d. Model Selection

The 17 covariates were scaled and correlation between the covariates was generated (Figure 5b). Covariates are commonly not used for further modelling if they show high correlation to each other .However none of the covariates showed very high correlation and were therefore used. Each covariate that we had used to construct our hypothesis, was then individually checked at the point level first (0m) for its effect on occupancy and the top 60% of these covariates were then taken based on their AIC. 12 covariates were included for dredging- Elevation, Nighlight, Slope, Roughness, Distance from Roads, Population, NDVI, Bioclim1, Livestock, Deciduous forest, mixed deciduous forest and dry teak forest. This was done to maximize output and minimise the run time for dredge function. These 12 covariates were then dredged for all the three spatial scales- 0m, 250m and 500m. The model selection was done using the dredge function from MuMIn, a package in R, which fits models through repeated evaluation and creates model subsets in the global model (Barton, 2020). NDVI and forest types were used for modelling detection, while all covariates were taken as occupancy factors. Ranking of candidate models and calculation of their Akaike weights was done using generated Akaike Information Criterion (AIC) values. The top models were then averaged and used for prediction modelling . The predicted values for occupancy were utilized for mapping. In order to select the most prominent factors model contribution was also conducted to understand which

of the covariates contributed most based on their averaged weight values on the occupancy of the sloth bears and would further be used for generating resistance surfaces.

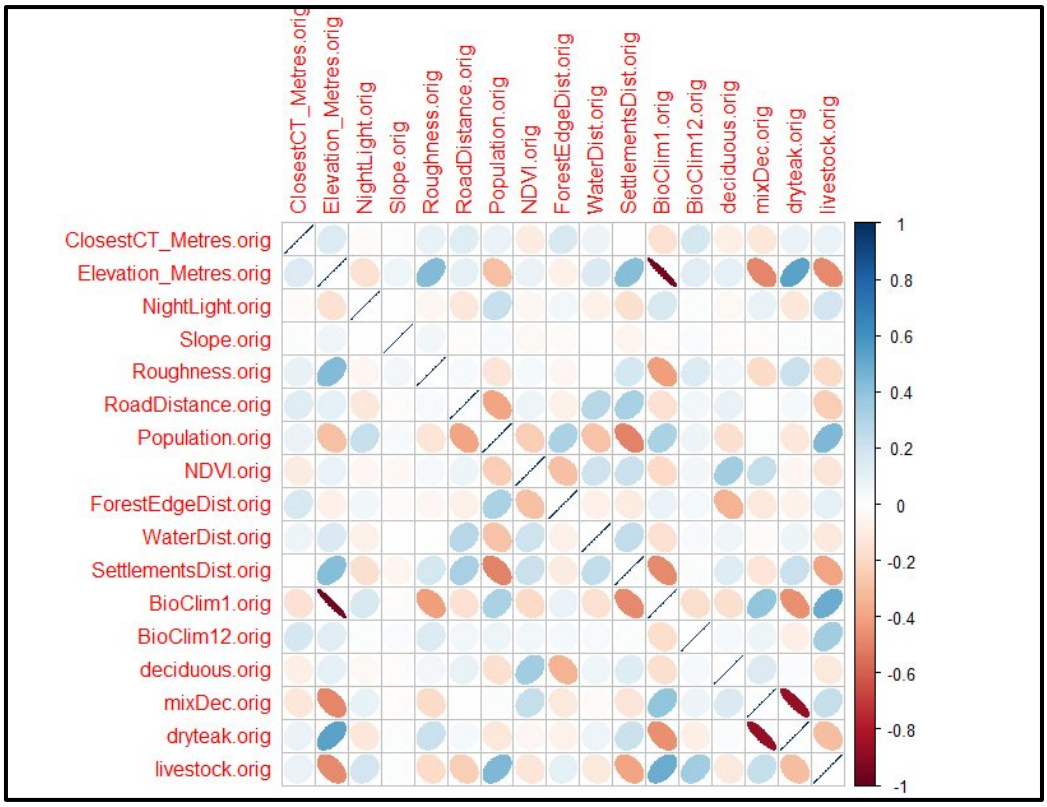


Figure 5b: shows correlation generated between the variables considered apriori

5.5. Results

5.5.a. Occupancy and detection

The naïve occupancy and detection values at the three spatial scales were calculated. For the point scale the occupancy estimation was 0.43 and p was 0.051. For 250 metres, occupancy was 0.46 and detection was 0.055, while for 500m the occupancy was 0.46 with 0.055 detection.

5.5.b. Effect of covariates

The null model, in which no covariate was included , did not perform well. It was seen that at 0m maximum number of covariates featured in the top models. i.e.8 covariates. In 250 m scale the number of variables was similar while for 500 m scale the number of covariates in

the top model decreased to 5. Hence, I considered the results of the 0m spatial scale to derive the surfaces for the landscape.

Variables	Occupancy Estimates at point scale (0m) showing maximum effects				
	AIC values	Estimate	SE	z	P(>|z|)
P(.)Psi(Bioclim1)	13866.17	0.00146	0.000204	7.14	9.14e-13
P(.)Psi(Elev)	13807.32	-0.00014	0.000377	-0.371	0.711
P(.)Psi(NL)	13804.88	-0.222	0.303	-0.735	0.462
P(.)Psi(Rough)	13796.37	0.00615	0.00229	2.684	0.00727
P(.)Psi(RD)	13885.63	7.32e-05	0.000031	2.361	0.0182
P(.)Psi(Pop)	13795.54	-0.246	-0.0897	-2.74	0.00607
P(.)Psi(livestock)	13890.1	5.48e-05	3.14e-05	1.746	0.0809
P(.)Psi(NDVI)	14264.13	0.000586	1.28e-05	46	0
P(.)Psi(FED)	13802.77	-0.0715	0.0467	-1.53	0.126
P(.)Psi(slope))	13886.43	0.000389	7.93e-05	4.90	9.48e-07
P(.)Psi(Set)	13897.29	0.000197	4.82e-05	4.08	0.000045
P(.)Psi(FT)	13802.53				
Southern Dry Mixed Deciduous Forest		4.57	10.3	0.4455	0.656
Very Dry Teak Forest		4.30	10.3	0.4191	0.675

Table 5a : The top 12 covariates with their AIC values. The covaries are (NL= nighlight, Elev= Elevation, RD= Distance from road, Pop= Population, density, FED- distance from Forest edge, Set-= Distance from settlement, FT= Forest Type

With the inclusion of covariates, the performance of the models increased. The 12 top covariates were dredged to give the top models ($\Delta AIC<2$) which were then averaged to obtain the best fitting models included a combination of different covariates.

Model Averaged for covariates at point scale

	Estimate	Std. Error	z value	Pr(>|z|)
psi(Int)	-0.1444494	0.06329062	2.28231981	0.0224705
psi(BioClim1)	0.15171977	0.05666386	2.6775402	0.0074165
psi(Elevation)	0.18526346	0.05974664	3.10081787	0.0019299
psi(RoadDist)	-0.1754114	0.09696224	1.80906897	0.0704403
psi(livestock)	-0.1421373	0.05908904	2.40547651	0.0161514
psi(Population)	-0.1404319	0.06103575	2.30081335	0.0214022
p(Int)	-2.8739813	0.03780277	76.0256778	0
p(DecFor)	0.08223911	0.03591472	2.28984447	0.0220303
p(NDVI)	-0.1171371	0.03202281	3.65792834	0.0002543
p(VDTF)	0.15777389	0.03207113	4.91949915	9e-07
psi(Nighlight)	0.03920171	0.05794474	0.67653625	0.4987002
psi(TeakForest)	-0.0089848	0.03669607	0.24484245	0.8065784

Table 5b: Model averaged for top covariates. The top contributing covariates are given below (below)

Top parameters contributing to models (by weight)	Elevation Nightlight Distance from Roads Population (human) NDVI Bioclim 1(mean Annual temperature) Livestock Forest Type (includes Deciduous, mixed dec and teak)

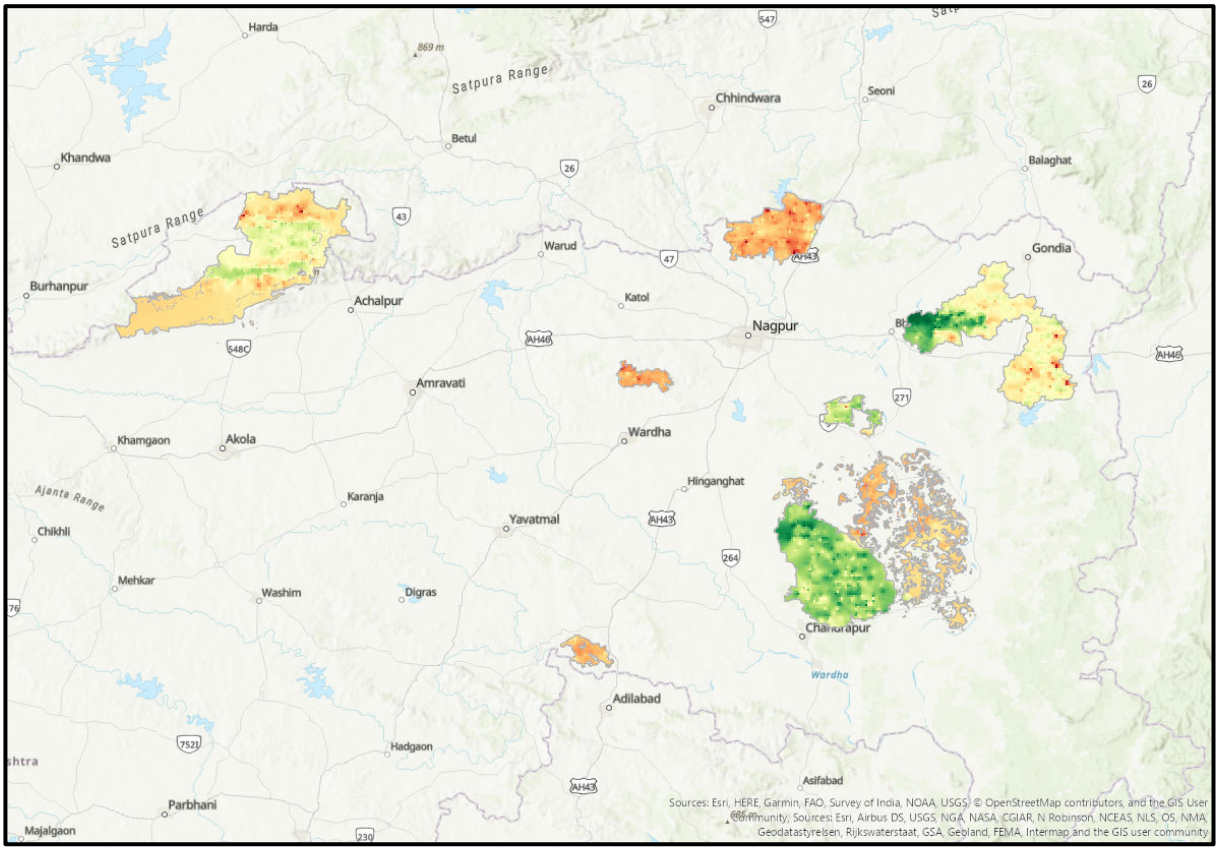


Figure 5c: Shows occupancy generated through IDW for point scale (0m)

5.6. Discussion

5.6.a. Occupancy

Our study is the first ever occupancy modelling and mapping study from the Eastern Vidharbha Landscape, Maharashtra. Sloth bears are extremely elusive and having used non-invasive sampling technique across all sampling areas, the probability of detection is low. The naïve occupancy estimation is 0.45. Taking imperfect detection (MacKenzie, et al., 2002) and effect of covariates in to account the occupancy predicted by the averaged model performed better.

5.6.b. Covariate Effects on model parameters

The occupancy and detection probability did not show major difference. We concluded that the covariates that we had selected had a considerable influence on sloth bear occupancy. The results we obtained on covariates effect at all three spatial scales was similar. However at the point scale, the effect was most prominent. Obtaining an understanding of the influence that each of these variables have on occupancy can be quite exigent. This is primarily because response to disturbances is associated with basic ecology, behaviour and evolutionary traits of the species and also on the extent and frequency of disturbance (Graham, Smith, & Freund, 2021). The individual variable models confirmed that disturbances such as population, roads and nightlight along with resources such as water and forest cover had to interplay and shape the habitat usage of sloth bears. It was interesting to see that occupancy was negatively affected by elevation and that Night light also affected sloth bear occurrence negatively. Other factors that had a negative impact on their occurrence are population and distance from forest edge. This confirms that even though bears tend to avoid humans, they may occur near the forest edges. Since, such areas may lead to unprotected areas as well, the landscape interspersed with adequate forest cover and resources could aid in movement of the sloth bear and serve as corridor to maintain genetic diversity (Athrey, Odden, Linnell, Krishnaswamy, & Karanth, 2013). From the models, it is clear that the prime determinants of sloth bear occurrence across all scales is not only driven by the forest type but also depends on disturbances attributed to distance from settlement and distance from road. Our results also show that land use especially (Appendix) in the form of plantation or cropping , negatively affects sloth bear distribution. In a landscape that is predominantly inhabited by humans, studies have shown that in shared landscapes, sloth bears tend to avoid areas that are frequented by humans and are highly used (Garshelis & Smith, 1999). Ratneyeke et al too showed that in Sri Lanka, bears avoided areas of high disturbance (Ratnayeke & Padmalal, 2007).

5.7. Selection of Variables for Connectivity

The variables that showed profound effect on sloth bear occupancy included disturbance covariates (population, livestock, nightlight and distance from road) and environmental factors (temperature, elevation, forest type and ndvi). These covariates were selected from their averaged weight conducted in R (ver 3.0) to understand the contribution of each variable on sloth bear occupancy. All these covariates were then optimized and used for further modelling and identifying corridors and areas of conservation as described in Chapter 6.

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CHAPTER 6

Genetic Connectivity and Connectivity Gradient across Eastern Vidarbha Landscape

6.1 Introduction

Habitat fragmentation is a pervasive and escalating issue in the world today, driven primarily by human activities that alter landscapes (Hanski 2011). Rapid urbanization, agricultural expansion, infrastructure development, and deforestation are among the key contributors to the fragmentation of natural habitats. As populations grow and economies expand, these activities lead to the conversion of large, continuous habitats into smaller, isolated fragments and is a potential threat to dispersal and movement. Metapopulation theory states that any such reduction could drastically decrease possibilities of local species to re-colonize an area from where it was pushed out or phased out, thereby leading to large loss of biodiversity at an even greater scale (Hanski and Ovaskainen 2003; Ozinga, et al. 2009).This global phenomenon has profound consequences for biodiversity and ecosystems. Fragmented habitats result in isolated populations, reducing genetic diversity and making species more vulnerable to environmental changes, diseases, and other threats.

Measuring protected area network connection through outcomes, such as genetic diversity or species occurrence (Baguette, et al. 2013; Opermanis, et al. 2012), is critical to understanding how landscape planning techniques conserve biodiversity. By studying functional connection patterns between different habitats and identifying these connections would help stake holder maange these remnant connection to ensure connectivity in the future. Understanding how biological systems respond to issues such as land-use change and habitat fragmentation requires a focus on connectivity. Structural and functional connectedness are well-established ideas in ecology (Vogt, et al. 2009). Functional connectivity is species specific and explicitly considers the ability of the species under focus to move across habitat patches (Crooks and Sanjayan 2006) and it makes it more prudent to study the functional connectivity

of sloth bears which unfurls how the species chooses to move through the landscape given the positioning and effects for different natural, man-made and environmental factors.

Landscape connectivity studies that include habitat variable, behavior and dispersal abilities of a species are deemed to be more realistic (Drielsma, et al. 2022). Most studies till date have focused on larger animals or important and charismatic animals such as tigers or elephants as a representation other taxa (Williams, et al. 2012). It is true that in some cases a single connectivity model mapping may serve the need of multiple species however, however such studies are mainly skewed to only the higher species neglecting the needs of mapping of other less-studied species (Cushman and Landguth 2012). Since in our study landscape, human-bear conflicts are not uncommon and with the rising cases of bear fatalities owing to roads, it has become important to obtain a connectivity map for sloth bears in the landscape.

For this study ,circuit theory was selected over least cost route. This is particularly because, in the case of least cost route, the algorithm just considers one route between a pair of locations ignoring the possibility of alternative routes or incorporating species-specific behavior (Moilanen, 2011). However, circuit theory overcomes such a drawback and considers all paths of movement possible in a landscape. In the Circuit Theory ,the landscape is depicted as a grid and a circuit (refer figure below). The terrain is made up of one "barrier" cell with infinite resistance (black), one dispersal habitat with finite resistance (gray), and two adjacent patches of 0-resistance cells (open). Nodes (tiny dots) are used in place of cells with finite resistance, and resistors are used to connect neighbouring nodes. Large dots represent the consolidation of patches of cells with 0 resistance into a single node. It is also possible to include connections between nonadjacent cells and diagonal neighbours, the latter of which indicate "hops" over intervening cells.

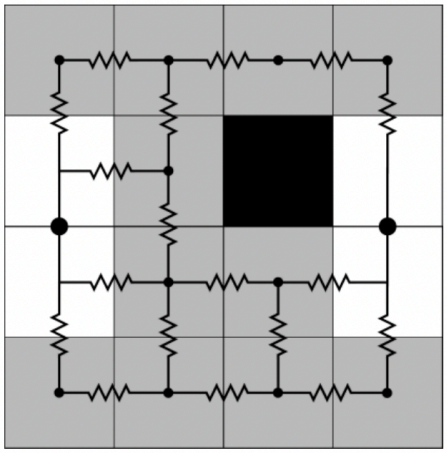

Figure 6a: Landscape depicted as an electric circuit with resistance and connectivity (McRae, et al. 2008)

In this chapter, I try to understand how sloth bears move across the landscape and the different factors that influence its geneflow. I hypothesize that forest cover and forest type would have a profound influence over other factors.

6.2. Methodology

The genetic dataset of sloth bears generated and used in chapter 4 was used for the study. Following the occupancy modelling of sloth bears conducted and explained in chapter 5 the variables and surfaces to be used for developing the resistance surfaces were selected. Landscape resistance surfaces to estimate influence of these variables on the sloth bears in the study areas was then developed.

6.2.a. Developing Resistance Surfaces

All surfaces were first converted to ASCII. I utilized ResistanceGA (Peterman, 2018) in Rstudio ver.105.1.2.5 to optimize the link between genetic distance and landscape resistance distance using GA R package (Scrucca, 2013), and pick Rmax for each landscape variable model. ResistanceGA determines the linear mixed-effects model fit with genetic

distance measured as actual distance and estimated using Genepop as the dependent variable and the scaled and centered resistance distance as the predictor variable (Rousset, 2008).

I used GenAlEx to do a mantel test to see if there was any relationship between pairwise codominant genotypic and geographical distance .Furthermore, I used the spatial autocorrelation function for each species to investigate the spatial extent of genetic structure. Mixed models were fitted using the maximum-likelihood population effects (MPLE) parameterisation (Clarke, Rothery and Raybould 2002), which was implemented in the R package LME4 , to account for the non-independence of pairwise genetic and ecological distances. I measured the cost distance using CIRCUITSCAPE v. 5.0.0 built in the JULIA computer language (Hall, et al. 2021).

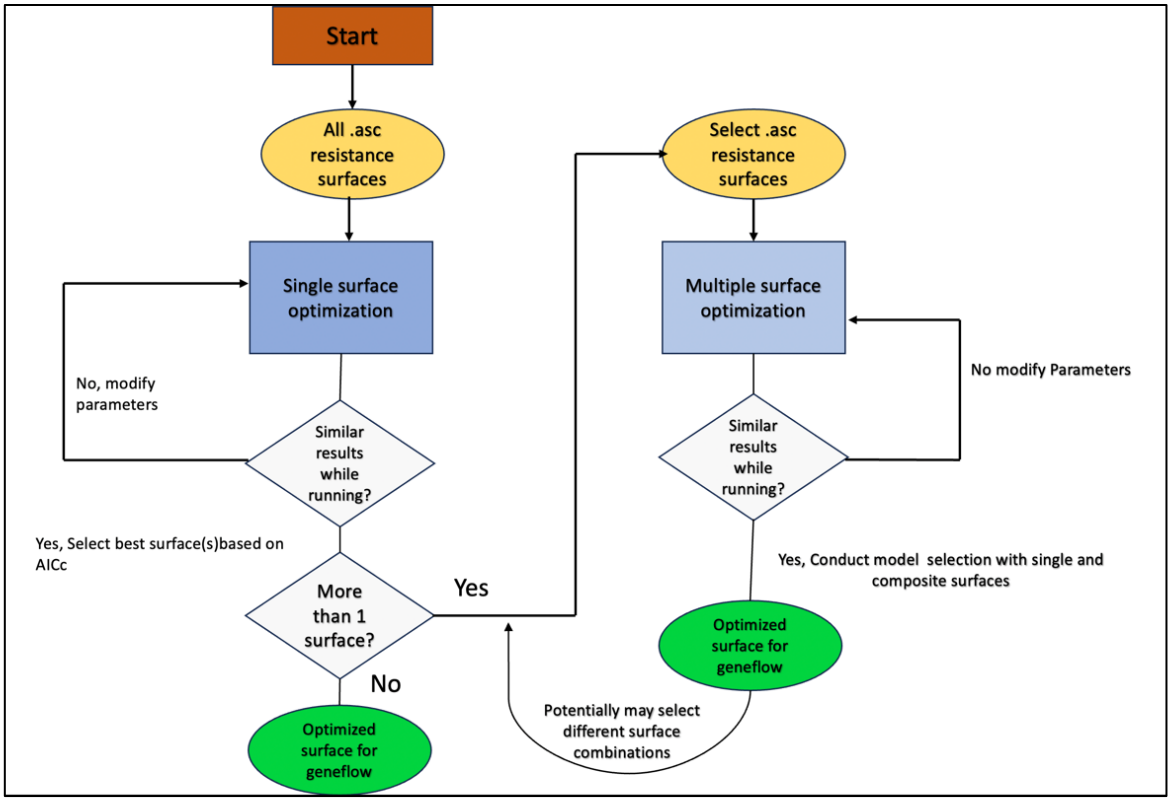

Figure 6b: Flowchart depicting the methodology for the optimization of the resistance surfaces

I examined nine alternative transformations for all continuous variables except LULC to get the best fit between the two variables. The pairwise geographic distance was used as a null variable, and I ran the optimization three times independently to ensure reliable results. AIC values from linear mixed effect models were used to determine model fit. The MLPE model's covariate structure was designed to account for non-independence of values within pairwise distance matrices (Clarke, Rothery and Raybould 2002).The random effect in these models indicates the dependencies between each pairwise distance. I resampled 75% of the samples without replacing them; each surface was fitted to a subset of the data using its optimized resistance surface to control for potential bias in the analyses. The technique was repeated 10,000 times to find the AICc-best model (π) and each model's marginal R2 value. Multi-surface optimization assessed the significance of each landscape feature by determining the percent contribution of each surface by dividing each transformed resistance surface by the total number of composite multi-surface resistance surfaces.

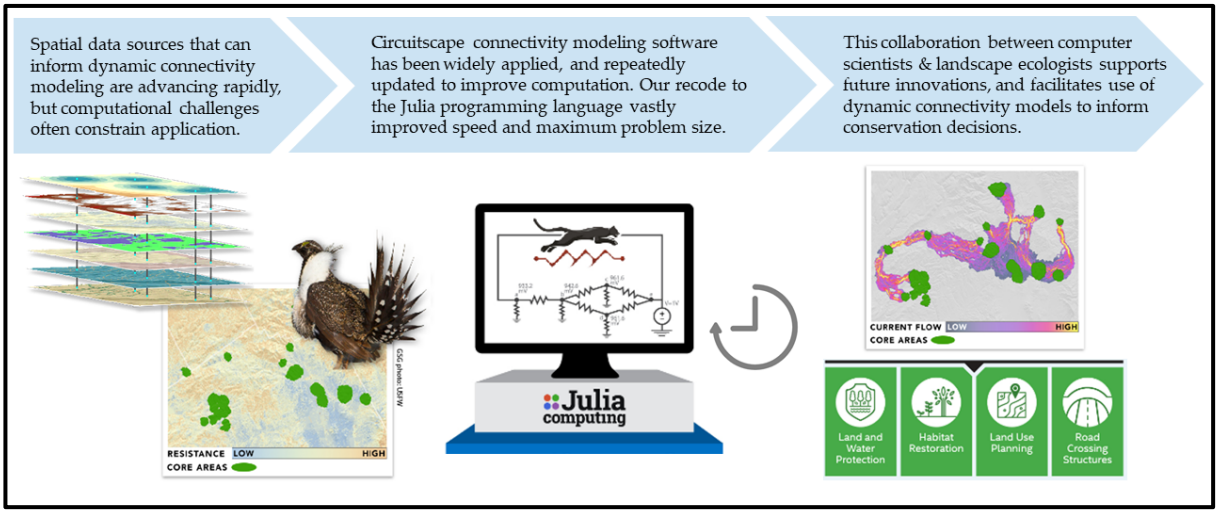

Figure 6c: Depicts Circuitscape in Julia Interface

6.2.b. Mapping Connectivity

To understand the visualize the flow of current and the possible corridors for sloth bear movement in the landscape under study Circuitscape and linkage mapper (1.0.2 ArcGis tool) was used.

Circuitscape models movement and gene flow in a landscape and helps in identifying corridors or areas that may require conservation or restoration. It works on the theory of electrical circuits and hence can seamlessly induct all possible “circuits” or paths in the landscape (McRae, Shah and Mohapatra 2013). The pinchpoint mapper of linkage mapper toolbox, both use Circuitscape to map and prioritize possible species corridors.

6.3. Results

6.3.a. Spatial Autocorrleation

The mantel tests shows significant correlation between the genetic and geographical distance r= o.13, p=0.11. The spatial autocorrelation showed significant autocorrelation for the first 100km after which it broke down and showed no correlation.

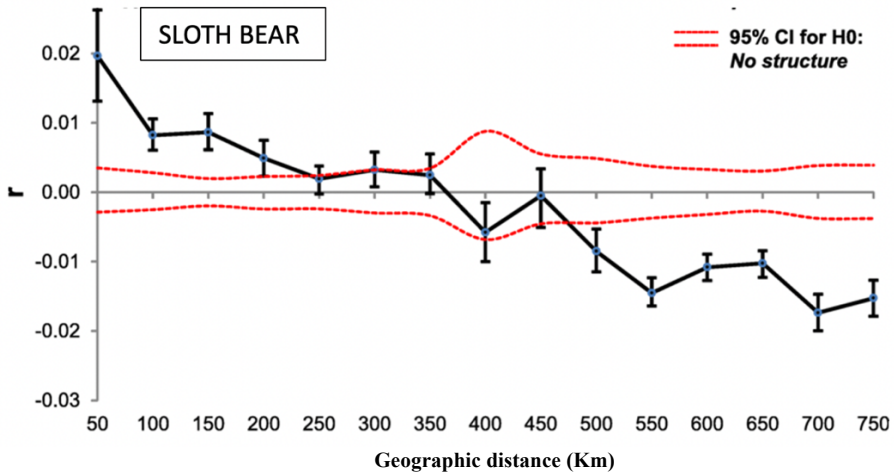

Figure 6d: Spatial genetic structure correlogram for sloth bears. The X axis depicts geographic distance and Y axis represents the coefficient between genetic and geographic distance

6.3.b. Univariate Optimization

Single surface optimization was conducted in Circuitscape. Overlapping or sample location that were in close proximity were removed to minimize the chances of occurrence of more than one sample in one pixel. Of the 265 samples that been used for genetic demographics, 187 samples have been used for surface optimization. The single surface optimization process is used to accurately identify the parameters that facilitates or impedes gene flow in the landscape. I took into account models that met both the marginal R2 and AICc requirements. Distance from roads and NDVI are the best-supported models for sloth bears (table below).

The graphs of resistance versus distance from road and resistance versus NDVI (refer figure 6e), showed that with increase in the distance from the roads the resistance value decreased while in the case of NDVI, with an increase in the vegetation cover, the resistance decreased.

Surface	K	Equation	AIC	Av Weight	Average Rank
Distance from Roads	2		-567.765	0.391	1.44
NDVI	4	Inverse monomolecular	-566.543	0.456	3.03
Population	4	Monomolecular	-589.820	0.256	4.56
Forest type	4	Inverse monomolecular	-567.878	0.142	5.98
Nighlight	4	Inverse-Reverse monomolecular	-585.887	0.096	6.07
DEM	4	Monomolecular	-568.712	0.045	6.89
Livestock	4	Inverse monomolecular	-.566.751	0.053	7.09
Mean Annual Temperature	4	Inverse Monomolecular	-577.901	0.076	9.58

Table 6a : Average weight and Rank of contributing resistance surfaces

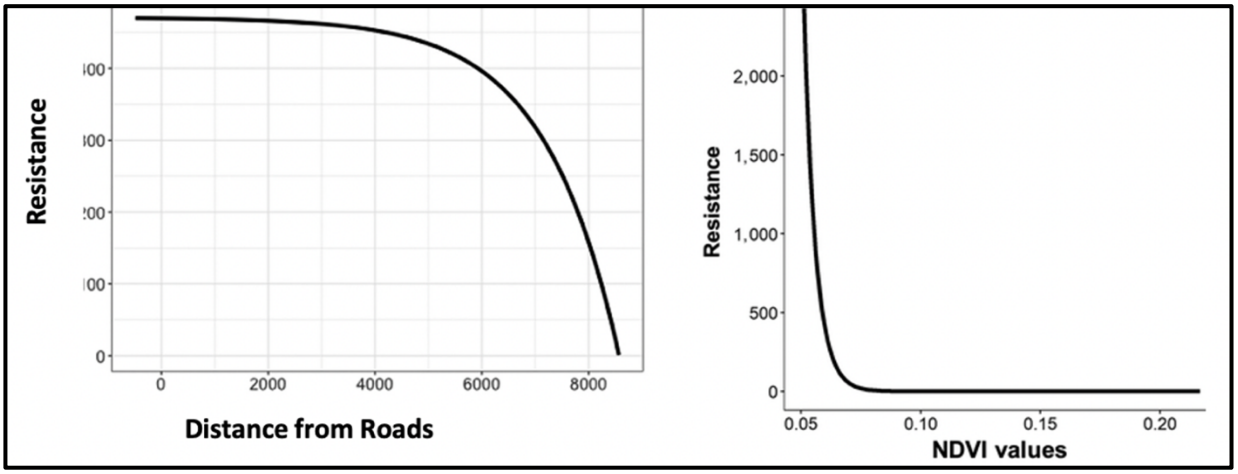

Figure 6e: Resistance curves of Distance from roads and NDVI values

6.3.c. Multivariate Optimization

The top four models for optimizing three species were used to create composite surfaces. Boot strap analysis for both single surface and composite surface showed distance from road as the best model (75%). This however is followed by interplay between the single surfaces. The top models have been given in the table below and are- Distance from road, population+NDVI, NDVI, Forest type+ distance from road, forming the first 5 best fit models.

It is concluded that sloth bears, in terms of model fitting show a slight difference for multivariate and univariate optimization and hence a connectivity map using the top 4 models of composite surface has been constructed.

SLOTH BEAR			
Surface	**K**	**AIC**	**Average Wt**
Distance from road	3	-706.898	0.426
Population+NDVI	4	-704.762	0.343
NDVI	4	-699.909	0.201
Forest type + Distance from road	4	-698.628	0.096
Population	5	-697.008	0.087
NDVI+ Distance from road	5	-696.653	0.081

Table 6b: Model selection results for single and composite surfaces

The heat map was generated using Circuitscape using combination of top 6 models to indicate their movement in the landscape. For sloth bears the feasibility of movement across the landscape ranged from high to low. Red indicates low movement and blue indicates high

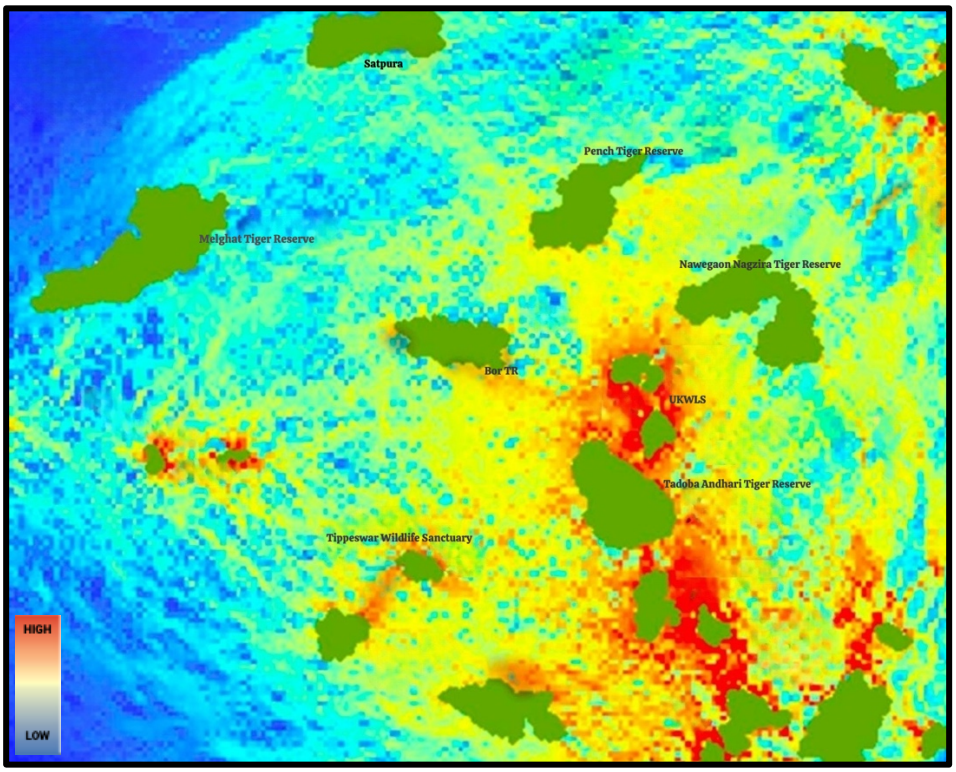

Figure 6f: Heat map of sloth bear movement across the landscape showing how permeable the landscape for sloth bear movement. Red depicts lowest permeability.

The pinch point mapper is given below. The map shows that even though the landscape seems permeable to movement of sloth bears, there are a number of pinch points and areas denoted by red, which act as impediment areas and are less likely to be used.

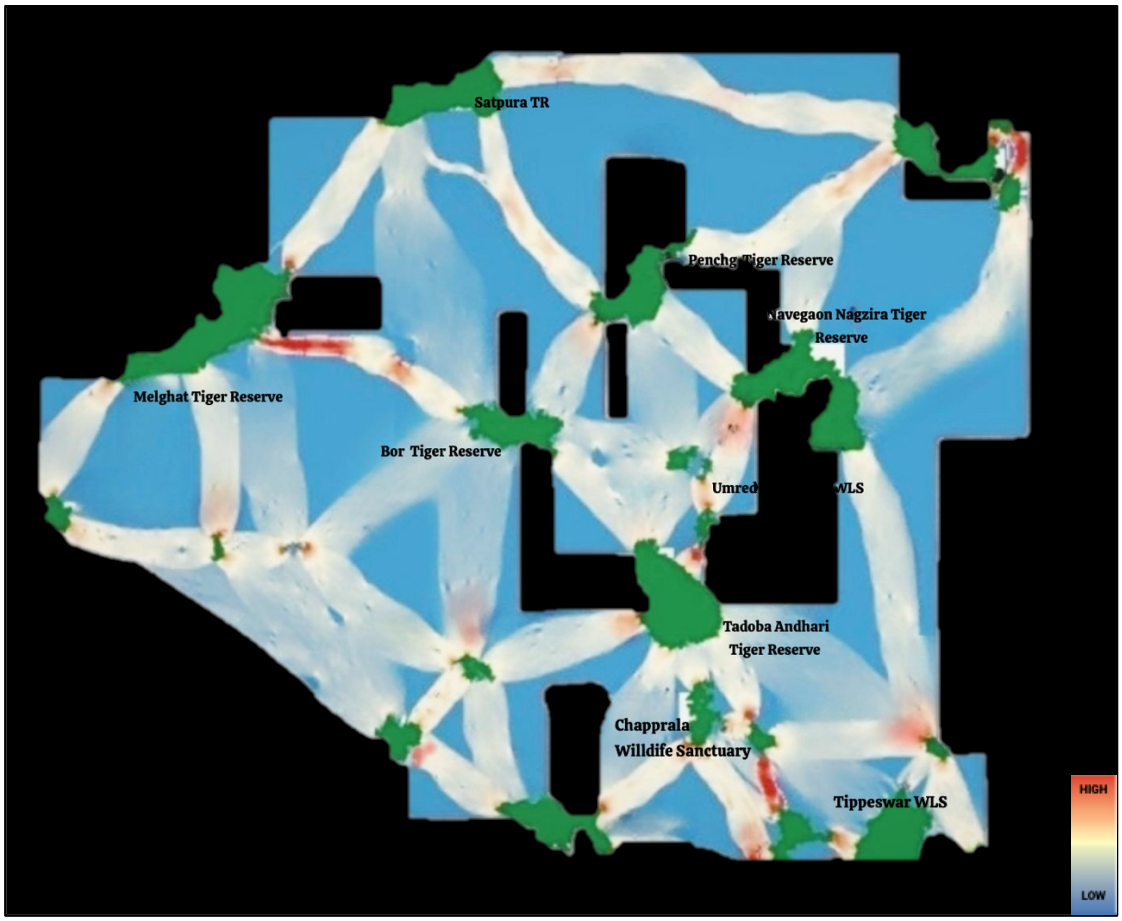

Figure 6g: Pinchpoint map of sloth bear connectivity showing movement areas and pinchpoints in the landscape

6.4. Discussion

Understanding habitat connectivity is crucial for preserving biodiversity and ecosystem health. It ensures the unimpeded movement of species, allowing for gene flow, migration, and adaptation to environmental changes. Connectivity promotes resilient ecosystems by enabling populations to recover from disturbances and climate shifts. Maintaining habitat connectivity is vital for preventing isolation of species, reducing the risk of extinction, and enhancing overall ecological stability. Additionally, it supports sustainable land-use planning, helping to minimize fragmentation and safeguard critical habitats.

Ultimately, a comprehensive grasp of habitat connectivity is essential for effective

conservation and sustainable management of ecosystems. The Vidarbha Landscape is a rapidly changing landscape with a number of developments . Given this scenario the number of bear-man conflicts is on the rise. The instances of sloth bears coming in to human habitation or being victim of road kills or retaliatory killings is also increasing. In such a landscape, balancing the existence of both is instrumental. Therefore, gaining an understanding about the possible sloth bear corridors is beneficial.

In this study, I modelled the connectivity of sloth bears in the landscape. Linear mixed-effects models that was fir using genetic and geographical distance was used. It was established that to some extent the geographical distance played a role, however beyond a limit (100 km) in this case, the effects of geographical distance breaks down. In the previous chapter, covariates that influenced occupancy in the landscape was obtained and had been used in this chapter to model for movement of sloth bears.

During modelling of both univariate and composite surfaces I noticed that distance from roads NDVI and population had the most impact on sloth bear movement. Therefore, these features are strong influencers of geneflow across the landscape. Sloth bears are adaptable when it comes to resources that includes fruiting trees, hives, termite and ant mounds. Therefore, forest cover and undisturbed forests serve as the best pathways for sloth bears. In contrast to my hypothesis, that forest cover and type would have more impact than other factors, I found that even though forest cover plays a role, the type of forest does not. This could be ,most of the fruiting is seasonal and the dependency of sloth bears on this is limited to a few months in a year. Since, the sloth bear is highly adaptable resource wise, the type of forests, hence, does not play a very pivotal role.

Additionally, it was also seen that sloth bears avoided disturbance in the form of human population and disturbance from roads or vehicles which is seen by their avoidance of

occupying spaces near to roads pr near to human settlements. Even though there are a number of instances where bears do come in to contact with people they tend to be shy by nature. Loud sounds and noises are often used to deter bears and hence we may safely say that these act as deterrents. The findings of this study is supports Thatte et al's findings about the landscape being highly permeable for sloth bear movement (Thatte, et al. 2020). However in contrast to Thatte's paper conducted on a similar landscape the most importance variable is distance from roads i.e. avoidant behavior for movement is shown here compared to the previously reported LULC alone.

Habitat fragmentation impacts are faced by multiple species and functional connectivity needs to be understood and needs to include behavioral patterns and habits of the species. In such cases it would be fruitful to obtain movement corridors of long ranging species and modelling a multi-species corridor to ensure corridor efficacy.

6.5. Conservation Implication

Sloth bears, despite their inherently shy nature, are regarded as one of the most dangerous species in the landscapes that I had selected for the conduction of my research. Their unpredictable behaviour often catches people off guard, leading to encounters that can be hazardous. This thesis aims to explore the concept of functional corridors for sloth bears, which are vital for their survival and well-being. The implications of this research extend far beyond academic interest; it holds significant value for stakeholders, wildlife managers, and planning bodies invested in the conservation of these remarkable animals. Additionally, the implications of this research study would also aid in reducing man-sloth bear conflicts in the landscape.

One of the primary outcomes of this research is the identification of occupancy patterns and connectivity among sloth bear populations. By layering this information with documented cases of sloth bear attacks, we can illuminate areas of concern, highlighting critical zones where human-bear interactions are most likely to occur. This approach allows for a more nuanced

understanding of the dynamics at play in regions where sloth bears roam, providing valuable insights for local communities and wildlife officials. Identifying hotspots of human-wildlife conflict can guide efforts to develop targeted mitigation strategies, fostering coexistence rather than conflict.

Moreover, the identification of movement corridors is essential for maintaining the ecological integrity of sloth bear habitats. These corridors facilitate the movement of bears between fragmented landscapes, enabling them to access food sources, mates, and other critical resources. Without these pathways, sloth bears may face isolation, leading to a gradual decline in their populations and, ultimately, local extinction. By pinpointing important habitat patches and corridors, conservation efforts can be more effectively directed toward restoration initiatives that will sustain these bears and their ecosystems.

Genetic monitoring is a crucial aspect of wildlife management, and while it has been widely applied to various bear species globally, it remains underutilized in our country, particularly concerning sloth bears. As highlighted in this thesis, genetic sampling methods and landscape genetics could serve as instrumental tools in understanding habitat connectivity. Genetic diversity is fundamental to the resilience of any population; thus, monitoring genetic variation among sloth bear populations can provide insights into their health and viability. By assessing gene flow between populations, we can identify critical corridors that facilitate genetic exchange, ultimately strengthening the overall population.

However, I acknowledge that genetic monitoring should not occur in isolation. Behavioral studies and telemetry tracking are equally important in understanding sloth bear ecology. Telemetry studies allow researchers to track the movements of individual bears in real time, revealing patterns of habitat use, seasonal variations, and responses to human encroachment. This behavioural data complements genetic findings, creating a comprehensive picture of sloth bear ecology that can inform conservation strategies.

It is essential that these various research methodologies—genetic monitoring, behavioural studies, and occupancy modelling—are not viewed as separate entities but rather as interconnected components of a holistic conservation strategy. By integrating these approaches, we can develop more effective management plans that address the multifaceted challenges sloth bears face. This comprehensive strategy will ensure that conservation efforts are well-rounded and tailored to the specific needs of sloth bear populations in our study area.

Stakeholder Engagement and Conservation Planning

Engaging stakeholders is a critical part of the conservation process. Local communities, government agencies, and conservation organizations must work collaboratively to implement the findings of this research. Educational outreach can raise awareness about the importance of sloth bears in the ecosystem and the need for coexistence strategies. Workshops and community forums can serve as platforms for sharing knowledge, fostering a sense of ownership among local residents, and encouraging them to participate in conservation efforts.

In addition, effective conservation planning should involve adaptive management strategies that are responsive to new data and changing conditions. This means that the research findings should inform policy development and wildlife management practices continuously. By creating a feedback loop where research informs action and action generates further research, we can create a dynamic conservation framework that adapts to the needs of both sloth bears and human communities.

Addressing Human-Wildlife Conflict

One of the significant challenges in conserving sloth bears is managing human-wildlife conflict. As sloth bears roam into agricultural areas in search of food, encounters with humans become more frequent, often resulting in negative outcomes for both parties. Implementing strategies to minimize these conflicts is paramount. This could include creating bear-proof enclosures for livestock, utilizing deterrents such as noise devices, and educating communities on how to react during bear encounters.

Additionally, developing compensation schemes for farmers who experience losses due to sloth bear interactions can help build goodwill and reduce animosity towards these animals. By addressing the economic concerns of local residents, we can foster a more positive attitude toward conservation initiatives and promote coexistence.

Understanding the complexities surrounding sloth bears requires a multifaceted approach that encompasses genetic monitoring, behavioural studies, and stakeholder engagement. The core outcome of this thesis—the identification of functional corridors—provides a framework for enhancing sloth bear conservation efforts. By shedding light on occupancy patterns and connectivity, we can develop strategies that prioritize habitat restoration, reduce human-wildlife conflict, and promote genetic diversity.

The conservation of sloth bears is not just about protecting a single species; it involves safeguarding the intricate web of life in which they play a vital role. As we move forward, integrating research findings into practical conservation strategies will be essential for ensuring the survival of sloth bears in our landscapes. By working collaboratively with stakeholders and adapting management practices based on ongoing research, we can create a sustainable future for these remarkable creatures and the ecosystems they inhabit.

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Article

The Bear Truth: Analyzing Genetic Variability and Population Structure in Sloth Bear across the Vidarbha Landscape Using Microsatellite Markers

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Abstract: Endemic to the Indian subcontinent, the sloth bear (*Melursus ursinus*) is a threatened species, present in fragmented habitats across India. Field techniques such as direct observation and camera trapping alone are not sufficient and may not be explicit enough to understand a monomorphic species like the sloth bear at larger spatial scales. In this study, we looked into the genetic structure, variability and population demographics amongst the extant sloth bear populations in the highly fragmented Vidarbha landscape, using a panel of 13 microsatellite markers with a cumulative PID value of 1.48×10^{-5} PID_{sibs}. Our results revealed genetic clustering (K = 5) and moderate structuring amongst the study populations. Despite being geographically distant and placed in two different genetic clusters, sloth bears from the Melghat Tiger Reserve and Sahyadri Tiger Reserve shared genetic signatures, indicating connectivity, while migration was detected amongst other study areas as well. The findings from this study can serve as baseline assessment for future genetic monitoring of the species in the human-dominated landscape and assist in managerial decisions to step up protection of fragmented forest patches and reduce human–bear conflicts without compromising on the genetic connectivity.

Keywords: *Melursus ursinus*; microsatellite; bear; primer; standardize; ursid; connectivity; structure

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1. Introduction

Stochasticity in an ever-changing habitat and its demographics has pronounced effect on the population status of any species [1]. In a fast-paced human world, inclined towards its own development and connectivity, factors such habitat fragmentation, population decline and range contractions have become a threat to animal populations everywhere [2–4]. Among the animals that are most affected are large-bodied and wide-ranging species [5,6]. Large-bodied animals require larger home ranges that are contiguous. However, in the absence of such intact habitats, animals are forced to adapt to their changing surroundings in order to persist [7]. One such species is the sloth bear (*Melursus ursinus*), a member of the Ursidae family. Sloth bears are medium-sized omnivores with physiological adaptations to feed on insects such as ants and termites [8]. Historically, sloth bears were widely distributed throughout the Indian subcontinent, and it was only recently, in 2013, that this wide-ranging carnivore was declared extinct in Bangladesh [9]. Such an event outlined the need to pay attention to them and fill the information gaps to ensure long-term species persistence. At present, sloth bears have been given the "vulnerable" status by the IUCN, with India housing 90% of the extant population [10]. Sloth bears are found in a variety of habitats across India, with Central India and Western Ghats being strongholds of the species [11]. Protected areas, large patches of contiguous forests and dry deciduous forests support large abundances of sloth bears [11]. However, their population has declined significantly over the years due to habitat loss and fragmentation caused by human activities

such as deforestation, agricultural expansion and urbanization [11,12]. These factors not only fragment landscapes but also act as impediments to genetic exchanges between animal populations. Studies have established that such isolated populations often undergo local extinction [13–15]. Such an exigency entails implementation of reliable molecular methods to understand and study populations of sloth bears in the wild.

Genetic monitoring methods are used extensively in other ursid species around the world [16,17] and serve as key tools for biodiversity monitoring approaches [18,19]; however, studying sloth bear populations using genetic methods is sporadic in India [20–22]. To date, there have been very few studies that standardized ursid microsatellite primers in sloth bears for individual identification [21,22], while no studies have used genetic tools to infer population structuring and variability in the Eastern Vidarbha landscape. Sharma et al. [21] used 7 microsatellite primers, yielding a probability of identity between siblings ($PID_{sibs)}$ value of 2.15×10^{-3}, but Wang et al. [23] showed that genotyping with fewer than 11 or 12 markers results in significantly changed estimates of population structure and genetic variation. Some studies have entrenched that the number of loci is more likely to enhance the power of population genetic inferences over the number of individuals being sampled [24,25]. In 2020, Thatte et al. [22] tested 13 microsatellite markers in non-invasive samples, with a PID_{sibs} value of 1.43×10^{-3}. Given that the rough estimation of sloth bears in India is at least 20,000 individuals [26], the marker panel standardized in this study gives a better resolution and understanding of genetic structuring and variability in the focal landscape. In this paper, we aimed to (i) standardize and use microsatellite panels for genotyping sloth bears, (ii) create a baseline dataset of sloth bear genetic parameters for the eastern Vidarbha landscape and (iii) understand the pattern of genetic variability and structure in the studied populations of sloth bears.

2. Materials and Methods

2.1. Sampling Area

This study was conducted in the Vidarbha landscape (VL) of Maharashtra, India. The landscape has a forest cover of 22,508 sq km, accounting for areas both inside and outside of protected areas [27]. The major protected areas of the VL in which sampling was conducted include the Melghat Tiger Reserve (MTR, 2768.52 sq km), Sahyadri Tiger Reserve (STR, 1166 sq km), Tadoba Andhari Tiger Reserve (TATR, 1727.59 sq km), Bor Tiger Reserve (BTR, 816.27 sq km), Navegaon-Nagzira Tiger Reserve (NNTR, 1894.94 sq km), Pench Tiger Reserve (PTR, 741.22 sq km) and Umred-Karhandla Wildlife Sanctuary (UKWLS, 189 sq km) (Figure 1).

2.2. Field Sampling

A total of 565 fecal samples of sloth bears were collected (NNTR n = 82, STR n = 268, PTR n = 35, MTR n = 83, TATR n = 69, UKWLS n = 28, BTR n = 1). Since only 1 sample was collected from the BTR, we did not use it for further analysis. All concerned permits for the collection and removal of fecal samples from protected areas were provided by the Maharashtra Forest Department (Permit No. 09/2016). The tissue reference samples were taken from the forensic department of the Wildlife Institute of India, Dehradun. Intensive sampling in the study sites was conducted from 2016 to 2019. Little is known about preferred defecating sites in the case of sloth bears, which made sampling both laborious and time-intensive. We focused our search mainly on animal trails, dry riverbeds, rocky plateaus, near fruiting trees or trees with beehives, trees with scratch markings and areas near bear-dug outs or termite mounds, each of which indicated the presence of bears. Sloth bear scats are remarkably different to scats of other large carnivores, lesser cats or langurs based on their size, shape, appearance and the presence of seeds, ants and termite remnants [28]. Once a scat was located, a bolus of the scat sample was collected and kept directly on a piece of butter paper and stored in an individual zip lock bag. Samples collected during fruiting season were sprayed with 99% ethanol to delay fungal growth on the undigested fruit matter. Details such as location, date, state of scat and locality-

associated details such as substrata or terrain type were recorded. Upon reaching the field station, all zip lock bags were stored in a box containing silica beads to minimize chances of fungal growth until further processing.

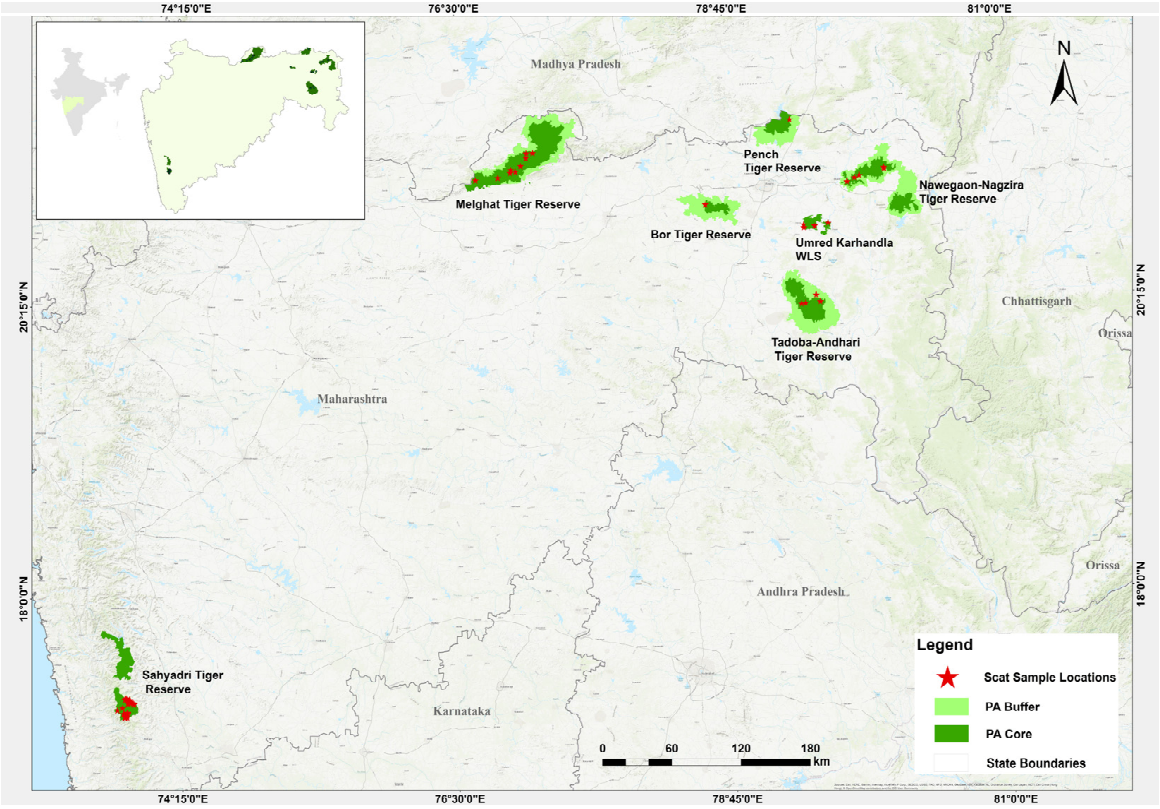

Figure 1. Study area map indicating sampling location used (NNTR n = 82, STR n = 268, PTR n = 35, MTR n = 83, TATR n = 69, UKWLS n = 28, BTR n = 1).

2.3. Primer Selection

No species-specific microsatellite markers exist for sloth bears; therefore, we screened a panel of 25 different cross-species markers developed on different species of bears and canids [29–34]. Following an initial screening of the marker panels, a final marker panel of 13 tagged microsatellites was selected for the population-level study. These markers were selected based on their polymorphic information content, heterozygosity and the number of alleles per locus and rates of allelic dropouts.

2.4. DNA Extraction

All laboratory procedures and sample storage were carried out in the genetic laboratory facility of the Wildlife Institute of India, Dehradun. The tissue and the scat samples were extracted following standardized protocols. Each scat sample brought to the laboratory was swabbed twice using sterile swabs (HiMedia, India) dipped in PBS buffer. The swabs were then placed in 2 mL Eppendorf tubes until extraction. Extraction of the swabbed samples was carried out using a DNeasy kit (QIAGEN, Germany) following the instructions stated in the kit's instruction manual with a few modifications. An overnight lysis was performed with 330 µL of InhibitEx buffer (QIAGEN) and 20 µL of Proteinase K, and DNA was extracted the following day according to the manufacturers' instructions. In the final step, the DNA was eluted twice with 100 µL of 1×TE buffer and stored at −30 °C for long-term storage. Care was taken to include negative controls during each set of 12 extractions to monitor the chances of possible contamination during handling and extraction steps. Genomic DNA was extracted from tissue samples using the DNaesy

Kit (QIAGEN, Germany) and from bone samples using the GeneiPureID DNA Isolation Kit-Bone (Merck, Germany) following the manufacturers' protocols.

2.5. PCR Standardization and Data Validation

The selected primers were first individually standardized using the confirmed tissue samples. The individual primers were then put into multiplex panels of 2–3 primers each based on their annealing temperature, dye and PCR amplicon size (refer to Table 1). A total of 6 panels were standardized to make data generation both cost-effective and time-efficient. The multiplex panels were checked for data accuracy by replicating the PCR conditions for different cycles—40, 50, 60 cycles to ensure that the multiplex panels were consistent. The panels were then used for PCR amplifications of all study samples. PCR for each eluted sample was performed using 3.5 µL of hotstart taq mix, 3 µL of BSA, 1 µM of primer mix (fluorescent-tagged forward primer and a reverse primer) and 2 µL of DNA extract under conditions that included initial denaturation (95 °C for 15 min); 55 cycles of denaturation (94 °C for 30 s), annealing (temperature as calculated for each primer for 30 s) and extension (72 °C for 30 s) and final extension (72 °C for 10 min). Negative controls were also included during the PCR process to ensure the quality of the process and monitor the chances of any cross-species amplification. The PCR products post amplification were then prepared using GeneScan 500 LIZ (Thermo Fischer, MA, USA), for genotyping, and the plates were run on an ABI 3500xl Genetic Analyzer (ABI, MA, USA). The results of the alleles were scored using GeneMarker v3.0.1 (Softgenetics Inc., State College, PA, USA). Allelic bins for each locus were created using the data generated from the tissues and scat samples. Each sample was amplified and genotyped three times for each locus, and a consensus final dataset was used for further analysis.

For data validation of the scat samples, a multi-tube approach was used [35]. Two additional rounds of PCR were performed on all samples that were amplified in 50% of the loci in the panel during the initial round. Each sample was amplified and genotyped three times for each locus, and a consensus final dataset was used for quality index analysis as described in Miquel et al. [36]. In this protocol, the alleles are scored. If the repeats are identical to the first allele call, then they are assigned '1', and if they are different due to non-amplification, allelic dropout or allelic slippage, then '0' is given. The quality index for each locus is calculated by adding the scores of each locus and dividing each by the number of repeats for that locus. If this value was equal to or above 0.75, the sample was then used for further downstream analyses [36]. Monomorphic markers and those not conforming to the index were excluded. The quality index was calculated for each sample using the scores assigned to each repeat and dividing by the total number of repeats [37]. Allelic dropout and false alleles were calculated using MICROCHECKER V2.2.3 [38]. False allele rates were calculated manually in both homozygous and heterozygous samples as the ratio between the number of false alleles detected at a locus and the total number of amplifications, following Broquet and Petit [37].

Table 1. Sequences and panels of selected ursid microsatellites.

Locus	Primer Sequence	Multiplex Panel Group	Repeat Type
UarT838	5′-3′ TCTCTACATCCTTGCCAGC CGCAAATCAAAACCACAATG	1	Tetra
UT1	5′-3′ ACAACTCTTCTCAGATGTTCACAAA CCCAGGTCAGCACTTGGCATAC	1	Tetra
UT38	5′-3′ ATTATTGATGAGCAGGGACAG CTAAAGCAACAACATGTGAATG	2	Tetra

Table 1. *Cont.*

Locus	Primer Sequence	Multiplex Panel Group	Repeat Type
UarT259	5′-3′ CTCTGGACTTCTGGCTCAGG TGAAGCCATCAACATTGCTC	2	Tetra
Umar2	5′-3′ TCACGGGTTTGTAGTAAACA CACAAAGTGGATGCTAAGAA	3	Di
UT4	5′-3′ GAGTTATTGGCACTAAAATCTAATG CTGCAAATCCCTGCTCAACTTTC	3	Tetra
UamD112	5′-3′ GAATCCTCTCCAAGACCTATG GTTTTCCTTATCCCTGAACTG	3	Di
G1D	5′-3′ GATCTGTGGGTTTATAGGTTACA CTACTCTTCCTACTCTTTAAGAG	4	Di
G10L	5′-3′ GTACTGATTTTATTCACATTTCCC GAAGATACAGAAACCTACCCATGC	4	Di
G10B	5′-3′ AAGCCTTTTAATGTTCTGTTG AGGACAAATCACAGAAACCT	5	Di
UT29	5′-3′ GACATTGCCTTTTACAGAGCAG GGGCAGATCTCAACCACCATAAGC	6	Di
CXX203	5′-3′ TTGATCTGAATAGTCCTCTGCG AGCAACCCCTCCCATTTACT	6	Tetra
Mu23	5′-3′ GCCTGTGTGCTATTTTATCC TTGCTTGCCTAGACCACC	5	Di

2.6. Data Analyses

The CERVUS 3.0.7 [39] module of identity analysis was used to identify genetic recaptures (identical genotypes) by comparing the consensus data generated from all loci for each sample. The recaptures were removed from the analysis. GIMLET was used to calculate PID values and genetic variations in the loci such as expected heterozygosity, observed heterozygosity, number of alleles, false alleles and allelic dropout [40]. Genepop was used to calculate F statistics and effective population (Nm) for all the samples [41–44]. TESS was used to infer population structuring using the Bayesian approach relying on the Markov Chain Monte Carlo algorithm under the no admixture model for K = 2 to 10 with 100,000 iterations [43,45]. TESS also accounts for spatial connectivity while assigning samples to clusters. The best value of K was selected based on the Deviance Information Criterion (DIC) value. BayesAss software version 3.0.3, which utilizes genotype information from multi-locus data, was implemented to estimate the recent migration rates between the protected areas in the study landscape [46]. The run parameters for the software included 5×10^6 iterations and 10^5 burn-in with sampling every 2000 iterations. The results from BayesAss were visualized using the "circlize" package version 0.34 in R ver 1.2.5 [47].

3. Results

3.1. Microsatellite Genotyping

A total of 565 scat samples of wild sloth bears were collected from the study area in Maharashtra for standardization purposes. In total, 14 confirmed tissue samples were provided by Nagpur Zoo and the Forensic laboratory facility of the Wildlife Institute of India, which were used as reference samples. During standardization and trial experiments conducted to group the primers into panels, a total of 25 primers were used, out of which 13 loci generated consistent data. No large allelic dropouts were seen. Genotyping results of the tagged primers showed clean peaks and fewer stutters. These 13 loci were then used to amplify and genotype the scat samples. Only those samples that generated data for at least seven loci were taken into consideration. In total, 256 of the 565 scat samples collected from the field were amplified and yielded adequate data for analysis and individual

identification. None of the loci used in the study deviated from the Hardy–Weinberg equilibrium, and no strong linkage disequilibrium was detected. The details of each locus and their measures of polymorphism are presented in Table 2. The amplification success rate of the scat samples can be attributed to their quality and the usage of InhibitEx buffer, which successfully removed PCR inhibitors in the scat samples that could be present owing to sloth bear's diet. Following our analysis in CERVUS, one genetic recapture individual was found. The cumulative values for the 13 panel primers were found to be 9.43×10^{-14} $PID_{unbiased}$ and 1.48×10^{-5} PID_{sibs} [48]. The number of alleles ranged from 4 to 14 alleles (Table 2).

Table 2. Details of microsatellite markers used for sloth bears in this study.

Locus	T_a	Allelic Size	Number of Alleles	H_o	H_E	P_{ID} [a]	$P_{ID(sibs)}$ [b]	$P_{ID(cum)}$ [c]	$P_{ID\ (Sibs\text{-}cum)}$ [d]	ADO	FA
UarT838	57 °C	97–145	4	0.66	0.80	1.777×10^{-1}	4.649×10^{-1}	1.777×10^{-1}	4.649×10^{-1}	0.183	0.024
UT1	57 °C	176–192	5	0.63	0.36	1.786×10^{-1}	4.825×10^{-1}	3.172×10^{-2}	2.243×10^{-1}	0	0.028
UT38	53 °C	196–232	12	0.68	0.24	1.118×10^{-1}	4.396×10^{-1}	3.548×10^{-3}	9.860×10^{-2}	0	0.038
UarT259	53 °C	153–177	10	0.46	0.18	2.923×10^{-1}	5.975×10^{-1}	1.037×10^{-3}	5.891×10^{-2}	0	0.038
Umar2	53 °C	185–227	10	0.65	0.31	1.472×10^{-1}	4.632×10^{-1}	1.527×10^{-4}	2.729×10^{-2}	0	0.056
UT4	55 °C	157–182	8	0.75	0.55	9.237×10^{-2}	3.984×10^{-1}	1.410×10^{-5}	1.087×10^{-2}	0.225	0.054
UamD112	55 °C	142–210	12	0.84	0.29	3.785×10^{-2}	3.411×10^{-1}	5.339×10^{-7}	3.708×10^{-3}	0	0.042
G1D	59 °C	176	5	0.60	0.47	2.031×10^{-1}	5.048×10^{-1}	1.084×10^{-7}	1.872×10^{-3}	0.072	0.035
G10L	59 °C	165	7	0.73	0.84	1.063×10^{-1}	4.149×10^{-1}	1.153×10^{-8}	7.766×10^{-4}	0.026	0.012
G10B	56 °C	133–143	14	0.89	0.45	1.910×10^{-2}	3.123×10^{-1}	2.20×10^{-10}	2.42×10^{-4}	0.063	0.051
UT29	58 °C	168–192	10	0.85	0.53	3.598×10^{-2}	3.353×10^{-1}	7.927×10^{-12}	8.131×10^{-5}	0.105	0.025
CXX203	58 °C	122–146	7	0.69	0.46	1.366×10^{-1}	4.404×10^{-1}	1.08×10^{-12}	3.581×10^{-5}	0.288	0.024
Mu23	56 °C	164–180	11	0.73	0.25	9.926×10^{-2}	4.093×10^{-1}	1.075×10^{-13}	1.46×10^{-5}	0.015	0.024

T_a—annealing temperature; [a]—probability of identity; [b]—probability of identity between siblings; [c]—cumulative probability of identity; [d]—cumulative probability of identity between siblings; ADO—allelic drop out; FA—false allele rate.

3.2. Population Structuring and Variability

The average value for Fis was 0.095 and that for Fst was 0.09 based on 13 loci [28]. The Nm after correcting for size was 2.69. TESS assigned a cluster value of 5 for the populations of sloth bears based on the lowest value of DIC before reaching a plateau (Figure 2). The TESS results showed that neighboring populations of the TATR and UKWLS were assigned to one genetic cluster, with UKWLS samples sharing some genetic signature with the adjacent NNTR population (Figure 2). The populations of the NNTR and PTR formed the next cluster, with PTR samples showing some links to the MTR population. The majority of samples from the MTR were assigned to a third genetic cluster, while a small part of the MTR population grouped with samples from the STR into a common cluster, and the remaining part of the STR population was assigned to a separate group.

The circus plot based on BayesAss analysis shows the pairwise migration rates obtained between each of the seven protected areas of the landscape. The plot depicts the source of migration for each migration movement (Figure 3). The thickness of the ribbons is proportional to the rate of flow of genes from the source population. The contemporary rate of migration was estimated to be 0.098 (range 0.009–0.21). The rate of migration represented by the proportion of individuals migrating was found to be the highest from the NNTR to PTR (0.214) and from TATR to UKWLS (0.177).

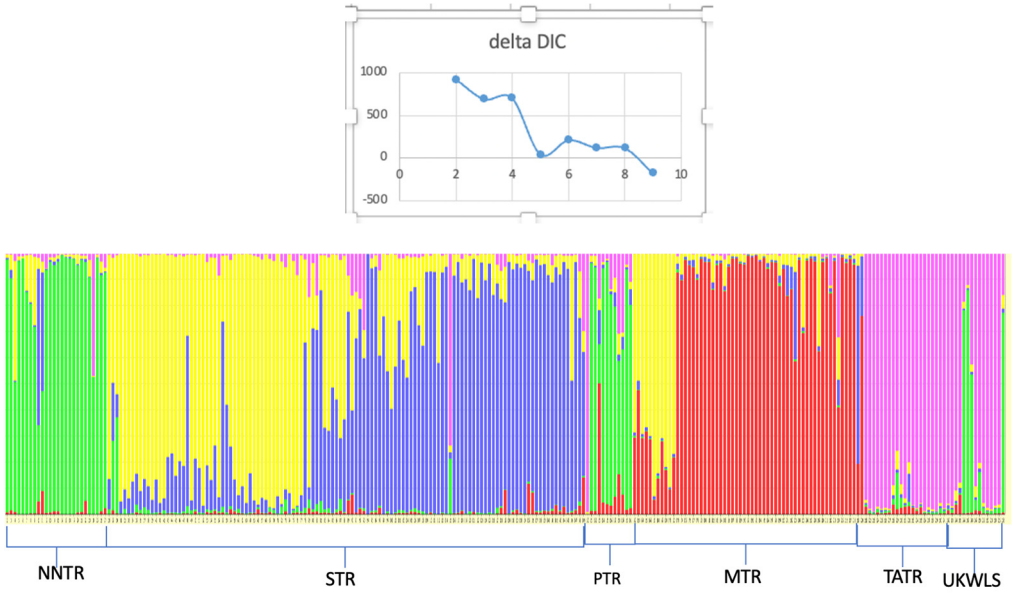

Figure 2. TESS assignment plot of sloth bears across the Vidarbha landscape, India, assigning the samples to 5 distinct populations.

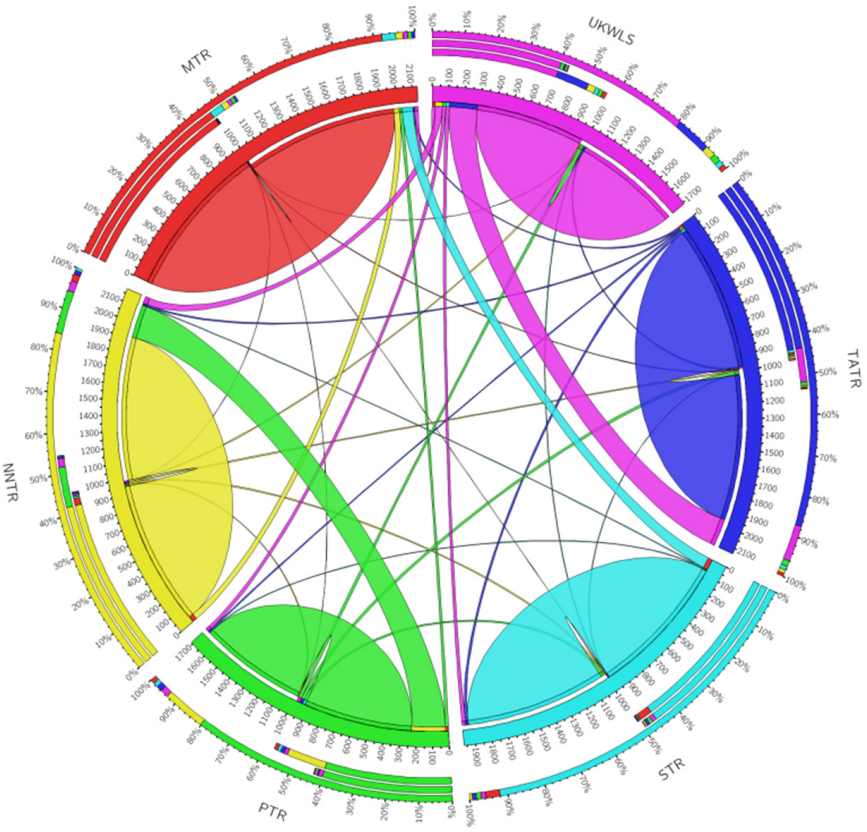

Figure 3. Circus plot visualization of migration rates between different protected areas of the Vidarbha landscape, India.

4. Discussion

4.1. Primer Standardization and Data Analysis

In this paper, we have tested and standardized a panel of 13 microsatellite tagged primers to infer population structuring and genetic variability. Using a larger number of

primers in the initial stage allowed us to test for appropriate panels to identify individuals with robust statistical bolstering. We have taken care to include both dinucleotide and tetranucleotide markers to balance stuttering from peaks and higher amplification success from degraded samples [29,33]. Based on reproducibility and consistency, 13 markers were selected for this study. The final panel was divided into six multiplex reactions to make the entire process cost-effective and efficient. For genetic estimation of the population size in each area, it is recommended that the PID_{sibs} value be double the expected population size [42]. Using 13 microsatellites, the PID_{sibs} value obtained in this study (1.48×10^{-5}) should be sufficient to conduct a large-spatial-scale study and is better compared to the values published in studies prior to this [20–22]. The amplification success of freshly collected samples has been well established [49]. For this study, however, we collected both fresh and old fecal samples to ensure extensive sampling. A few samples that were either very old or very dry did not yield any data. It was also noticed that samples that might not have been adequately sprayed with alcohol had fungal growth, which may have also degraded sample quality. Therefore, it needs to be highlighted that the data generated in this study were from the 45.3% of the total scat samples collected that were reliable. Only one genetic recapture was found in the samples. This low recapture rate could potentially be an offshoot of our sampling strategy wherein we focused on collecting samples from as many individuals as possible to capture the genetic variability in the landscape. The difference in study design and number of markers used for studying sloth bear populations is an obstacle to comparing our study with other sloth bear studies conducted by Dutta et al. [20] and Thatte et al. [22]. This study used 13 microsatellite primers in six panel combinations to obtain a PID_{sibs} value of 1.48×10^{-5}, which is better than the studies previously conducted and has the scope to be used for future population studies and monitoring.

4.2. Population Structuring and Variability

The result from this study showed that the observed heterozygosity for most loci was greater than the expected heterozygosity (see Table 2) [41]. The population structure visualized in TESS divided the sloth bear populations into five distinct genetic clusters. The value of K was selected as the lowest value in DIC before reaching consistency. The plot shows moderate structuring in the six populations of sloth bears that were sampled (Figure 2). The UKWLS, which is a known connecting link between [50] the PTR and TATR, was not assigned any distinct genetic cluster and showed mixed genetics signals from two major populations (NNTR and TATR). It was interesting to see that the MTR and STR, although grouped in different genetic clusters, shared some genetic signatures as well. This could be attributed to two plausible explanations. Firstly, owing to the higher dispersal capability of sloth bears, the genetic differentiation is not very high since the exchange of genes is facilitated [22]. Similar trends have also been seen in other long-ranging carnivore species such as tigers [50–52] and leopards [53] of the same landscape. Secondly, it is also possible that the STR and MTR had migrating populations through other forest patches such as the present day Yawal Wildlife Sanctuary and Gautala Autramghat Sanctuary before the landscape was fragmented. Studies using mtDNA may give insight into this. The BayesAss plot shows migration from one population to another [46]. The most prominent migration occurs from the PTR to NNTR and from the UKWLS to TATR. The low Fis value of 0.095 and Fst value of 0.09 [40] show that the sloth bear populations are not inbred and they exhibit moderate genetic differentiation. In their paper, Thatte et al. (2020) pointed out that since sloth bears were the most widely distributed species, their genetic distribution would be low based on the fact that long-ranging species, like the sloth bear, would have higher gene flow when compared to short-dispersal species [22]. Although our study does show moderate levels of genetic differentiation, there are no baseline data to compare our results to. The findings from this study may be used as the baseline data to understand and compare demographic trends for future studies.

The most fascinating, and to some extent, daunting explanation for the genetic clustering and the moderate genetic differentiation that has been obtained from this study is the absence of contiguous forests. Such that, as a result of adaptive behavior and in order to persist, smaller patches of forests are used as stepping stones to facilitate movement. The moderate genetic variation observed also supports the fact that corridors used to be functional, but with ongoing developments and alterations in the landscape, the persistence of such functionality comes under question [52]. In the study landscape of eastern Vidarbha, patches of forests are often intercepted by developmental and anthropogenic factors such as linear infrastructure and upcoming townships or developmental projects, which curb movement and dispersal of species. About 567 barriers with 30 linkages were enumerated in a study focused on a larger landscape that also included the EVL [54], and such barriers are potential impediments to movement and connectivity. In such a scenario, one of the plausible modes of movement would be that of using stepping-stone patches of forests and other similar habitats. Compared to other long-ranging ursid species such as polar bears or brown bears, whose movements have been widely studied, very few studies have investigated the movement and range of sloth bears. On average, the home range of sloth bears ranges between 2 sq kms and more than 100 sq kms [11,55,56] based on VHF collaring data, whereas dispersal studies have not been conducted. Studies have shown that stepping stones are capable of the dispersal of long-ranging species and can aid in connectivity [57], and the role of such fragments in the Vidarbha landscape cannot be overlooked. The best possible way to establish the use of corridors or stepping-stone patches of forests is to combine molecular studies with telemetry. However, this is beyond the scope of this paper. This study, however, shows genetic exchange between sloth bear populations, which is enough to establish the need to identify and protect corridors and intact forest patches. Other large-bodied bear species like the polar bear, the brown bear and the Asiatic black bear have suffered the brunt of shrinking habitats, causing severe inbreeding and population decline [19,58–60]. But the sloth bear is much less studied, and therefore, more studies focused on the species, investigating the changing demographics across landscapes and the effect of fragmentation, are warranted.

5. Conclusions

This study is the first in the Vidarbha landscape to use genetic tools to understand population structuring and genetic variability. Landscapes that are the stronghold of some large-bodied species, especially in the tropics, have been undergoing rapid alterations and degradation, making it imperative to understand the ill-effects of these changes on the species inhabiting these landscapes [61]. At a larger spatial scale, especially for unmarked species, the utilization of non-invasive genetic methods to understand genetic variation and population dynamics is one of the most robust and reliable methods. The results from this study can be used as a baseline for subsequent studies to understand the population dynamics over time and adopt appropriate conservation measures for the protection or restoration of fragmented forest patches.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16020074/s1. Table S1, Sample Details of Sloth Bear Scats of Vidarbha Landscape.

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CERTIFICATE
of Appreciation

This is to acknowledge

Lynette Gomes

for her remarkable contribution as a Volunteer during the 50 Years of Project Tiger & First Indian Conservation Conference 2023, held from 9-11 April 2023 at KSOU, Mysuru.

Her dedication, hard work, and unwavering support have been instrumental in the successful organization of the event.

We extend our sincere appreciation and gratitude to **Lynette Gomes** for her valuable contribution, without which this event would not have been possible.

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Wildlife Institute of India

Society for Conservation Biology

CERTIFICATE OF PRESENTATION

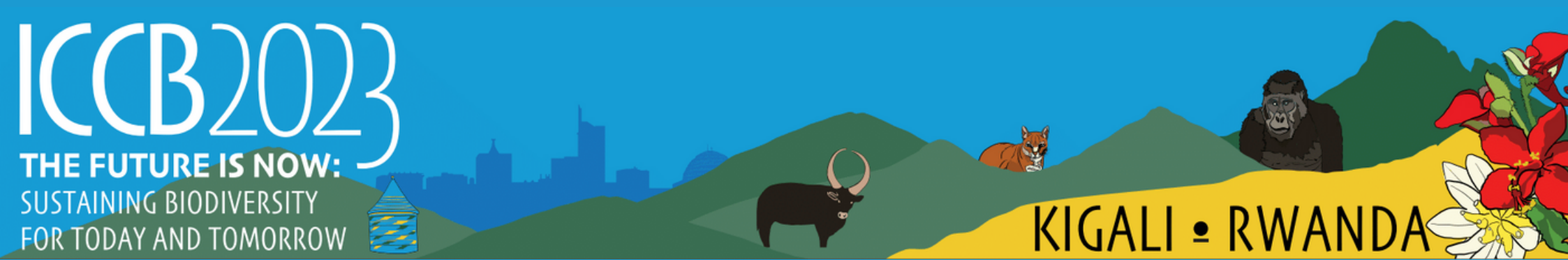
THIS CERTIFICATE IS AWARDED TO

Lynette Gomes

For presenting: BEARing the effects of fragmentation: Using Non-Invasive Genetic studies to understand geneflow and population structure of Sloth Bears across EVL (Oral Presentation) and Sharing with the Bears: Occupancy modelling of sloth bears across central India Landscape (Poster Presentation)

on July 25 and 27, 2023 at the International Congress for Conservation Biology: 23-27 July, 2023 in Kigali, Rwanda

Tony Lynam
President, Society for Conservation Biology



Dear Covid 19

You had brought in times that I have mixed feelings about. The lock down periods took a toll on me especially since I was living alone in Flat 502. Design Arcade.

I had everything planned – 2018–2019 would be field . 2020–2021 would be lab work. analysis and writing and by 2022 I planned on writing down my entire thesis. But. No! you had other plans not only for me but for the entire world. And here I am still writing in 2023! The period that was supposed to be the most productive of my doctoral life was spent indoors. I could not process my samples for 2 years with the laboratory all closed down. My deadlines were pushed back. my entire planning was trashed and like everyone else. I was just existing and praying hard for the safety of my family and friends.

.....But. Covid 19. these times that you catapulted at us. gave me a lot to think. plan. re_plan_ I had never had the time to do so much thinking. I learnt cycling. I baked. I looked at the Mussoorie mountains from my balcony. I studied not only things related to academics but beyond it. You gave me time and taught me its importance. The bonding I developed with friends around. is something I will treasure all my life. My life took new turns and I achieved other unplanned milestones in life like adopting Idlee. Appam and Leo..

So. Dear Covid 19. you were what you were. not completely good or bad. but most definitely an unforgettable time that touched and changed lives – you surely changed mine. So. here you are. an entire page in my thesis dedicated to you

Thank you