EVALUATION OF POPULATION STRUCTURE OF TIGERS (*Panthera tigris*) IN CENTRAL INDIA USING GENETIC MARKERS

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By

BIBEK YUMNAM

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

Dehradun – 248 001, Uttarakhand, India

March 2014

March 7, 2014

CERTIFICATE

This is to certify that the thesis titled "**Evaluation of population structure of tigers (*Panthera tigris*) in Central India using genetic markers**" submitted for the award of degree of Doctor of Philosophy in Wildlife Science to the Saurashtra University, Rajkot, is a record of original work carried out by **Mr. Bibek Yumnam** under our guidance. This thesis has been not submitted either in whole or in part, to this or any other University/ Institution for the award of any other degree or diploma. It fulfills all the requirements laid down in the ordinance governing award of the Ph.D. degree by Saurashtra University.

Yadvendradev V. Jhala, Ph.D.,
Supervisor,
Conservation Genetics Laboratory,
Department of Animal Ecology
and Conservation Biology,
Wildlife Institute of India,
Dehradun,Uttarakhand 248001, India
Ph: 91-135-2640111 (extension 223)
Fax: 91-135-2640117
jhalay@wii.gov.in

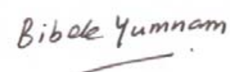
Jesus E. Maldonado, Ph.D.,
Co-supervisor,
Center for Conservation
and Evolutionary Genetics,
National Zoological Park,
Smithsonian Institution,
Washington, DC 20008, USA
Ph: 1-202-633-4198
Fax: 1-202-673-0040
maldonadoj@si.edu

Forwarded
P. K. Mathur
7/3/2014

संकाय अध्यक्ष /Dean
भारतीय वन्यजीव संस्थान
Wildlife Institute of India
देहरादून / Dehradun

DECLARATION

I hereby declare that the thesis titled "**Evaluation of population structure of tigers (*Panthera tigris*) in Central India using genetic markers**" submitted for the award of degree of Doctor of Philosophy in Wildlife Science to the Saurashtra University, Rajkot, is a record of original research carried out by me under the supervision of **Dr. Yadvendradev V. Jhala**, Professor, Department of animal Ecology and Conservation Biology, Wildlife Institute of India, and **Dr. Jesus E. Maldonado**, Research Scientist, Center for Conservation and Evolutionary Genetics, Smithsonian Institution, USA. This thesis has not formed the basis for the award of any other degree or diploma at this or any other University/Institution. All work described in this thesis was carried out by me at the Conservation Genetics Laboratory of the Wildlife Institute of India and at various field sites in Central India. I also declare that the thesis embodies my own work, observation and analyses, and in this respect contributes towards advancement of knowledge in the subject.



Dated: March 7, 2014 (Bibek Yumnam)

Place: Wildlife Institute of India, Dehradun Candidate

Countersigned:

(Dr. Yadvendradev V. Jhala)

Supervisor

TABLE OF CONTENTS

	Page No.
APPROVAL PAGE	iii
DECLARATION	iv
ACKNOWLEDGEMENTS	viii
EXECUTIVE SUMMARY	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF APPENDICES	xx
CHAPTER 1. GENERAL INTRODUCTION	
1.1. Research Aims	2
1.2. Population Fragmentation	3
1.3. The Study Species	5
1.3.1. Conservation Need	6
1.3.2. Genetic studies	9
1.4. The Study Area	11
1.4.1. Physical characteristics	12
1.4.2. Ecological background	12
1.4.3. Land use and conservation history	13
1.4.4. Human habitations and forest dwellers	14
1.4.5. Conservation significance	15
1.5. Research Objectives and Thesis Organization	19
CHAPTER 2. IDENTIFICATION OF TIGERS FROM SYMPATRIC CARNIVORE SCATS	
2.1. Introduction	21
2.2. Methods	23
2.2.1. PCR primer design	23
2.2.2. PCR standardization on reference samples	23
2.2.3. Restriction enzyme digestion	24
2.3.4. PCR and restriction digestion on scat sample	24
2.3. Results	25
2.4. Discussion	26

CHAPTER 3. INDIVIDUAL IDENTIFICATION FROM TIGER SCATS AND MICROSATELLITE LOCI GENETIC VARIATION

3.1. Introduction 31
3.1.1. Probability of identity (P_{ID}) 33
3.2. Methods 34
3.2.1. Field sampling 34
3.2.2. Laboratory work 35
3.2.3. Microsatellite loci amplification and allele scoring 35
3.2.4. Gender identification 37
3.3.5. Individual discrimination and descriptive statistics 37
3.3.6. Detection of genetic bottleneck 38
3.3. Results 39
3.3.1. Tiger scat and individual identification 39
3.3.2. Genetic diversity statistics 42
3.3.3. Genetic bottleneck 46
3.4. Discussion 48

CHAPTER 4. POPULATION STRUCTURE OF TIGERS IN CENTRAL INDIA

4.1. Introduction 54
4.2. Methods 58
4.2.1. Sampling and laboratory work 58
4.2.2. Data analyses 59
4.2.2.1. Multidimensional ordination of individuals 59
4.2.2.2. Bayesian clustering of individuals 60
4.2.2.3. Descriptive statistics of population subdivision 61
4.2.2.4. Isolation-by-distance 61
4.3. Results 62
4.3.1. Multidimensional ordination of individuals 62
4.3.2. Bayesian clustering of individuals 65
4.3.3. Descriptive statistics of population subdivision 68
4.3.4. Isolation-by-distance 70
4.4. Discussion 71

CHAPTER 5. GENE FLOW AMONG TIGER POPULATIONS IN THE LANDSCAPE

5.1. Introduction 78

5.2. Methods 81

5.2.1. Samples and microsatellite loci genotyping 81

5.2.2. Detection of migrants 81

5.2.3. Estimation of contemporary and historic migration rates 84

5.3. Results 86

5.3.1. Migrants 86

3.3.2. Contemporary and historic gene flow rates 91

5.4. Discussion 96

CHAPTER 6. SYNTHESIS 111

APPENDIX 124

LITERATURE CITED 142

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EXECUTIVE SUMMARY

Habitat fragmentation has played a key role in the range contraction of large carnivore populations. Restricted to fewer than 7% of their historic range, habitat fragmentation and isolation bedevil conservation of existing tiger (*Panthera tigris*) populations, though they garner wide support as charismatic flagship species. Most tiger populations are critically endangered as they are confined to increasingly fragmented and isolated populations in human-dominated landscapes throughout their extant range. Small and isolated populations are prone to local extinctions and managing such populations in a metapopulation framework by connecting them through habitat corridors so that individuals occasionally disperse, establish and reproduce, reduces the overall risk of extinction. A prerequisite to conservation is to understand the role of habitat and population fragmentation on the long-term demographic and genetic viability of metapopulations that have arisen due to increased human activities.

In the present study, I investigated patterns of spatial genetic structuring to identify habitat corridors for gene flow between populations within the fragmented tiger habitats in Central India. The Central Indian landscape is a globally recognized area for tiger conservation, with significant potential for long-term persistence of the species. It supports one of the largest global concentrations of tiger populations (~20% of an estimated 1,700 adult Indian tigers) in patchily connected habitats. Though populations were historically connected, rapid infrastructural development and urbanization in recent years threaten to form permanent barriers to dispersing tigers by isolating tenuously connected small populations, thereby reducing long-term metapopulation persistence. In this context, I wanted to explore the relationships between differential tiger densities and patchy habitat connectivity among source populations to understand metapopulation structure and gene flow across this fragmented human-dominated landscape. This study explores a strategy that utilizes extensive landscape-scale noninvasive genetic sampling and statistical assignment methods to detect population genetic structuring and determine which populations are exchanging migrants.

I collected 587 carnivore scats and 17 tissue samples across seven tiger reserves in the Central Indian Landscape during 2006 to 2011. After verifying275 (out of 587) scats as tiger scats using mitochondrial DNA cytochrome *b*(mtDNA cyt *b*) markers, I identified 169 individuals from the combined scat and tissue datasetfor tigers by using a panel of eleven autosomal microsatellite loci. The match probability statistic for the panel of eleven microsatellite loci that was used for individual discrimination had low cumulative sibling probability of identity ($P_{ID\text{-}sib}$) of 1.5×10^{-4}. In other words, only 1 tiger in a population of 6,666 siblings (which share half the alleles at each locus) has a chance of being misidentified as the same individual.

Genetic diversity was high across the population with a high mean number of alleles (9.1 ± 2.2) and high heterozygosity, with similar estimates for expected (75.4% ± 3.9) and observed (70.1% ± 5.9) values. These values are comparable to extant variation in Indian tigers in different tiger conservation landscapes. Past demographic contracting events as evidenced from genetic bottleneck tests of heterozygosity deficiency, mode-shift in allele distributions and *M* ratio showed that most populations in the area did not exhibit signatures of bottleneck, except in Bandhavgarh which had low *M* ratio than the critical threshold for bottlenecked populations (<0.68). The Kanha and Pench populations on the other hand showed evidence of population expansion, as evidenced from significant heterozygosity excess values.

Assessment of population structuring using multidimensional principal coordinate analysis (PCoA) and a model-based Bayesian clustering of individual microsatellite genotypes (STRUCTURE) showed the seven tiger populations in the study area were grouped into four genetic clusters, viz. (i) Bandhavgarh; (ii) Melghat-Satpura-Tadoba; (iii) Pench; (iv) Kanha, Achanakmar and individuals from the Pench-Kanha corridor. Except for Bandhavgarh which formed an isolated genetic cluster, the other three population clusters had some degree of population mixing. On the other hand, population pairwise genetic distance estimators such as F_{ST} and Analysis of Molecular Variance (AMOVA) showed higher level of hierarchical structuring with significant differentiation (p<5%) across each of the seven sampled localities, which suggest on-going population fragmentation in the area was leading to increased genetic structuring among populations.

Next, I used the four population clusters previously identified by the Bayesian individual based method above to detect gene flow (or migration) patterns between population units. By using a combination of Bayesian (STRUCTURE, BAYESASS) and likelihood based assignments (GENECLASS), and further corroborating with parentage tests (CERVUS), I detected a total of seventeen individuals with putative immigrant ancestry, representing almost 10% (17 out of 169) of the total sampled individuals in the area. Of these, five individuals represented by four males and a female were identified as first generation migrants. The remaining twelve individuals had assignment patterns suggestive of admixed ancestry. The low number of first generation migrants relative to the higher amount of admixed individuals suggests that contemporary dispersal events are low in the area in comparison to gene flow events which occurred within the last one to two generations. Also the low number of female migrants compared to males is indicative of sex-biased dispersal in the species.

The highest number of migrants was observed between the Kanha and Pench localities, where four first generation migrants and seven likely admixed individuals were detected. Three individuals were detected in Melghat with migrant ancestry from Pench. One admixed individual was detected in Satpura with likely origin from Pench. Two individuals with admixed ancestry were detected in Tadoba to have cross-assignments with Kanha and Pench. Bandhavgarh represented an isolated population with no evidence of contemporary migrants from the other populations in the study area. The pattern suggests that available structural forest cover which constitutes habitat corridors along with levels of fragmentation dictate relative intensities of gene flow between populations.

The above pattern of migration events was corroborated by estimation of migration rates, which showed low levels of contemporary gene flow in the area. The highest estimated rates of gene flow were observed from Pench to Melghat (9%) and Kanha to Pench (7%) populations. Asymmetric gene flow rates suggested that Pench and Melghat likely represented source-sink relationships, as migration from Melghat to Pench was correspondingly low (<1%). Kanha and Pench likely represented the largest source populations in the area as confirmed by the high number of migrants

originating from these two localities and the gene flow results. The historic pattern of gene flow was also low, with Kanha and Pench having the highest amount of ancestral effective population sizes compared to the other populations. Further, the historic gene flow rates reiterated that Kanha and Pench were the largest source populations in the area.

The overall results of my study affirm the role of habitat fragmentation, structural connectivity and demographics (tiger populations in the area have differing densities) ongenetic structuring and important metapopulation processes of gene flow in the area. The structuring patterns showed that the identified genetic population clusters are clearly beyond the boundaries of most protected areas. Sites like Kanha, Pench and Achanakmar which share migrants and have forest connectivity need to be managed as a metapopulation. The study identified critical corridors such as the ones between Kanha and Pench, Kanha-Tadoba, Melghat-Satpura, Pench-Satpura-Melghat, which are still functional and need to be protected. Other corridors which did not have evidence of recent migrants need to be restored and protected for maintaining metapopulation linkages. The eventual loss of connectivity between populations in the foreseeable future, as observed currently in Bandhavgarh, is likely for most populations in the area if present trend of fragmentation continue unabated. The genetic clusters identified in this study can be useful in long-term monitoring, and translocation efforts can easily enrich demographic and genetic diversity by moving animals among each of the four genetic clusters. It would be particularly effective in the localities of Melghat, Satpura and Achanakmar given their small population sizes, low contemporary migration and susceptibility to drift.

LIST OF TABLES

Table 3.1 Primer sequences of microsatellite and sex-chromosome linked loci used in individual and gender discrimination…..…………………..…36

Table 3.2 Locality-wise estimates showing sampling effort and number of tiger individuals identified in the study area……………………………….41

Table 3.3 Summary of alleic diversity statistics of theeleven microsatellite loci used in the study …………………………...………..…………….43

Table 3.4 Summary of population-wise Hardy-Weinberg Equilibrium (HWE) tests and null allele frequencies at each locus……………………......44

Table 3.5 Overall loci summary statistics of population-wise mean genetic variation and bottleneck tests……………………………………...45

Table 3.6 Results of population-wise bottleneck tests at each locus under the assumption of the two-phase (TPM) and stepwise (SMM) mutation models……………………………………………………..……47

Table 4.1 Principal coordinate (PCo) loadings of covariates relevant for inferring population structure, Eigen values of the coordinates, and the percent variation of the original data explained by the first six PCo axes……………………………………………………………..….63

Table 4.2 AMOVA results showing estimates of differentiation based on various genetic distance estimators………………………………………...69

Table 4.3 Population pairwise F_{ST} and R_{ST} estimates of genetic differentiation……………………………………………………...…69

Table 4.4 Results of standard and partial Mantel tests for correlation between pairwise genetic and geographic distance metrics……..…………….…70

Table 5.1 Results of migrant assignments based on STRUCTURE, GENECLASS and BAYESASS analyses..……..……………………89

Table 5.2 Locality-wise contemporary migration rates, *m*, estimated using BAYESASS showing means (± standard deviation) of the posterior distributions along with the 95% confidence intervals in parenthesis...92

Table 5.3 Contemporary migration rates, *m*, among STRUCTURE identified population clusters estimated using BAYESASS showing means (± standard deviation) of the posterior distributions along with the 95% confidence intervals (CI) in parenthesis…………………...……..93

Table 5.4 Means of posterior distributions ofmutation scaled immigration rate, *M* (m/μ), along with the 95% CI limits and mean number of migrants ($4N_em$, i.e. product of theta and *M*) per generation estimated from MIGRATE analysis………………………………...……..……94

LIST OF FIGURES

Figure 1.1 Map showing tiger occupied forest, individual populations and their habitat linkages in the study area……………………..………...……17

Figure 1.2 Map of human habitations, and major road networks overlaid on tiger populations in the study area………………………………………18

Figure 2.1 MtDNA cyt *b* sequence alignment with a complete tiger mtDNA genome sequence (GenBank Acc. No. JF357967), showing the presence of *Bam*HI restriction enzyme site, G/GATCC, in tiger but not in leopard...27

Figure 2.2 Gel image of PCR products showing amplification by PtiL-14,716 and PtiH-14,901 primers…………………………………………...….28

Figure 2.3 Capillary electrophoresis output from a DNA sequencer machine of DNA band profiles of *Bam*HI digested cytochrome *b* PCR products…………………………………………………...……….29

Figure 3.1 The study area in Central India showingforest cover, tiger reserve boundaries, and sampling location of individually genotyped tigers (*n*=169)……………………………………………………….…40

Figure 4.1 Two dimensional plot showing population relationships on the two most informative coordinate axes, which cumulatively account for ~ 45 % of the total variation in the data…………………………………...63

Figure 4.2 Three dimensional PCoA plot showing partitioning between different populations based onPhi_{PT} co-dominant genetic distance among individuals……………………………………………..….….…….64

Figure 4.3(a) STRUCTURE results showing the difference in delta *K* and mean *Ln P(K)* for an estimated number of *K* populations, in models run with

(locprior=1) and without (locprior=0) prior sampling location information……………………………………………….………..66

Figure 4.3(b) Summary barplots depicting results of sequential STRUCTURE runs (assumed *K* = 2 to 8), of sampled populations in central India clustered according to individual *Q* values, in models run with (locprior=1) and without (locprior=0) prior sampling location information……..……66

Figure 4.4 Summary of STRUCTURE Bayesian clustering run at *K*=4 showing population assignments for each individual……..……...……………67

Figure 5.1 Posterior distributions of individual assignment to nonimmigrant, and first and second generation immigrant ancestry states……………….90

Figure 5.2 The study area showing tiger habitat, protected areas, human habitation, major roads, and least-cost habitat corridors connecting tiger reserves. Individually genotyped tigers (*n*=169) are shown as colour coded dots at their sampled locations with their colours matching their genetically assigned population……......…...………95

LIST OF APPENDICES

Table A1 PCR results in reference species, with PtiL-14,716 and PtiH-14,901 primers showing amplified (+) and not amplified (-) samples……...124

Table A2 Information on scats (*n*=65) as identified by PCR and *Bam*HI restriction enzyme digestion……..………………………………….125

Table A3 Microsatellite loci genotype data of individual tigers, including information on gender (M-male; F-female; U – unknown), sampling site and GPS coordinates……………………………………………127

Table A4 Mean *Q* values of population assignments to each of the four STRUCTURE clusters for all tiger individuals in this study...……..137

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

1.1. Research Aims

The goal of my research was to investigate patterns of genetic structuring and gene flow among tiger (*Panthera tigris*) populations in the Central Indian landscape, to gain an understanding of the overall populationfragmentation and landscape connectivity in this regional metapopulation located in fragmented human dominated habitats. I chose the tiger as a study species to investigate my research goals, because of the need for fine-scale genetic studies to assist in long-term population monitoring and conservation of the species. Also, a suitable study system was available across the patchily distributed tiger populations in Central India where differential tiger densities and patchy habitat connectivity among source populations provided a practical model to test my research questions on metapopulation structure and gene flow. Importantly, the non-invasive genetic techniques were well established, with numerous polymorphic microsatellite loci already identified in other felid species for conducting population genetic studies.

This introductory chapter consists of four further sections. First, I provide a general overview of the process of habitat and population fragmentation in large carnivore range contraction and the techniques used to study metapopulation genetic structure and gene flow among fragmented populations. Second, I present the tiger in light of current understanding of evolutionary and population history in the species. I address the prospects, priorities and problems in conserving fragmented tiger populations. I also present a review on important conservation genetic studies on tigers. Third, I describe the study area and its importance for long-term global conservation efforts to protect the tiger. Finally, I list the objectives of my study and provide a summary of the contents of the remainder of the thesis.

1.2. Population fragmentation

Habitat fragmentation has been recognized as the most pervasive threat to biological diversity and largely responsible for the present extinction scenario (WCMC 1992). Threatened and endangered species are at elevated risk of extinction as they are present in reduced numbers in fragmented and degraded habitats. Understanding the effects of population fragmentation and gene flow on the long-term viability of species is becoming increasingly important for conservation due to ongoing loss of habitat (Lande 1988; Simberloff 1998; Hanski and Gilpin 1997; Allendorf *et al.* 2013). Dispersal and gene flow maintain population connectivity, permit range expansion and increase evolutionary adaptation in species (MacArthur and Wilson 1967). Small isolated populations which occur in fragmented ecosystems with limited to no migration between local subpopulations, face heightened risk of extinction due to mortality from deterministic demographic (Woodroffe and Ginsberg 1998), and stochastic genetic processes (Frankham *et al.* 2002). The ability to evaluate spatial genetic structure and measure inter-population dispersal and gene flow enhance our understanding of population dynamics in fragmented species.

Population fragmentation has played a major role in the range contraction of large carnivores (Woodroffe and Ginsberg 1998). Large carnivores are apex predators and by virtue of their high resource requirements, they occur at low densities, have large home ranges and require vast areas to harbour viable populations (Purvis *et al.* 2000; Gittleman *et al.* 2001). Since historical times, large carnivores have competed with humans for food and resources (Gittleman *et al.* 2001). Habitat destruction and excessive hunting by humans in lieu of real or perceived threat to humans and livestock, and the use of body parts for traditional medicine, have extirpated many populations (Woodroffe 2000; Clark *et al.* 1996; Check 2006) while severely reducing, fragmenting, and isolating most others to varying degrees (Crooks 2002; Proctor *et al.* 2005). Small and isolated populations are prone to local extinctions (Wilcox and Murphy 1985; Woodroffe and Ginsberg 1998).Conservation of large carnivores is fraught with the dilemma of protecting sufficient habitat and resources that fulfill the energetic needs of the species, as well as

reconciling the needs of people (Karanth and Gopal 2005; Woodroffe *et al.* 2005), as the land base that currently supports carnivore and prey populations is under threat from competing human land uses and development activities (DeFries *et al.* 2010). Since habitat fragmentation is inherently tied to local population extirpation, efforts to resist range contraction and population fragmentation require an understanding of demographic processes affecting gene flow and metapopulation function (Proctor *et al.* 2005).

With the application of molecular genetic approaches in conservation, researchers are increasingly resorting to genetic techniques to infer demographic parameters and population structure in elusive and threatened species (Proctor *et al.* 2005; Bergl and Vigilant 2007; Sharma *et al.* 2012). Despite recent advances in spatial and statistical genetic tools (Pritchard *et al.* 2000; Chen *et al.* 2007; Corander *et al.* 2008), measuring the genetic effects of habitat and population fragmentation can be challenging especially in recently disturbed systems (Whitlock and McCauley 1999). This is because genetic changes are not synchronous with the rapid timeline of habitat fragmentation events, as the former occurs on a longer time-scale compared to the latter. Genetic distances are governed by the time since disturbance of the population, migration effects, generation time and life history traits in the species (Frankham *et al.* 2002). Hence there is a time lag before the effects of fragmentation on genetic distance are visible. Classical genetic analyses based on F_{ST} and N_m estimates require equilibrium between the forces of drift, mutation and immigration (Slatkin 1985). However, these equilibrium assumptions are rarely met with in fragmented natural systems, and therefore estimates of genetic structuring and migration rates are not reliable (Whitlock and McCauley 1999). Due to the drawbacks of classical methods, individual based clustering approaches (Pritchard *et al.* 2000; Piry *et al.* 2004) to evaluate population genetic structuring and gene flow have gained popularity. Empirical studies on a range of species (wolverine*Gulo gulo*, Kyle and Strobeck 2002, Schwartz *et al.* 2009; bobcat*Lynx canadensis*, Campbell *et al.* 2006, Row *et al.* 2012; bobcat *L. rufus*, Croteau *et al.* 2010; grizzly bear*Ursus arctos*, Proctor *et al.* 2005; lowland gorilla*Gorilla gorilla diehli*, Bergl and Vigilant 2007; California tiger salamander *Ambystoma californiense*, Wang *et al.* 2009; eastern massasauga rattlesnake *Sisturus catenatus catenatus*, Chiucchi and Gibbs 2010; leopard*Panthera pardus*, Dutta

*et al.*2013; tiger, Sharma *et al.*2013,Joshi *et al.* 2013) have shown the power and the promise of genetic methods to infer patterns of population structuring and migration in recently fragmented systems.

1.3. The Study Species

The tiger is the largest felid species in the *Panthera* lineage of roaring big cats, and has one of the widest geographical distributions of any big cat species, stretching from the equatorial forests of Sumatra to the temperate boreal forests of Siberia and the dry tropical forests in Western India(Seidensticker *et al.* 1999). It is also the largest obligate terrestrial carnivore among all of the mammalian assemblage throughout its range of distribution. Like other members of the *Panthera* genus, the tiger has evolved as specialized predator on large ungulate prey. It's very success as a dominant predator at the top rung of the trophic pyramid and high energetic needs, on the otherhand poses a dilemma for its continued survival in the current scenario of fragmented human-dominated habitats widespread throughout its extant range (Seidensticker *et al.* 1999).

The evolutionary history of the tiger has been constructed based both on fossil records and from recent molecular phylogenetic studies. DNA evidence suggests that the *Panthera* lineage evolved from an ancestral panther-like felid, that lived around 10.8 million years ago (MYA) in the late Miocene of south-east Asia (Johnson *et al.* 2006). The tiger and other cats of the genus *Panthera* first appeared during the pantherine divergence and migration events that began about 3.7 MYA in south-east Asia (Johnson *et al.* 2006). Paleontological evidence also lends credence to an east Asian origin for the tiger (Mazak 1981; Hemmer 1987; Kitchener 1999), as they are widely represented from the late Pliocene - early Pleistocene epoch (nearly 2 MYA) fossil records of northern China (Hemmer 1971, 1987) and Java (Brongersma 1935; Hemmer 1971; Swisher *et al.* 1994). Elsewhere, in the Indian subcontinent and in Central and northern Asia, the earliest tiger fossils were only detected during the late Pleistocene (Hemmer 1971, 1987). The late arrival of the tiger in the Indian subcontinent around 12,000 years ago (Kitchener and Dugmore 2000) is consistent with the late Pleistocene record, the absence

of tigers in the fossil record of Sri Lanka (except for one record by Manamendra-Arachchi *et al.* 2005) and genetic evidence (Luo *et al.* 2004; Johnson *et al.* 2006).

The broad distribution of the tiger encompasses a wide range of phenotypic (Kitchener and Dugmore 2000) and genetic variation (Luo *et al.* 2004) in the species. A total of nine subspecies, three of them extinct, are recognized (Luo *et al.* 2004). The earliest tigers appeared in south-east Asia and range expansion likely followed the radiation of cervids and bovids in the region during the Pleistocene (Flerov and Biron 1960; Geist 1971). The alternating climatic fluctuations of the Pleistocene probably caused repeated contractions and expansions leading to serial bottlenecks in tiger populations and which resulted in relatively low population genetic diversity in the six surviving subspecies (Luo *et al.* 2004). Coalescent simulations have timed the expansion of the modern tiger genome to 72,000 to 108,000 years ago (Luo *et al.* 2004). This period coincides with the catastrophic Toba volcano super-eruption in Sumatra, Indonesia about 72,500 years ago (Rampino and Self 1992). The devastating climatic effects of this eruption which have been linked with a major late Pleistocene bottleneck in human evolution (Ambrose 1998) and a northward thrust in Asian elephant populations (Fleischer *et al.* 2001) perhaps also contributed to prehistoric range reduction in tigers (Luo *et al.* 2004).

1.3.1. Conservation Need

The tiger acts as a flagship species for the conservation of forested ecosystems throughout its extant fragmented range in southern and north-eastern Asia (Tilson and Seal 1987). Conserving the tiger typifies the prospects and challenges inherent in the current paradigm of fragmented small populations and landscape based conservation models. There are only ~3,000 tigers left in the wild, reduced from probably over 100,000 a century ago, and extant populations are confined to fewer than 7% of their historical range (Sanderson *et al.* 2006; Dinerstein *et al.* 2007). Throughout its global range, tiger populations sizes number from less than 20 to less than 200 adults (Nowell and Jackson 1996), which makes these populations vulnerable to the detrimental effects of poaching, disease and inbreeding (Frankham *et al*. 2002). A recent assessment has

revealed an estimated 1,700 individuals (accounting for >50% out of the entire global population of ~3,000 wild adult tigers), residing within India in six global priority tiger conservation landscapes with significant potential for long-term population persistence (Jhala *et al*. 2011b). They are (i) Western Ghats, (ii) Eastern Ghats, (iii) Central India, (iv) Sunderbans, (v) Shivalik-Gangetic Plain and (vi) North East India. These landscapes contain from one to several fragmented populations which are linked by patchy habitat connectivity (Jhala *et al*. 2008). The best connected habitats are located within the Western Ghats and the North East, while highly fragmented in the Shivalik-Gangetic Plain and the Central Indian landscapes (Jhala *et al.* 2011b). In addition, these Indian populations are important for global recovery as they also harbor more than 60% of the global genetic variation in the species (Mondol *et al.* 2009a).

The high genetic variation seen in Indian tigers could be attributed to historically high population sizes, numbering about 50,000 individuals till *c.* 200 years ago, and habitat contiguity that permitted genetic exchange between the various regional tiger populations in the area (Mondol *et al.* 2009a). Due to change in land ownership and forest use policy in the mid nineteenth century during the British rule and again during the early years of India's independence a century later, much of the forest was cleared for timber and agriculture needs (Rangarajan 1996, 2001). This change in land use combined with organized trophy hunting and bounty driven extermination resulted in severe decline, fragmentation and isolation of tiger populations throughout India (Rangarajan 2001, Narain *et al.* 2005). Legislation (Wildlife Protection Act 1972) and conservation initiatives such as Project Tiger in 1973 provided some measure of protection to the tiger and its habitat by securing critical source populations under the federally protected tiger reserve system (Panwar 1987).

A tiger reserve is legally mandated to designate a critical core area wherein human habitation and resource extraction is not permitted (Wildlife Protection Act 1972, amendment 2005). This core is surrounded by a buffer zone, which is essentially a multiple use area, wherein conservation objectives are to be given precedence over other land uses. Breeding populations of tigers are mostly located in the critical core area of

tiger reserves, while the buffers usually serve as habitat sinks (Karanth and Gopal 2005; Jhala *et al.* 2011a, 2011b). The size of these tiger reserves vary between 344 km^2 to 3,150 km^2 (average 1,321 km^2), with tiger densities ranging from about 1 to 20 individuals per 100 km^2 (Walston *et al.* 2010; Jhala *et al.* 2011a, 2011b). For long-term viability of tigers, a minimum of 20 to 25 breeding units is believed to be essential (Chapron *et al.* 2008; Gopal *et al.* 2010; Walston *et al.* 2010). As such many extant tiger populations are by themselves inadequate for long-term persistence (Kenny *et al.* 1995; Linkie *et al.* 2006), either due to habitats' harboring low number of breeding tigers, small size of the protected area and/ or ecological isolation from other populations.

Due to the relatively high *K* selected life history traits of the tiger in comparison to other large cats, dispersal and immigration play a vital role in long-term viability of tiger populations (Chapron *et al.* 2008). Small tiger populations that become isolated are likely to face extinction due to demographic stochasticity, inbreeding depression and deterministic factors such as poaching (Kenny *et al.* 1995; Chapron *et al.* 2008). Incidentally, two tiger reserves Sariska and Panna that recently suffered from local extinction events were small and isolated (Check 2006; Gopal *et al.* 2010). Habitat connectivity is integral to sustain regional populations of tigers as they require contiguous forest connectivity for dispersal and genetic exchange between populations (Smith 1993). Due to high spatial genetic structuring observed in modern Indian tiger populations, preserving even small populations is an important strategy for ensuring conservation of the extant tiger gene-pool (Mondol *et al.* 2013).

Securing critical source populations, and delineating functional corridors and those habitats that require restoration is crucial in the landscape-level scheme of conservation. However, most of the connecting habitats are not within the legal domain of protected areas and are often lost to burgeoning development demands of a growing economy and attrition by human consumptive uses. In India, the transfer of forest-land to other land uses requires approval from the Federal Government (Forest (Conservation) Act 1980). Since Federal Government approvals are usually sought on a case-by-case basis and rarely are cumulative impacts of projects or landscape scale spatially explicit

conservation significance of forest patches factored into decision making, such permissions are frequently granted (Fernandes 2012). However, when the Supreme Court and Federal Government Committees were presented with concrete data on the conservation significance of forest patches, projects even of National Interests have been stalled (Vattakaven 2010; Pinjarkar 2011; Ramchandran 2011). Unfortunately, scientific data rarely exist to substantiate claims of landscape scale conservation significance of forest patches that constitute habitat corridors and crucial areas are often lost. Studies on genetic structure and gene flow to document population differences and migration between patches can provide a quantitative and formal assessment of corridor function and identify priority populations for conservation action.

1.3.2. Genetic studies

Early molecular genetic studies revealed diminished genetic variation in tigers. Except for moderate monophyly in the Sumatran subspecies (*P. t. sumatrae*), little evidence of genetically distinct groupings was observed among the continental mainland subspecies (Cracraft *et al.* 1998, Wentzel *et al.* 1999, Hendrickson *et al.* 2000). Several factors such as inadequate sampling complicated these early efforts in the study of tiger genetic variation. Also, the discovery of a large 13kb *Numt* (Lopez *et al.* 1996; Kim *et al.* 2006), nuclear pseudogene insertions of the cytoplasmic mtDNA in tiger autosomal DNA made it difficult to use universal primers to amplify mitochondrial genes without co-amplifying *Numt* regions (Cracraft *et al.* 1998). The paucity of information necessitated the sequencing of a large portion of the mtDNA gene, and target rapidly evolving nuclear loci to assess variation.

In the past decade, the application of molecular genetic techniques has provided new insights into the taxonomic status, phylogeograhy, demographic history and population parameters of the tiger. Foremost was a landmark study by Luo *et al.* (2004) on revising extant tiger phylogeny by using (i) carefully designed mtDNA primer sets to amplify a total of 4 kb cytoplasmic mtDNA sequence, (ii) a panel of 30 highly polymorphic microsatellite markers, and (iii) a highly variable MHC class II DRB gene sequence. By

combining information from these three marker systems, the study provided a comprehensive phylogeographic assessment of extant tigers and updated taxonomy from five to six existing subspecific groupings. The traditional Indochinese tiger (*P. t. corbetti*) was split into two subspecies: the northern Indochinese and a peninsular Malayan subspecies (*P. t. jacksoni*), with each being represented by unique mtDNA haplotypes and signature microsatellite alleles.

Advances in ancient DNA techniques have made it possible to obtain DNA from archival samples (Paabo *et al.* 2004). Eight mtDNA primers that target short fragments <200 bp and which collectively amplify 1140 bp were designed by Driscoll *et al.* (2009) for use in Caspian tiger specimens. The markers were based on a subset of the mtDNA system of Luo *et al.* (2004), and designed to avoid *Numt* sequences as well as being situated in conserved regions across tiger subspecies. The extinct Caspian tigers (*P. t. virgata*) differed by only a single haplotype substitution with the Amur tiger (*P. t. altaica*), indicating a close evolutionary relationship between the two subspecies.

Non-invasively collected samples, such as hair and scat from wild animal species, represent an important source of genetic samples that are abundant and relatively easy to obtain. Several studies have been collected using noninvasively collected faecal samples from wild tigers. Mondol *et al.* (2009a) designed nine mtDNA primer sets, which amplified a total of 1,263 bp sequences and represented a subset of the 4 kb sequence amplified by Luo *et al.* (2004). The study improved previous non-invasive genetic sampling methods (Bhagavatula and Singh 2006; Sharma *et al.* 2009) and presented a comprehensive assessment of population variation in the Indian tiger subspecies.

Many microsatellite loci are especially suited for the identification of individuals, assigning parentages and population due to their high variability, neutrality and co-dominance (Goldstein and Schlotterer 1999). Criteria to consider in selecting reliable microsatellite markers for amplification include the polymorphic information content, and its consistency and robustness in amplifying degraded scat DNA extracts. Since all cat species rapidly diverged from a common ancestor less than 11 MYA, and because the

genomic composition is highly conserved in the Felidae, the domestic cat genomic resources can be readily applied to wild species, including the tiger (Luo *et al.* 2004). Many microsatellite markers spanning the complete genome in the domestic cat (Menotti-Raymond *et al.* 1999, 2003), including a limited number of microsatellite markers from tiger (Williamson *et al.* 2002; Zhang *et al.* 2006; Bhagavatula and Singh 2006; Sharma *et al.* 2008; Wu *et al.* 2008) have been isolated. These markers have been increasingly used in genotyping of tiger faecal samples to assess genetic variation (Mondol *et al.* 2009a, Henry *et al.* 2009), estimate population size (Bhagavatula and Singh 2006; Mondol *et al.* 2009b; Borthakur *et al.* 2011), evaluate metapopulation genetic structure (Reddy *et al.* 2011; Sharma *et al.* 2012) and landscape genetics (Joshi *et al.* 2013). With the exception of the Amur tiger study by Henry *et al.* (2009), all the other studies were carried out on the Indian tiger subspecies.

These molecular genetic tools are starting to unravel many conservation questions in tigers that were previously intractable with only traditional field based research. Recently the complete genomes of the Amur and Indian tiger were sequenced (Cho *et al.* 2013). This significant breakthrough will provide a rich and diverse genome resource that could be used in future studies of conservation and population genomics.

1.4. The Study Area

The study was carried out in the global priority tiger conservation landscape of Central India which is located within the states of Madhya Pradesh, Maharashtra, and Chhattisgarh. The study area (20.1-23.5˚ N and 76.5-81.5˚ E) covered the seven tiger reserves of Melghat, Satpura, Pench, Kanha, Tadoba, Achanakmar, and Bandhavgarh along with their buffer zones, corridor habitats and adjoining forested habitats (Figure 1.1).The area is home to some of India's largest forest tracts and wildlife populations, as well as a myriad number of indigenous people who have been living in the forests here since time immemorial.

1.4.1. Physical characteristics

The region is bounded by the Aravalli Range in the north-west, the Satpura Range in the south, Chota Nagpur plateau in the north east and the Orissa hills in the south-east.The topography varied from about 200 meters above sea level (m a.s.l.) in the low lying hills to the *dadar* plateaus and meadows in Kanha (500 m a.s.l.) and the rugged Satpura ranges (highest elevation 1,352 m a.s.l.). The region is a catchment area of major Central and South Indian rivers, Narmada, Tapti, Mahanadi and Godavari. The Vindhyas in the north and the Satpuras in the south form a wide valley through which the river Narmada flows, and divides the Central India from the Indo-Gangetic Plains (Mani 1974).There are three distinct seasons in the area (Gopal and Shukla 2001). Summer: March to mid-June (the hottest period extends from late April to the first weekof June). Monsoon: mid-June to late September (July and August are the wettest months, and theaverage annual rainfall is around 1200 mm.).Winter: November to February (with night temperature dropping to as low as -3˚C duringDecember and January).

1.4.2. Ecological background

The study area corresponds to the biogeographic province 6A (the Central Highlands) of the Deccan zone with the adjoining parts of 6D (Central Plateau) in the classification of Rodgers and Pawar (1988), extending across the states of Maharashtra, Madhya Pradesh, and Chhattisgarh. The region is principally the zone of deciduous forests (Champion and Seth 1968). The study sites covered different types of tiger habitats found in Central India ranging from the tropical moist-deciduous sal (*Shorea robusta*) forests in Kanha and Bandhavgarh to tropical dry-deciduous teak (*Tectona grandis*) dominated forests in Pench, Tadoba and Melghat. Grasslands in the area are few, and now lost to agriculture.

The important mammals found in the region are tiger (*Pantheratigris*), leopard (*P. pardus*), jungle cat (*Felis chaus*), rusty-spotted cat (*Prionailurus rubiginosus*), striped hyena (*Hyaena hyaena*), sloth bear (*Melursus ursinus*), dhole (*Cuon alpinus*), jackal (*Canis aureus*), wolf (*C. lupus pallipes*), chital (*Axis axis*), sambar deer (*Rusa unicolor*),

barasingha deer (*Rucervus duvaucelli branderi*), barking deer (*Muntiacus muntjak*), chousingha antelope (*Tetracerus quadricornis*), nilgai antelope (*Boselaphus tragoocamelus*), gaur (*Bos gaurus*), wild pig (*Sus scrofa*) and langur (*Semnopithecus enetellus*). Most wildlife species are widespread throughout the entire area, e.g. chital, sambar, nilgai, and barking deer. Species such as gaur, dhole and sloth bear which have been severely fragmented or extirpated from other landscapes in India have their global stronghold in this region (Jhala *et al.* 2008). Some species have relict distributions such as the hard-ground barasingha which survive only in few small populations have their only home in this landscape.

1.4.3. Land use and conservation history

Historical records dating back to the 5th and 14th centuries, state that the region was extensively forested (Forsyth 1919). Even during British periods, reclamation of most parts of this forested country was a challenge with large tracts remaining unexplored until as late as 1853 (Forsyth 1919). Development and exploitation of resources occurred only in the 14th century during the Mughal period (Rangarajan 2001). Large areas of forest were cleared especially in the low lying valleys to construct cities and roads which connected the region with Northern India (Forsyth 1919). Henceforth, subsequent periods saw large immigration by people from the thickly populated belt of Northern India, leading to the displacement of many local tribes to the remoter forested highlands. There they continued with their traditional subsistence level agriculture and hunting-gathering practices. State controlled commercial forestry operations started by the British in the 1860s and continuing post India's independence have exploited the vast tracts of timber in these areas with most government forests being Reserved Forests (Rangarajan 2001). Commercial habitat exploitation acted in concert with large-scale hunting in the region to fragment wildlife populations, during the British period.Some idea of the abundance of wildlife in this region can be drawn from the shooting records of erstwhile rulers and British in this region, where 1116 tigers and about 2000 leopards were shot by a single ruler from a single province (Rangarajan 2001). Large-scale bounty hunting wiped out species such as the Lion (*Panthera leo persica*) and the Cheetah (*Acinonyx jubatus*) from

the area (Divyabhanusinh 1999). Hunting was widespread and unregulated in the area till the late 1960s, and the tiger and other wildlife populations were only saved from the brink of extinction due to committed conservation action in the early 1970s (Panwar 1987). The period of recovery and relative stability has since been under continual threat from a commercial poaching, and development activities since the early 1990s (Kumar and Wright 1999).

1.4.4. Human habitations and forest dwellers

Administratively, the Central Indian Landscape is distributed over 32 districts (24 in Madhya Pradesh, 1 in Chhattisgarh and 7 in Maharashtra). According to the 1991 census, the total human population is 51,290,238 with a mean density of 169.57/sq.km. The region is also home to India's largest scheduled tribe population most of who are amongst the poorest in the country. Major tribal communities living in Central India (with number of districts where they are predominant) are: Gonds (15), Kols (6), Bhils (5), Korkus (4), Baigas (3), and Saurs (2) (Bhatt 1997).Agriculture and collection of non-timber forest products (NTFPs) are the two primary occupations of these peoples, and is mainly restricted to subsistence farming, as most farmers are marginal cultivators with per capita land holding less than 2 acres. Slash-and-burn farming, which was widespread in the region in the past, is now limited to few localities e.g. Bori-Satpura. NTFPs form the mainstay of the local economy in Central India and the annual net revenue is estimated to be worth well over 4000 million rupees (Dobhal 1994). Indiscriminate harvesting and over-exploitation of these forest produces have been found to have adverse effect on regeneration of the NTFP species and forest structure (Koliyal 1997). Incidentally, this is also the area with the highest concentration of minerals and thus mining interests (Narain *et al.* 2005). These large mineral deposits often result in the conflict of interest between conservation and revenue sources (Fernandes *et al.* 2012).

1.4.5. Conservation significance

The Central Indian Landscape contains several Tiger Conservation Units (TCUs, Dinerstein *et al.* 1997), with significant potential for long-term persistence of the species (Walston *et al.* 2010; Jhala *et al.* 2011b).The area supports one of the largest global concentrations of tiger populations (~20% of an estimated 1,700 adult Indian tigers, Jhala *et al.* 2011) located in patchily connected habitats. Apart from being one of the most important areas for tiger conservation in the country, several range restricted and relict populations of critically endangered species are found in the area. This region also encompasses several biosphere reserves, such as the Achanakmar-Amarkantak biosphere reserve, which is part of the UNESCO's Man and Biosphere Programme (MAB).

Jhala *et al.* (2008) identified the Kanha-Pench, Satpura-Melghat as important metapopulations for long-term tiger survival. To strengthen such areas and reduce the impact of human disturbance in the tiger breeding zones (core areas of tiger reserves) would be necessary to protect the biodiversity of these regions while reducing cases of human-wildlife conflict.Though populations were historically connected, rapid infrastructural development and urbanization within the last decade threaten to form permanent barriers to dispersing tigers by isolating tenuously connected small populations (Figure 1.2). This will effectively diminish long-term metapopulation persistence. Recent population and spatial genetic studies in the Central Indian landscape have observed that the tiger populations in the area exist as a metapopulation with low genetic structure (Sharma *et al.* 2012), have variable levels of gene flow depending on the structural integrity and human disturbance in connecting habitat corridors (Joshi *et al.* 2013; Sharma *et al.* 2013). Population fragmentation was also detected in other species which are relatively more tolerant of disturbed habitats, like leopards (Dutta *et al.* 2012).

A brief account of the major protected areas (PAs) in my study is outlined below. Density estimates of tigers were obtained from the recent country-wide tiger monitoring exercise (Jhala *et al.* 2011b).

(i). Pench-Kanha-Achanakmar landscape - Covering a total area of over 20,000 km^2 within the states of Madhya Pradesh, Maharashtra and Chhattisgarh, this landscape contains relatively intact forest connectivity and is classified as a level I Tiger Conservation Unit (TCU). Tiger densities vary from 8.7 per 100 km^2 in Kanha to 3.6 per 100 km^2 in Pench and 0.1 per 100 km^2 in Achanakmar It has two major source populations (Kanha and Pench Tiger Reserves) existing as a metapopulation. Multilaning of national highways threaten to fragment habitat connectivity between Pench and Kanha (Vattakaven 2010).

(ii). Satpura Melghat landscape - The area has low population densities (2.7 per 100 km^2 in Satpura and 3.0 per 100 km^2 in Melghat) and covers 12,700 km^2 of geographic area. A mixture of relatively intact and degraded forests located between these two reserves provide patchy stepping stone connectivity.

(iii). Bandhavgarh Tiger Reserve - The PA has an area of 2,000 km^2, and the forest patch here is fragmented, with scope for a corridor linkage with Achanakmar in the southern half of the reserve. Bandhavgarh is an important source population with a high density of tigers (16.3 per 100 km^2).

(iv). Tadoba-Andheri Tiger Reserve – With tiger densities of 5.3 per 100 km^2, this 2,000 km^2 reserve holds potential to be an important source population of tigers in the landscape, with scope for corridor linkage through degraded habitats with the Kanha-Pench landscape.

(v). Intervening isolated tiger habitats - These include all areas which are not covered in the PA network, but could serve as vital corridors for linking isolated source populations throughout the landscape.

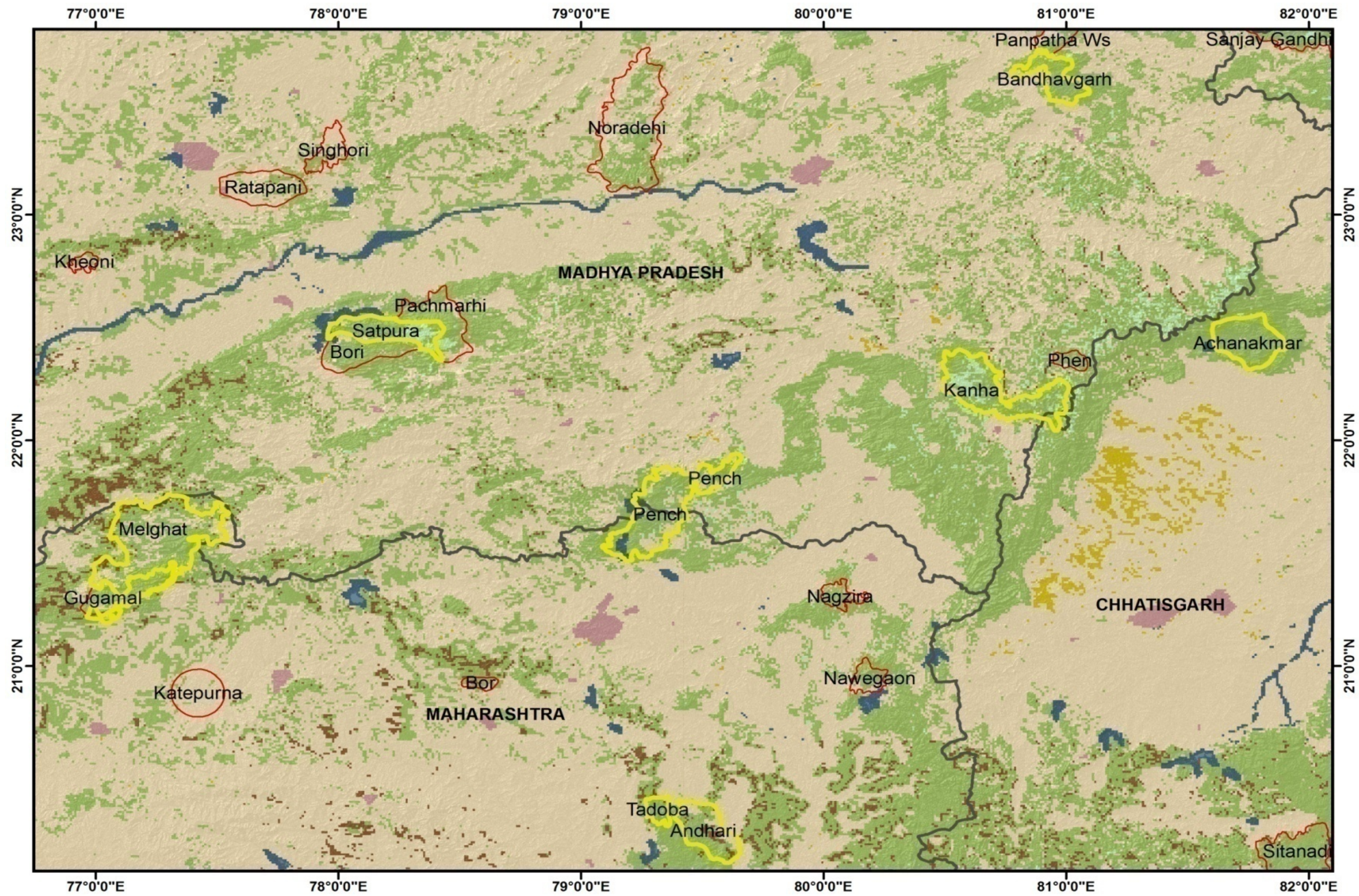

Figure 1.1. Map showing forest habitat (green patches)and tiger reserves (encircled in yellow) in the study area.

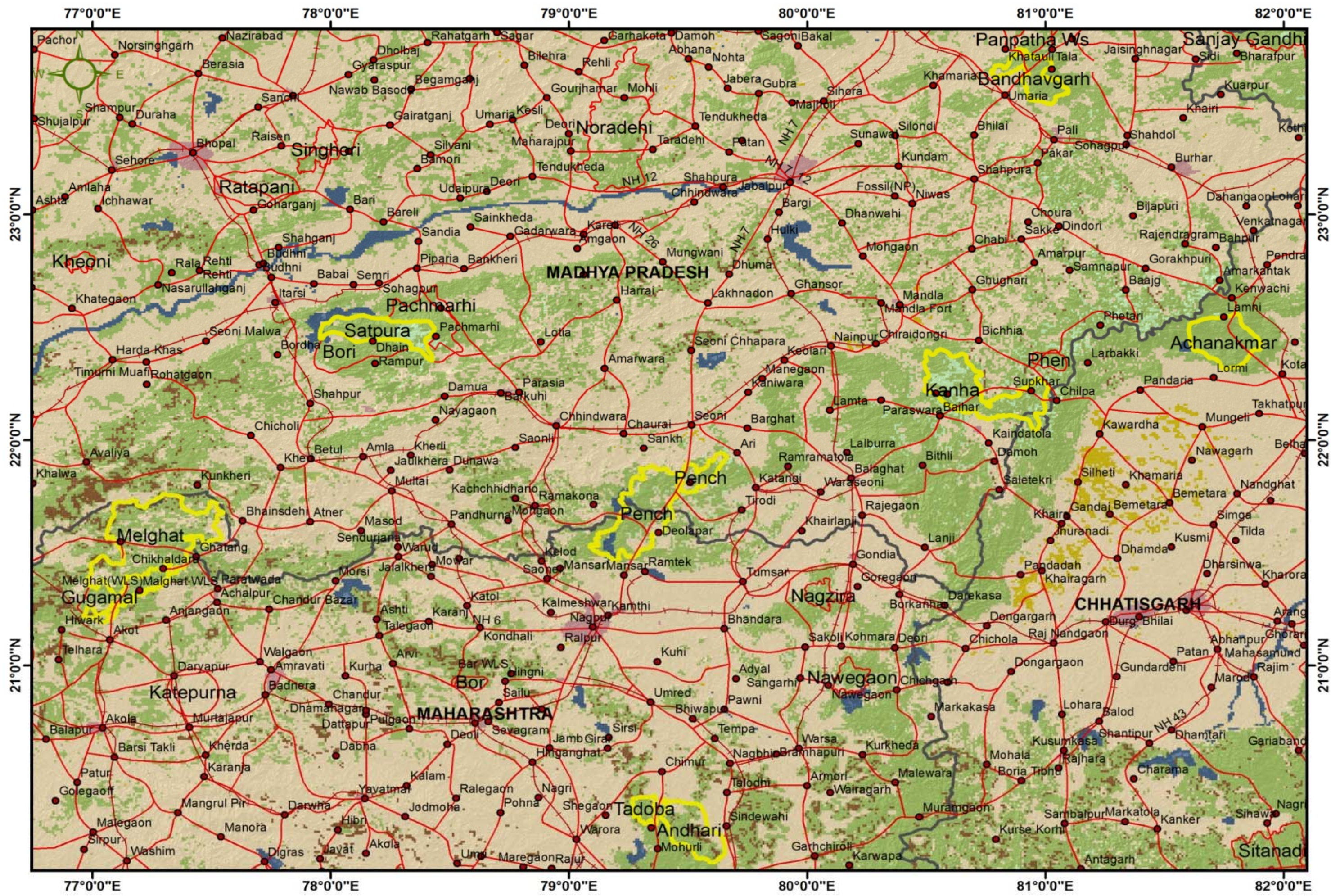

Figure 1.2. Low (dark red dots) and high-density (pink patches) human habitations, and road networks (red lines) disrupting habitat linkages (in green) in the study area.

1.5. Research Objectives and Thesis Organization

The primary goal of my study was to investigate the effects of population fragmentation on the genetic structure of local populations located in the fragmented tiger habitats of Central India, by using genetic data from individual tigers to detect population differentiation and gene flow among populations.

The specific objectives of the study were.

i. To carry out genetic identification of individual tigers in the study area, from scat DNA extracts (Chapters 2 and 3).

ii. To assess genetic structure of the metapopulation using microsatellite loci genotypes (Chapter 4).

iii. To detect migrants and estimate rates of gene flow between source populations in the area (Chapter 5).

This thesis is organized in four main chapters. Chapters 2 and 3 deal with the first objective of assigning tiger individuals to field collected scats. Chapter 2 describes a species identification test to identify tiger and leopard scats. It is important to carry out a genetic identification procedure between the two sympatric species as field identification can sometimes be confusing and inaccurate. Chapter 3 deals with the validation of a microsatellite loci panel used for assigning individual identity to tigers for population genetic analyses. The chapter also provides an overview of genetic diversity statistics and genetic bottlenecks observed across the populations. In chapter 4, I use individual based multivariate analysis and Bayesian clustering approaches on the individual tiger genotypes to detect and identify the number of genetic population clusters by grouping individuals to populations. Chapter 5 discusses the detection of gene flow among populations in the area through the identification of individuals having migrant and admixed ancestry. I use Bayesian and likelihood assignment tests to detect migrant individuals, and a parentage test to identify putative parent-offspring pairs. The chapter ends with a section on estimation of contemporary and historic rates of gene flow in the overall metapopulation.

CHAPTER 2

IDENTIFICATION OF TIGERS FROM SYMPATRIC CARNIVORE SCATS

CHAPTER 2

IDENTIFICATION OF TIGERS FROM SYMPATRIC CARNIVORE SCATS

2.1. INTRODUCTION

The goal of my study was to use genetic data to gain insights into the population genetic history and patterns of gene flow among tigers in the Central Indian landscape. Since carnivore scats formed the basis of my sampling, it was important that robust and accurate genetic detection methods were incorporated in the analysis and identification of tiger from degraded scat DNA extracts. Due to the presence of sympatric large cats like the leopard in the study area which yield morphologically similar scats, the specific identification of tigers can be misleading. Hence, genetic methods are useful to differentiate similar scats between closely related species. The objective of this study was to develop a rapid and cost-effective protocol for the reliable identification of tiger scats from sympatric carnivore scats.

Since the majority of felid species are rare and elusive, genotyping using scat (faecal) DNA extracts has increasingly been used as a tool to aid in population monitoring (Ernest *et. al.* 2000; Janečka *et al*. 2008; Mondol *et al*. 2009b; Borthakur *et al.* 2011) and in studies on dietary habits (Farrell *et al.* 2000)**.** However, reliable identification of the true defecator species can be misleading and undermined by the co-occurrence of closely related species which yield morphologically similar scats. Due to similarity in scat morphology and the sympatric distribution of tigers and leopards, field identification can at times be erroneous (Sugimoto *et al.* 2006). Molecular genetic methods based on Polymerase Chain Reaction (PCR) to amplify mtDNA markers from scats have increasingly been used to identify species where several closely related species live in sympatry. However, successful genetic identification can be compounded by experimental failures in amplifying molecular markers from degraded scat DNA extracts (Mukherjee *et al.* 2007). The genetic techniques are all based on PCR, viz. using species-specific primers (Sugimoto *et al.* 2006; Mukherjee *et al*. 2007), DNA sequencing of the

PCR products (Farrell *et al.* 2000; Janecˇka *et al*. 2008) or PCR-RFLP (PCR-Restriction Fragment Length Polymorphism), which is a combination of PCR followed by digestion of the PCR product with diagnostic restriction enzymes to yield species-specific banding patterns (Cossíos and Angers 2006; Nagata *et al.* 2005).

DNA sequencing of PCR products, though by far the most precise method, is inherently time consuming and costly compared to rapid gel-based screening or PCR-RFLP methods. The species-specific primer approach which is based on the simultaneous amplification of short segments of multiple mtDNA markers is a robust method to exclusively amplify tiger samples. It has the twin advantages of being fast and cost-effective, and with high amplification success rates even from old scats (Sugimoto *et al.* 2006; Mukherjee *et al*. 2007). However, the method being entirely PCR based, has the drawback of not taking advantage of DNA sequence information, particularly related to robust inter-species sequence differences at restriction enzyme sites, which can be useful taxon-specific markers. In comparison, the PCR–RFLP method combines the speed of PCR together with robust species specific sequence information at restriction enzyme sites obtained from DNA sequencing. Band patterns obtained after restriction enzyme digestion of PCR products are useful in assigning species identity, and is especially suited to identifying carnivore species from a spectrum of field collected scats (Bidlack *et al*. 2007; Mukherjee *et al.* 2010). Nagata *et al.* (2005) used the restriction enzyme *Hin*fI, to digest mtDNA cyt *b* PCR products in order to identify scats of Amur tiger (*Panthera tigris altaica*) from Amur leopard (*P. pardus orientalis*) in Far Eastern Russia. However, their targeted region for PCR was slightly large (280 bp) for scat DNA extracts obtained in the hot and humid tropical latitudes of Southern Asia , where the decomposition rate of scats is fast compared to the cold desiccating environment of the Russian Far East (Goossens and Salgado-Lynn 2013).

In lieu of the difficulties associated with amplifying molecular markers from degraded scat DNA extracts and to distinguish tiger scats from scat samples originating from other sympatric carnivores and closely related felid species, I designed primers to amplify a short ~187 bp stretch of the mitochondrial DNA cytochrome *b* (mtDNA cyt *b*) gene. This

segment contains a *Bam*HI restriction enzyme site which is present in tigers but absent in leopards, to enable a species identification procedure which relies on PCR followed by restriction enzyme digestion, to distinguish scats originating from the two closely related big cat species.

2.2. METHODS

2.2.1. PCR primer design

MtDNA cyt *b* sequences of tiger (JF357967, AF053019, JF357974, AF053047, AF053050) and leopard (EF551002, EF056506, EF056507 and EF199742) were aligned(Figure 2.1) using Clustal W, implemented in the program Bioedit (Hall 1999). Restriction enzyme sites on the cyt *b* gene in tiger and leopard were identified by sequence analysis using Webcutter (*http://rna.lundberg.gu.se/cutter2/*). Sequences flanking species-specific restriction enzyme sites between tiger and leopard were selected as potential primer binding sites. Primer3 (*http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi*, Rozen and Skaletsky, 2000) was used to design two new primers, PtiL-14,716 ([6-FAM]5'-ACTTCATCCTTCCGTTTATCGTCT-3') and PtiH-14,901 ([6-FAM]5'-GGACGAGTAGTATGAGGGTTAGGAT-3') at positions 14,716-14,901 bp, of the mtDNA cyt *b* gene in tiger and leopard, which flanked a diagnostic *Bam*HI restriction enzyme site, GGATCC, at 14,782 bp position of the tiger cyt *b* gene, but not in leopards. This was to enable species identification between scats of tiger, and the species with which it is most likely to be confused with in field conditions, the leopard. The 5'end of both primers were labeled with the fluorescent dye 6-Fam™, to enable band resolution of the *Ba*mHI restriction enzyme digested PCR products, on a DNA sequencer.

2.2.2. PCR standardization on reference samples

The PtiL-14,716and PtiH-14,901 primers were initially tested on reference blood, tissue, hair and known scat samples, available at the lab (Table 2.1). DNA was extracted from tissue samples using the DNeasy Blood and Tissue kit (QIAGEN, Germany). PCR amplification of DNA extracts was carried out using the above primer pair. PCR was

performed on DNA extracts obtained from eighteen radio-collared tigers, nine leopards, six striped hyenas (*Hyaena hyaena*), three wolves (*Canis lupus pallipes*), three domestic dogs (*C. lupus familiaris*), three golden jackals (*C. aureus*), and one each of sloth bear (*Melursus ursinus*), domestic goat (*Capra hircus*), wild pig (*Sus scrofa*) and a human sample (my blood DNA extract). Negative control (without DNA extract) was included during each PCR run to monitor contamination. A conserved universal primer pair targeting a 309 bp band of the cyt *b* gene across diverse taxa (L14841, 5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGA-AA-3' and H15149 5'-AAACTGCAGCCCCTCAGAATG-ATATTTGTCCTCA-3';Kocher *et al.* 1989) was used to set up a control reaction with all samples, in order to ascertain whether the non-amplification with other non-felid carnivores and prey species were not artifactual results. PCR was performed with 3 µl of the DNA extract in a total volume of 20 µl, using the Multiplex PCR kit (QIAGEN). The thermocycling parameters were - initial denaturation at 95°C for 15 minutes, followed by 30 cycles at 94°C for 20 seconds, 55°C for 20 seconds and 72°C for 40 seconds. The final soak time was kept for 10 minutes at 72°C.

2.2.3. Restriction enzyme digestion

Restriction enzyme digestion was carried out by incubating 5 µl of the PCR product with 10 units of Fast Digest *Bam*HI enzyme (MBI Fermentas, USA) in a total reaction volume of 10 µl, for 15 minutes at 37°C. After digestion was completed, enzyme digested bands were resolved on a DNA sequencer (ABI3130, Applied Biosysmtems, USA) by loading a mixture of 1 µl of the enzyme digested PCR product with 0.25 µl of Genescan 500 LIZ size standard and 9 µl of deionized formamide. Genemapper v3.7 (Applied Biosystems, USA) was used to size the fluorescent dye labeled enzyme digested bands.

2.2.4. PCR and restriction digestion on scat samples

A pilot experiment was carried out to assess amplification efficiency and species identification success on field collected carnivore scat samples (*n*=65), which were likely to have originated from either tiger or leopard based on scat morphology. These samples were collected variously in either 75% ethanol or stored dry with silica as preservative. Scats of small carnivore species including jungle cat (*Felis chaus*) and rusty-spotted cat

(*Prionailurus rubiginosus*), which are sympatric in the study area, were visually discerned from those of tiger and leopard by virtue of differences in size and morphology, and excluded at the initial stage of field sampling itself. All putative tiger or leopard scats were kept and transported at ambient temperature from the field and stored at 4°C upon arrival at the laboratory. Faecal DNA extraction was carried out in a room dedicated to low-copy DNA extraction and devoid of concentrated PCR products. DNA extraction was performed according to the guanidine isothiocyanate - silica based extraction protocol (Boom *et al.* 1990). Each extraction set was accompanied by blank extraction controls without scat sample to monitor contamination. DNA extracts from scat samples were amplified and the PCR products digested with *Bam*HI restriction enzyme as per the methods adopted for tissue DNA. Confirmed tissue samples of tiger (*n*=15) and leopard (*n*=7) were used as controls.

2.3. Results

Amplification of a 187 bp cytochrome *b* gene fragment using the PtiL-14,716and PtiH-14,901 primers was successful in both tigers and leopards, but amplification failed in other non-felid carnivores and all potential prey species (Figure 2.2;Table A1 in Appendix). The control 309 bp universal cyt *b* primers showed amplification with all samples proving that non-amplification of the 187 bp band in non-felid species is because of sequence differences at the priming site and not a result of artifacts or stochastic error in amplification. (Figure 2.2; Table A2 in Appendix). *Bam*HI digestion of the 187 bp long PCR product yielded two diagnostic bands of 120 and 67 bp sizes in tiger, but not in leopard (Figure 2.3). Since leopards lack the restriction site at the particular position of the cyt *b* gene compared to tigers, the band profile of the species on enzyme digestion of the PCR products was identical to the undigested PCR product. The tests were conducted using blood samples and demonstrate the reliability of the PCR-restriction digestion protocol to distinguish between the two sympatric species (Table A1 in Appendix) Performance of the primers was good in scats samples, with 61 out of 65 scats showing positive amplification (94% amplification success); of which 51 were identified as tiger

based on *Bam*HI digestion profiles, while the remaining 10 samples were assigned to leopard (Table A2 in Appendix).

2.4. DISCUSSION

The success of the PCR-RFLP method in obtaining high amplification rates from scat DNA extracts and the precise identification of tiger scats can be attributed to the selection of short amplicons for PCR and the species-specific location of the particular *Bam*HI restriction site in tigers but not leopards. Furthermore, tagging the 5'ends of both forward and reverse primers with the blue fluorescent dye, 6-Fam^{TM}, enhanced the sensitivity in detection of PCR products and accurate sizing of bands, with improved capacity for high-throughput analysis on capillary electrophoresis instruments. This would not have been possible with gel-based methods. The increased sensitivity attained through the use of dye labeled primers is especially useful when amplifying markers from degraded and trace DNA templates such as scats. Though it can be argued that the reliance of the above method on dye labeled primers for resolving enzyme digested bands on the DNA sequencer entails an added step which makes it more time-consuming compared to exclusively gel-based methods (Nagata *et al.* 2005; Mukherjee *et al*. 2007). However, the benefits of attaining enhanced sensitivity on the fluorescence method to detect trace amplification signals combined with consistent and accurate base pair resolution far outweigh the cons, making it a first-rate screening protocol for identification of species from sub-optimal DNA source material. Furthermore, data storage and archival of the results outputted from the DNA sequencer instrument is straightforward, resulting in a more streamlined approach, which is easily amenable for high-throughput technologies compared to gel based methods. The method being presented here enables quick identification of tigers from sub-optimal sources of DNA and serves to complement numerous field studies currently involved in estimating habitat use and occupancy by this species.

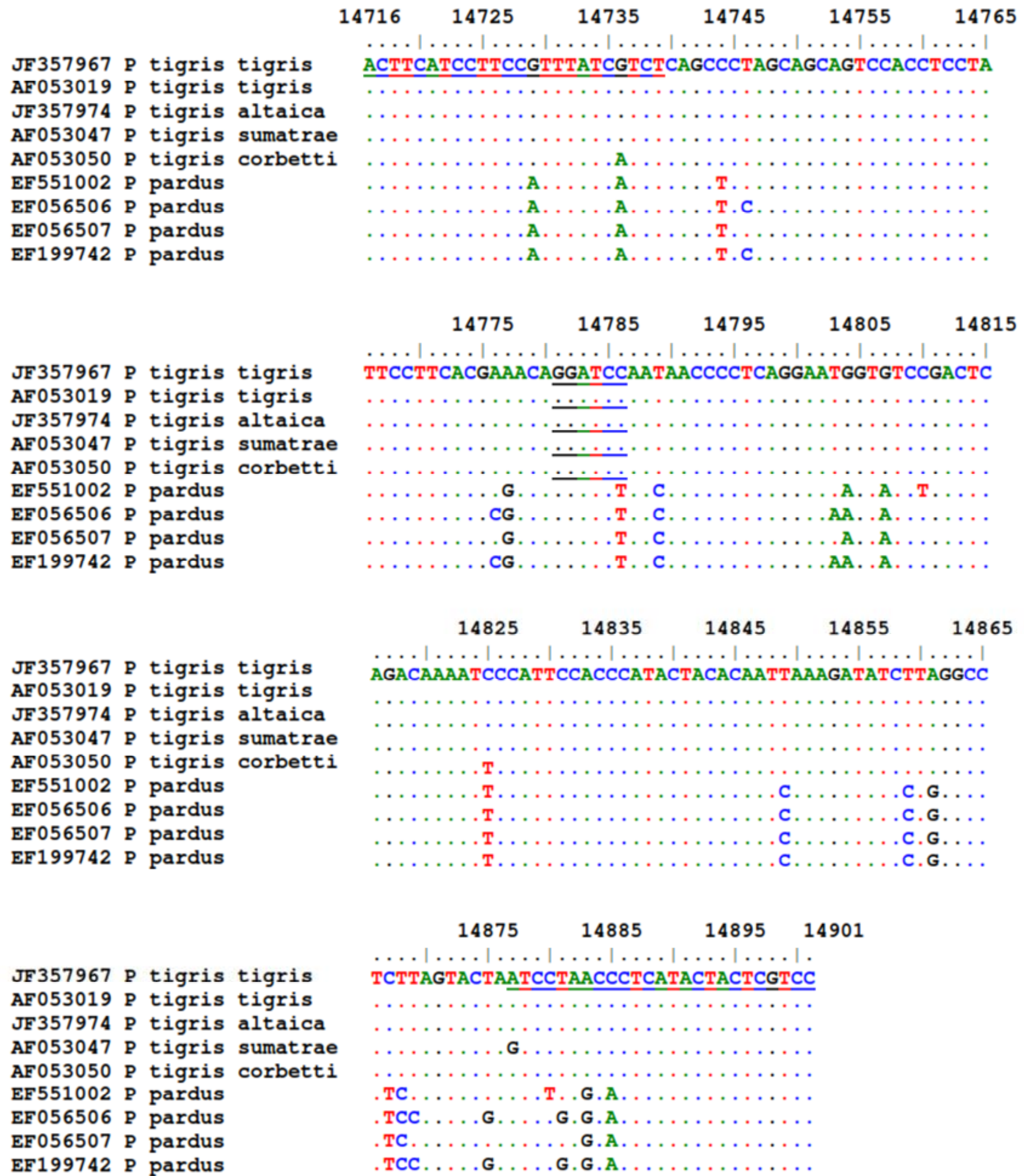

Figure 2.1. MtDNA cyt *b* sequence alignment with a complete tiger mtDNA genome sequence (GenBank Acc. No. JF357967), showing the presence of *Bam*HI restriction enzyme site, G/GATCC, in tiger (underlined sequence) but not in leopard. Forward (14716 – 14739) and reverse (14877 – 14901) priming sites are depicted.

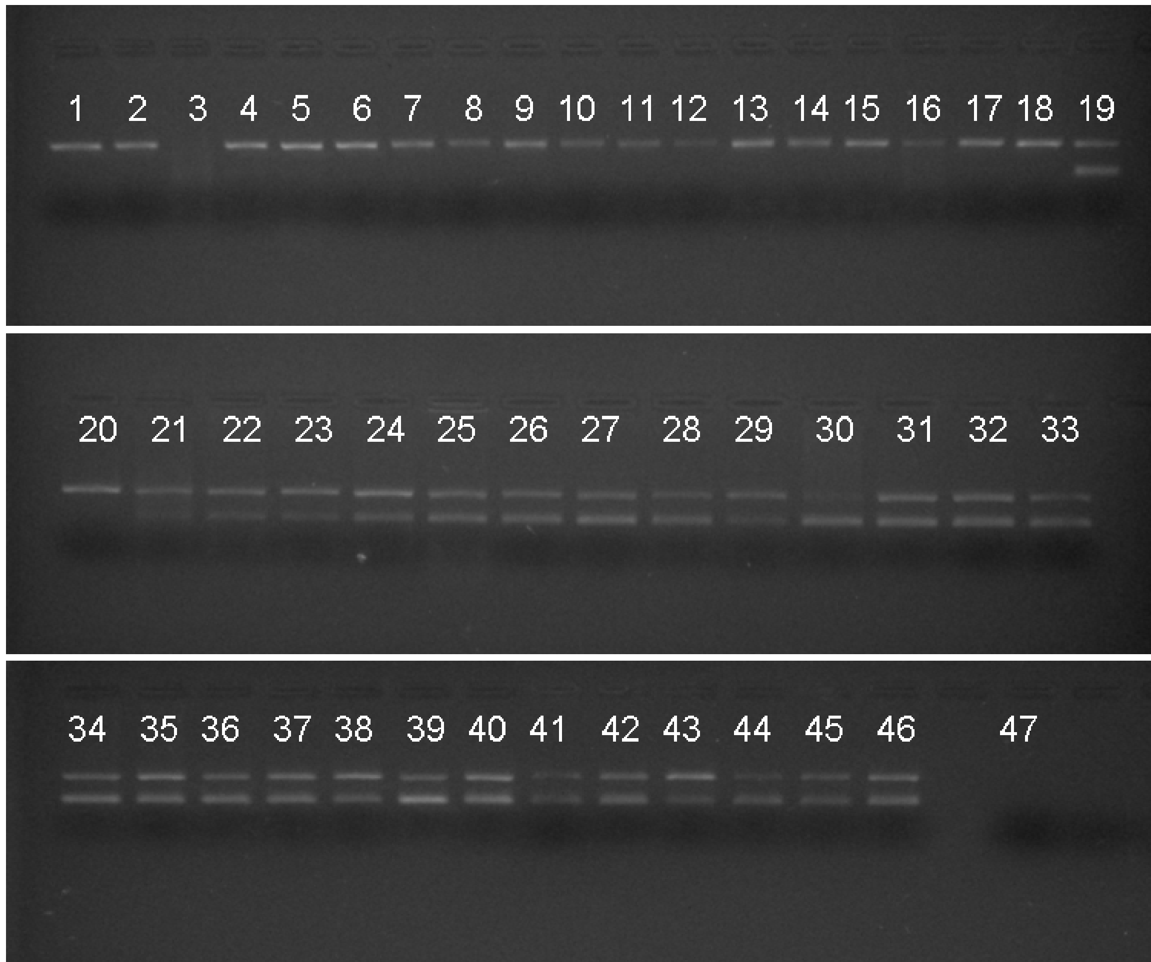

Figure 2.2. Gel image of PCR products showing amplification by PtiL-14,716and PtiH-14,901 primers (bottom band) in felid species only, but not in other carnivore or prey species. Universal cyt *b* primers targeting a 309 bp PCR product (top band) has been used as a control and shows amplification in all species tested (except in a hyena sample in lane 3, attributed to PCR sample failure). Striped hyena (1-6), wolf (7-9), domestic dog (10-12), golden jackal (13-15), sloth bear (16), wild pig (17), domestic goat (18), human (20), leopard (21-29) and tiger (19, 30-46), and water control (47).

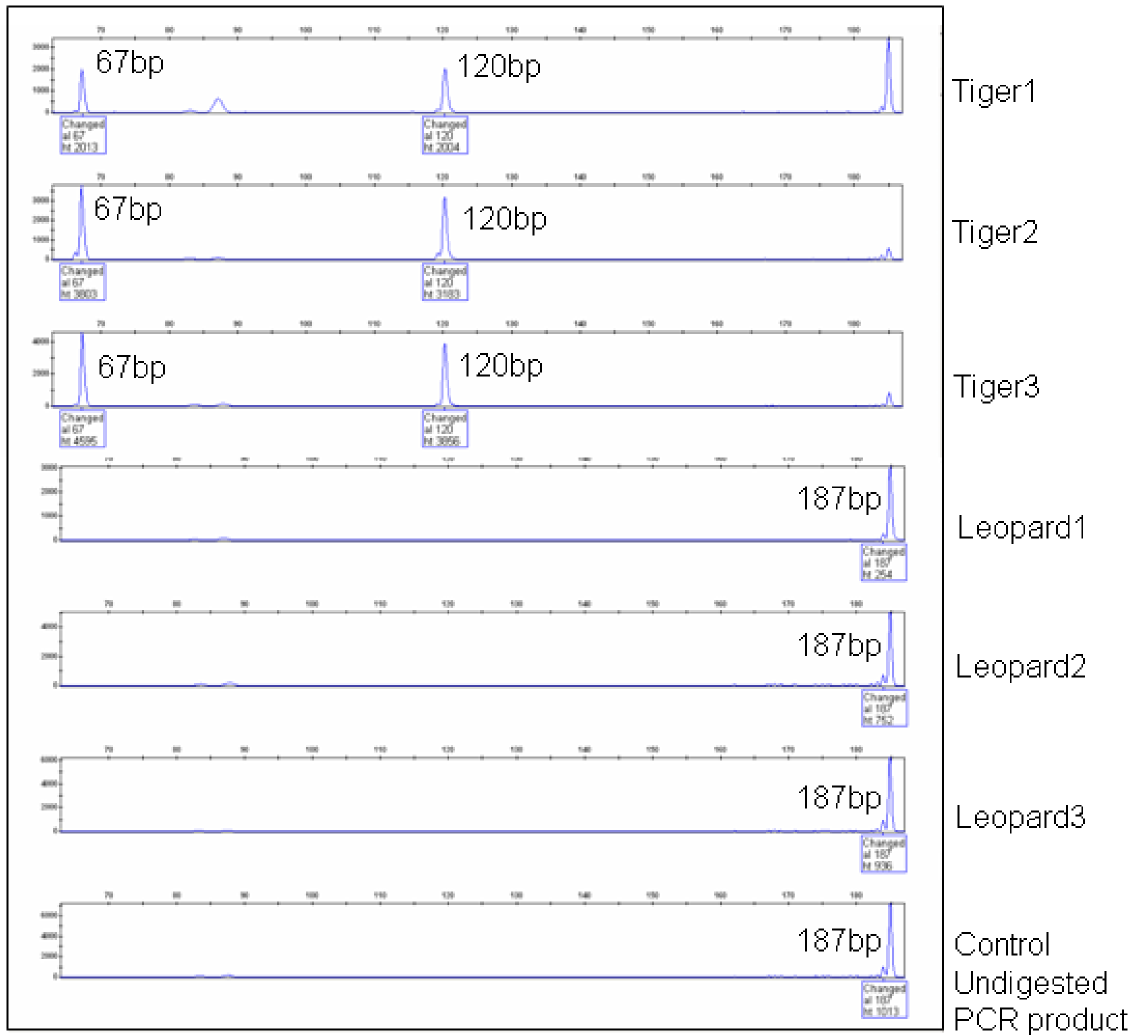

Figure 2.3. Capillary electrophoresis output from a DNA sequencer machine of DNA band profiles of *Bam*HI digested cytochrome *b* PCR products. Note the presence of two enzyme digested bands (67 and 120 bp) in tiger but a single undigested 187 bp band in leopard.

CHAPTER 3

INDIVIDUAL IDENTIFICATION FROM TIGER SCATS AND MICROSATELLITE LOCI GENETIC VARIATION

CHAPTER 3

INDIVIDUAL IDENTIFICATION FROM TIGER SCATS AND MICROSATELLITE LOCI GENETIC VARIATION

3.1. INTRODUCTION

This chapter presents the results of microsatellite loci genotyping for identifying individuals from tiger scats.Since scats formed the bulk of samples in my study, the unambiguous identification of individuals is important because of the high possibility of some tigers being sampled more than once. Along with identification of individuals from tiger scats, I provide an assessment of genetic variation and demographic fragmentation across the different tiger populations in the area. This information is important for understanding the population genetic history and conservation of tigers in Central India.

Microsatellites are the most widely used class of markers for population genetics for molecular ecology and conservation genetics. Due to their high polymorphism and Mendelian patterns of inheritance; microsatellite loci are well suited for analyses pertaining to identifying individuals, assigning parentages and in estimating gene flow (Goldstein and Schlotterer 1999). Microsatellites markers are also called VNTRs (variable number of tandem repeats), STRs (short tandem repeats) or SSRs (simple sequence repeats) and consist of a tandem repeat of short sequence motif of 1 to 6 oligonucleotides (e.g. "CAGCAGCAGCAGCAG" which can be represented by $(CAG)^n$where $n = 5$). The number of repeats at a polymorphic locus may range from 2 to 100. By designing PCR primers to amplify the conserved DNA sequences flanking the variable repeat units, one can assess genetic variation across taxa down to the individual.

For purposes of individual identification, the least amount of the most polymorphic and heterozygous microsatellite loci that give the lowest probability of identity (P_{ID}) value must be identified. The inclusion of more markers adds to the power of test as the

differences in genotype and allele frequencies that exist among relatives and among parental populations for hybrid individuals increase (Waits *et al.* 2001). However, as more markers are added, more genotyping errors are inadvertently introduced in the study (Dewoody *et al.* 2006). Therefore, utmost care must be observed in detecting and minimizing genotyping errors. The final panel of markers is a trade-off between incorporating more loci for achieving high resolving power in assignment and parentage tests, and reducing the inadvertent error in the data through addition of further loci.

The readily available information of a large amount of polymorphic microsatellite loci in the domestic cat (Menotti Raymond *et al.* 1999), together with a few loci identified in tigers (Williamson *et al.* 2002; Zhang *et al.* 2006; Bhagavatula and Singh 2006; Sharma *et al.* 2008; Wu *et al.* 2008) have spurred a flurry of research on noninvasive genetic studies on tigers (Mondol *et al.* 2009a, 2009b; Borthakur *et al.* 2011; Reddy *et al.* 2012; Sharma *et al.* 2012; Joshi *et al.* 2013). These studies have reliably demonstrated the applicability of using microsatellite markers on tigers to identify individuals and obtain information about population parameters. A common thread with most of these published noninvasive tiger genetics studies (Mondol *etal.*2009a, 2009b; Reddy *et al.* 2012; Sharma *et al.* 2012) is the preponderance of dinucleotide loci, so selected because of their high levels of polymorphism compared to other repeat classes. However, the consistently high levels of stuttering error associated with most markers of this class have caused intractable problems in scoring alleles, which is especially severe when using degraded scats DNA extracts (Pompanon *et al.* 2005; Dewoody *et al.* 2006). Often tri and tetra-nucleotide repeats (Wu *et al.* 2008) arepreferred as they have lower amplification errors and clear stutter peaks compared to dinucleotide repeats (Dewoody *et al.* 2006).

Here I present the results of genetic identification of tiger individuals obtained by amplifying microsatellite markers from scat DNA extracts. I used a panel of eleven highly polymorphic microsatellite loci to generate multilocus allelic profiles for unambiguous individual identification of tigers. Further, I assessed genetic variation and demographic contracting events in the populations using summary statistics of allelic variation. The assessment of genetic variation is important in context to my overall study

goal of understanding population fragmentation, genetic structuring and gene flow among the tiger habitats in the landscape. Recent field assessments have revealed that the tiger habitats in the region are patchy and though few habitats support thriving numbers of the species, in most habitats tiger occur in low numbers (Jhala *et al.* 2011b). By using microsatellite data, I wanted to find out relationships between the estimated animal densities with the number of genetic individuals, patterns of genetic variation, and past demographic events which could have impacted these populations.

3.1.1. Probability of identity (P_{ID})

In any study that uses microsatellite loci for genetic profiling, it is possible for different individuals to have identical profiles, if an insufficient number of loci have been used. It is therefore important to quantify the number of loci necessary to have sufficient power to distinguish between individuals with say 99% certainty. This can be achieved by computing probability of identity (P_{ID}) statistic. Bruford *et al.* (1998) defined P_{ID} "the probability that two individuals in a population will share a genotype by chance". $P_{ID}$is first calculated for a *single* locus and the *overall* probability of match for any two individuals in a population is obtained by multiplying the P_{ID}values across all loci that have been analysed (Paetkau *et al.* 1995). For a single locus the probability of identity is calculated as follows (Paetkau and Strobeck 1994):

$$P_{ID} = \Sigma\, p_i^4 + \Sigma\Sigma\, (2\, p_i p_j)^2 \qquad (1)$$

where p_i and p_j are the frequencies of the *i*th and *j*th allele respectively. The first part (Σp_i^4) of the equation gives the sum of squares of the homozygous genotypes, while $\Sigma\Sigma(2p_i\, p_j)^2$ gives the same result for the hetrozygous genotypes. The overall probabality of match between any two individuals is obtained by multiplying the P_{ID} values across all loci that thave been analysed (Paetkau *et al.*1995).

The estimation of match probabilities assumes independence of alleles at particluar lci, and thus the genotypes must be in genotypic equilibrium, signifying independence of the

alleles from different loci (Donelly 1995). However, these assumptions are often violated in natural populations due to factors such as poulation substructure, non-random mating, genetic drift and natural selection, which deviate from linkage and Hardy-Weinberg equilibrium (Taberlet and Luikart 1999). Most often, the P_{ID} values obtained with the above statistics usually result in underestimation of the actual match probabilities in natural populations (Waits *et al.* 2001). Due to the potential for relatives to be present in the sample, it is best to use the conservative estimate of P_{ID} among siblings ($P_{ID\text{-}sib}$, Evett and Weir 1998; Waits *et al.* 2001). $P_{ID\text{-}sib}$ for a single locus is computed thus:

$$P_{ID\text{-}sib} = 0.25 + (0.5\,\Sigma\, p_i^2) + [0.5\,(\Sigma\, p_i^2)^2] - (0.25\,\Sigma\, p_i^4) \qquad (2)$$

where p_i is the frequency of the ith allele. The overall $P_{ID\text{-}sib}$ is the upper limit of the possible ranges of P_{ID} in a population and will thus provide the most conservative estimate of the number of loci required to resolve all individuals, including relatives.

3.2. Methods

3.2.1. Field sampling

The work presented in this and the remaining chapters was conducted on a total of 603 samples (587 scats/ 16 blood/ 1 tissue) obtained across tiger habitats in the Central Indian landscape (Table 3.1). The 16 blood samples were represented by radio-collared tigers from Kanha (n = 12), Pench (n = 3) and Bandhavgarh (n = 1). One tissue biopsy was obtained from a dead sub-adult male in Satpura. 587 putative tiger scats were collected between 2006 and 2011 from Kanha, Pench, Achanakmar, Bandhavgarh, Satpura, Tadoba and Melghat Tiger Reserves and a few intervening forest corridors in the area. Scats were stored, either dry with silica or in 75% ethanol, and kept at ambient temperature, prior to laboratory analysis. For each sample, a Global Positioning System (GPS) reading was taken and transferred into a Geographic Information System (GIS) map to establish their map location.

3.2.2. Laboratory work

All scat DNA extractions were carried out in a room devoid of concentrated DNA or PCR products, and aerosol-barrier pipette tips were used during all steps. Scat DNA extractions were carried out using the guanidine thiocyanate-silica extraction protocol (Boom *et al.* 1990). For every extraction, negative controls composed of reagent only without the scat sample were included to monitor contamination. Extractions from blood and tissue samples were carried out using the DNeasy blood and tissue kit (QIAGEN Ag, Germany). Tiger scats were identified from sympatric carnivore species using the previously described PCR-RFLP procedure (see Chapter 2 *ibid*).

3.2.3. Microsatellite loci amplification and allele scoring

A panel of eleven microsatellite loci, derived from domestic cat (Menotti Raymond *et al.* 1999) and tiger (Williamson *et al.* 2002, Wu *et. al.* 2008) was used for individual discrimination. The loci consisted of three dinuceotide (Fca304, Fca954, 6Hdz700), three trinuceotide (Pati01, Pati09, Pati15) and five tetranucleotide repeat markers (Fca441, F85, F53, F124, Pati18). The 5' end of the forward primer for each microsatellite marker was variously labeled with the fluorescent dyes 6–FAM (blue), PET (red), VIC (green) or NED (yellow), while the reverse primers were not labeled (Table 3.1). PCR amplifications were carried out in 10 µl reactions with a multiplex panel of 3 to 4 loci using the Multiplex PCR kit (QIAGEN) according to the manufacturer's instructions. PCR amplified products were resolved on the ABI3130 Genetic Analyzer and GENEMAPPER v3.7 (Applied Biosystems, USA) was used to score allele sizes. Allele sizing was carried out by combining automated allele calling and visual inspection of each sample. This process provides a balance between the efficiency and consistency of automated allele- calling software (GENEMAPPER v3.7, Applied Biosystems, USA) and the accuracy provided by human inspection in detecting novel alleles outside of the expected range of a locus, stochastic amplifications within the size range, and potential mistypes due to stutter or large-allele dropout (Pompanon *et al.* 2005; Dewoody *et al.* 2006). To limit genotyping error due to allelic dropout (stochastic non-amplification of

one allele in a heterozygote), multiple PCR replicates were conducted as in Navidi *et al.* (1992). Heterozygotes were confirmed across at least two independent replicates and five replicates in case of homozygotes. The genotype data were checked on MICROCHECKER (van Oosterhout *et al.* 2004) for identifying and correcting genotyping errors such as those that arose from stuttering patterns, null alleles and small-allele dominance.

Table 3.1. Primer sequences of microsatellite and sex-chromosome linked loci used in individual identification and gender discrimination.

Locus	Orientation	Primer Sequence (5' – 3')	Label	Reference
Pati15	Forward	CGAACCTTCCTGCAAAACAAA	6-Fam	Wu *et. al.* 2008
	Reverse	GACTCCAAAGCCCAAACTCT		
Pati18	Forward	CGTGTTTGGCTATAACCATT	Ned	Wu *et. al.* 2008
	Reverse	GTAACCCAGTGTCTCCTTGT		
Pati09	Forward	CGAGCCAATCATCCAATCAAA	Vic	Wu *et. al.* 2008
	Reverse	GCAAGGACAGGAGCCAGTTA		
Pati01	Forward	TATGTTCAAAGTCACTGGGAGG	Pet	Wu *et. al.* 2008
	Reverse	GTTAGGCTGCGTGGTTCTGG		
6Hdz700	Forward	TCCTCCTTCCAGGATGCCA	Vic	Williamson *et al.* 2002
	Reverse	AGGATGGGGGAAAATCTCTC		
F124	Forward	TGTGCTGGGTATGAAGCCTACTG	Vic	Menotti Raymond *et al.* 1999
	Reverse	GTGTCTTCCATGCCCATAAAGGCTCTGA		
F53	Forward	CCTATGTTGGGAGTAGAGATCACCT	Pet	Menotti Raymond *et al.* 1999
	Reverse	GTGTCTTGAGTGGCTGTGGCATTTCC		
Fca304	Forward	TCATTGGCTACCACAAAGTAGG	6-Fam	Menotti Raymond *et al.* 1999
	Reverse	GCTTCTGCATGCCATTGGGTAA		
Fca441	Forward	ATCGGTAGGTAGGTAGATATAG	6-Fam	Menotti Raymond *et al.* 1999
	Reverse	GCTTGCTTCAAAATTTTCAC		
Fca954	Forward	ATGTTTTAAGTGCCAACGCC	6-Fam	Menotti Raymond *et al.* 1999
	Reverse	CTTGACCGAGGTCAGAATTACC		
F85	Forward	TAAATCTGGTCCTCACGTTTTC	Ned	Menotti Raymond *et al.* 1999
	Reverse	GCCTGAAAATGTATCCATCACTTCAGAT		
Fca651	Forward	CAGGGGCCCTGATTTCTAG	Pet	Menotti Raymond *et al.* 1999
	Reverse	GGCCTACAAATTGGCAAAGA		
SRY	Forward	GCGAACTTTGCACGGAGAGTC	Vic	Butler *et al.* 2002
	Reverse	GCGTTCATGGGCCGTTTGACG		

3.2.4. Gender identification

Gender of tiger scat samples was determined using primers to amplify the Y chromosome linked SRY (sex determining region) loci as demonstrated in the domestic cat individualization panel, MEOWPLEX (Butler 2002; Butler *et al.* 2002). Fca651 (Menotti Raymond *et al.* 1999), which is an X-chromosome microsatellite locus ranging in allele size between 130 to 140 bp was used as a positive control together with the SRY (PCR product size, 99bp) in a multiplex PCR reaction (Table 3.1). Male samples were identified based on amplification of both X (Fca651) and Y linked (SRY) markers. Samples which yielded amplification at the X-linked Fca651 locus, but showed non-amplification of the Y-linked SRY band were designated as females. PCR amplifications were carried out following manufacturer's instructions using the QIAGEN Multiplex PCR kit.

3.2.5. Individual discrimination and descriptive statistics

The conservative sibling probability of identity ($P_{ID\text{-}sib}$) statistic (Taberlet and Luikart 1999; Waits *et al.* 2001) was computed in GIMLET 1.1 (Valiere 2001) to determine that the panel of microsatellite loci had sufficient information to discriminate between two closely related individuals in the same population. Unique multilocus genotypes were identified using the *Identity* analysis option in CERVUS 3.0 (Kalinowski *et al.* 2007; Marshall *et al.* 1998). Samples which showed mismatches at upto two loci were re-examined for possible genotyping errors and allelic drop-out, and again amplified thrice in order to confirm the multilocus genotypes before assigning them as unique individuals. Multiple replicates of the same individual (from the same site) were discarded and only unique multilocus genotypes were used for all further analyses. Estimates of heterozygosity, allele frequency, and exact tests for deviation from genotypic and Hardy-Weinberg equilibrium (HWE) were calculated using GENEPOP 4.1 (Rousset 2008; Raymond and Rousset 1995). Significance values for linkage disequilibrium (LD) among all pairs of loci in the dataset were tested using 1,000 permutations implemented in FSTAT 2.9.3 (Goudet 2001).

3.2.6. Detection of genetic bottleneck

To detect signatures of genetic bottleneck in the sampled populations, I evaluated three summary statistics - (i) Wilcoxon's sign rank test and (ii) mode-shift test, implemented in the program BOTTLENECK 1.2.02 (Piry *et al*. 1999), and (iii) the *M* ratio test (Garza and Williamson 2001) implemented in ARLEQUIN 3.1 (Excoffier *et al*. 2005). Wilcoxon's test detects bottlenecks based on the probability of heterozygosity excess over that expected at mutation-drift equilibrium in a population. It is most effective at detecting historic bottlenecks occurring over approximately 2 - 4 N_e generations in the past. The mode-shift test is more suited for detecting bottlenecks within the last few dozen generations (Luikart *et al*. 1998; Cornuet and Luikart 1996). This test is based on the premise that a stable population at mutation-drift equilibrium will have a large proportion of alleles at low frequency and a smaller proportion at intermediate frequencies and few at high frequencies. The resulting allele proportions results in an L-shaped distribution. In bottlenecked populations the mode is shifted because of the rapid loss of alleles present at low frequency. I ran 10,000 simulations across all populations (excluding Achankmar due to low sample size) using the program BOTTLENECK under both the stepwise mutation model (SMM) and the two-phase mutation model (TPM) with 95% single step mutations and 5% multi-step mutations and a variance of 12 as recommended by Piry *et al.* (1999). *P*-values from the Wilcoxon's test were used to examine bottlenecks at each timescale and were assessed at the alpha 0.05 level. The *M* ratio ($M = k/r+1$) was calculated from the mean ratio of the number of alleles (k) to the allele size range (r). Assuming loci follow a generalized stepwise mutation model, the loss of rare alleles would diminish the value of k at a faster rate than r thereby a drop in the *M* ratio below a threshold of 0.68 would be suggestive of populations which experienced recent bottleneck (Garza and Williamson 2001).

3.3. RESULTS

3.3.1 Tiger scat and individual identification

PCR success on scats was low with only 56% successful amplification (330 out of 587 total scats) using the mtDNA cyt *b* felid specificPtiL-14,716and PtiH-14,901 primers, and a further 275 (out of the 330 cyt *b* PCR positive) scats were identified through *Bam*HI restriction enzyme digestion as tiger scat (Table 3.2). Microsatellite loci amplification on the 275 tiger scats produced 250 analyzable samples which yielded microsatellite loci data at a minimum of 7 markers. These 250 scat genotypes together with 17 genotypes (blood=16; tissue=1) were considered for *identity analysis* in CERVUS. Both the scat and blood/tissue genotype data were pooled for individual identification because of the fact that replicate individuals identified from the analysis could have originated from multiple sample sources. I identified 169 individuals with 81 recaptures from a total of 267 microsatellite loci genotypes (250 scats, 16 blood and 1 tissue). The total number of individuals identified here constitutes roughly 49% of the total tiger population in the entire sampled area (Table 3.2 and Figure 3.1). Gender identification yielded nearly equal proportions of male (74 out of 169) and female (85 out of 169) tigers. Genotype data of the 169 individuals were variable (Table A3 in Appendix). All blood and tissue DNA extracts were successfully genotyped at all eleven microsatellite loci, whereas typing success was variable in scats and varied from as low as 7 to all 11 loci. On average, individual multilocus genotypes were 92.8% complete (Table 3.3). Ninety seven percent (164 out of 169) of samples either had complete eleven genotypes or with a maximum of one or two missing loci. Two Tadoba individuals with three missing loci (73% complete) and three individuals from the Kanha Pench corridor with four missing loci (64% complete) were also retained since they were obtained from under-sampled areas and represented unique individuals. The panel of eleven microsatellite loci that was used for individual discrimination had very low cumulative sibling probability of identity ($P_{ID\text{-}sib}$) of 1.5×10^{-4} (Table 3.3). Even in the samples with the least amount of genotype information (four missing loci), the cumulative $P_{ID\text{-}sib}$ value (1.6×10^{-3}) of the samples was low enough to be considered as unique individuals.

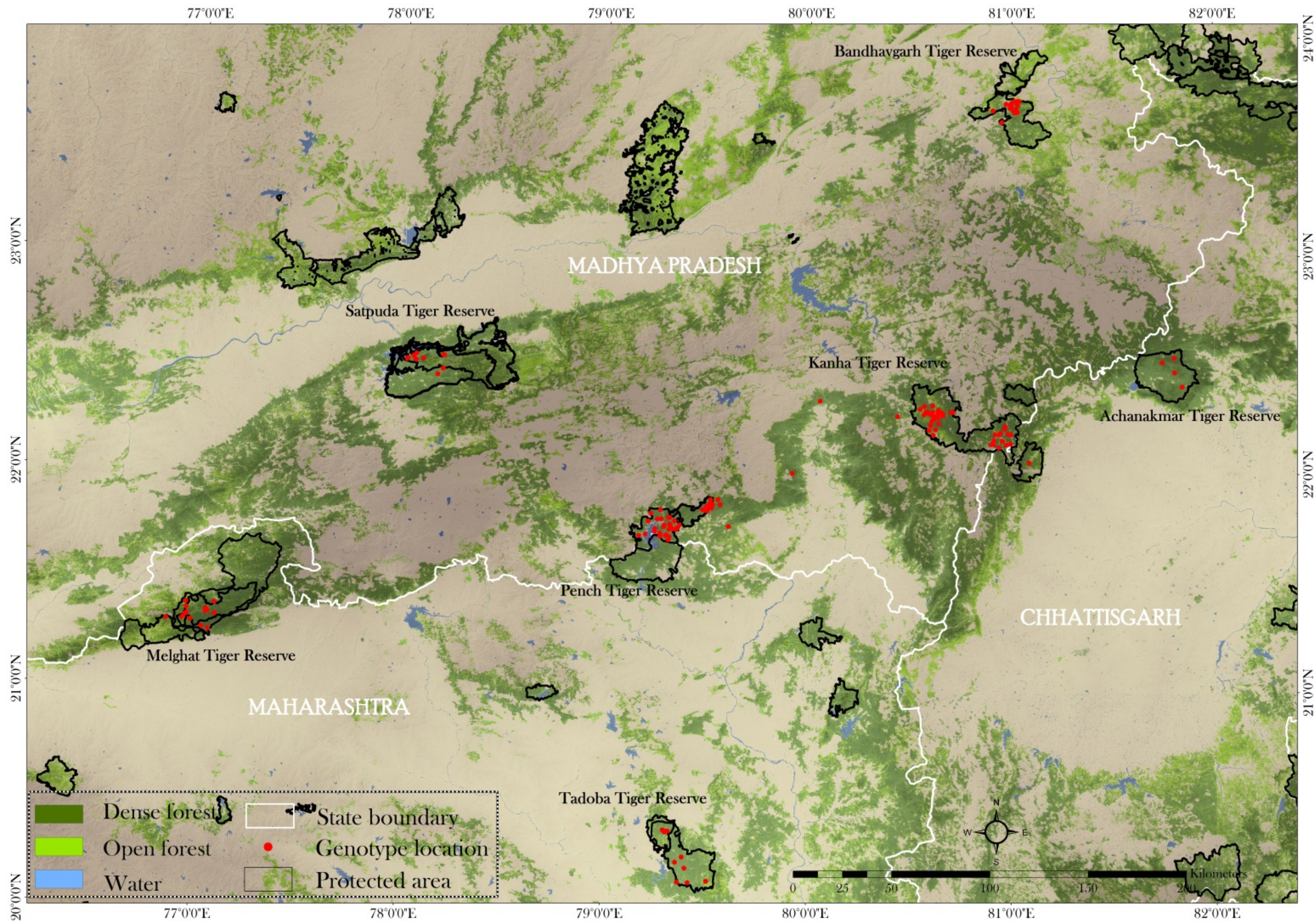

Figure 3.1. The study area in Central India showingforest cover, tiger reserve boundaries, and sampling location of individually genotyped tigers (*n*=169).

Table 3.2. Locality-wise estimates showing sampling effort and number of tiger individuals identified in the study area.

sampling location	area (km^2)	total scats/ cyt*b* PCR amplified/ tiger scats/ STR genotypes	other samples - blood (b), tissue (t)	all samples/ tiger STR genotypes/ individuals	estimated population size (SE range) *	% populationsampled	identified tiger individuals		
							male	**Female**	**unknown**
Melghat	1,677	66/ 47/ 44/ 35	0	66 / 35/ 15	35 (30-39)	43	6	8	1
Satpura	1,502	46/ 21/ 17/ 17	1 (t)	47 / 18/ 11	43 (42-46)	26	3	6	2
Pench	1,308	137/ 94/ 86/ 82	3 (b)	140 / 85/ 51	65 (53-78)	78	25	24	2
KPC**	16,000	26/ 24/ 5/ 5	0	26 / 5/ 5	*NA*	*NA*	2	2	1
Kanha	1,945	159/ 67/ 65/ 56	12 (b)	171 / 68/ 50	60 (45-75)	83	22	27	1
Achanakmar	552	23 / 16/ 5/ 5	0	23 / 5/ 4	12 (11-13)	33	2	2	0
Tadoba	775	32 / 20/17/176	0	32 / 17/ 11	69 (66-74)	16	5	3	3
Bandhavgarh	695	98 / 41/ 36/ 33	1 (b)	99 / 34/ 22	59 (47-71)	37	9	13	0
total		**587/ 330/ 275/ 250**	**17**	**604 / 267/ 169**	**343**	**49**	**74**	**85**	**10**

* in Jhala *et al.* 2011b, ** KPC– Kanha Pench corridor, *NA* - not available

3.3.2. Genetic diversity statistics

A summary of genetic diversity statistics across these 169 individuals, genotyped at eleven microsatellite loci (Table 3.3), reveals a high mean number of alleles (9.1 ± 2.2) and high heterozygosity, with similar estimates for expected (75.4% ± 3.9) and observed (70.1% ± 5.9) values. No significant evidence of linkage disequilibrium (LD) among all pair-wise loci combinations was observed when all sampling locations were pooled (p>0.05 at 1,000 permutations). Except for deviation at three loci, Fca441, Pati09 and Pati18 (p<0.001), all other loci were in Hardy-Weinberg equilibrium (HWE) in the pooled population after adjusting the critical P-value using the Bonferroni correction procedure (Table 3.3). Loci not in HWE or with null allele frequencies >5% appear to be random with respect to population (Table 3.4). Pati09 and Pati15 showed significant deviation from HWE across four and three populations respectively, while four loci (Pati01, Fca441, Fca954 and F124) each had significant deviation in only two populations. Such departures from HWE could indicate the possibility of genetic structuring among populations and likely presence of related individuals in the data (Lukas *et al.* 2004). Estimates of null alleles were >5% in five loci (Fca954, F53, Pati01, Pati15 and Pati18), with a maximum frequency of 10% in Pati18 while estimates in the other four loci were <8%. MICROCHECKER tests did not show any evidence of scoring errors due to stuttering or small allele dominance in the dataset.

Table 3.3. Information on the eleven microsatellite loci used in this study.Allele diversity statistics, observed (*Ho*) and expected (*He*) heterozygosity, Hardy-Weinberg equilibrium (HWE) tests, null allele frequencies and sibling probability of identity ($P_{ID\text{-}sib}$) values obtained across 169 tiger individuals. Null allele frequencies > +0.05 are italicized.

Locus	**Number of alleles**	**Allele size (basepairs)**	**Number of individuals typed(%)**	**Observed heterozygosity (*Ho*)**	**Expected heterozygosity (*He*)**	**HWE (*p* value)**	**Null allele frequency**	**Individual $P_{ID\text{-}sib}$ value**	**Cumulative$P_{ID\text{-}sib}$ product**
F124	9	250-282	152 (89.9)	0.711	0.742	ns(0.069)	+0.014	$4.11 x10^{-1}$	$3.82 x10^{-1}$
Fca304	10	115-145	169 (100)	0.686	0.717	ns(0.128)	+0.024	$4.24 x10^{-1}$	$1.51 x10^{-1}$
F85	11	123-176	163 (96.4)	0.712	0.782	ns(0.107)	+0.029	$3.79 x10^{-1}$	$6.2 x10^{-2}$
Fca954	13	169-196	157 (92.9)	0.701	0.793	ns(0.075)	*+0.060*	$3.71 x10^{-1}$	$2.7 x10^{-2}$
Fca441	6	136-157	167 (98.8)	0.790	0.698	*(<0.001)	-0.079	$4.32 x10^{-1}$	$1.2 x10^{-2}$
Pati15	13	190-241	147 (87.0)	0.667	0.789	ns(0.024)†	*+0.074*	$3.72 x10^{-1}$	$5.5 x10^{-3}$
6Hdz700	11	131-153	167 (98.8)	0.725	0.797	ns(0.088)	+0.049	$3.76 x10^{-1}$	$2.6 x10^{-3}$
F53	11	128-161	139 (82.2)	0.612	0.729	ns(0.938)	*+0.075*	$4.15 x10^{-1}$	$1.2 x10^{-3}$
Pati18	7	205-229	136 (80.5)	0.618	0.771	*(<0.001)	*+0.104*	$3.93 x10^{-1}$	$5.8 x10^{-4}$
Pati09	6	114-129	164 (97.0)	0.799	0.710	*(<0.001)	-0.078	$4.32 x10^{-1}$	$2.9 x10^{-4}$
Pati01	8	188-209	165 (97.6)	0.685	0.796	ns(0.456)	*+0.073*	$3.66 x10^{-1}$	$1.5 x10^{-4}$
Average	9.1 ± 2.2		156.9 (92.8)	0.701 ± 0.059	0.754 ± 0.039		+0.032		

ns – not significant, * significant (p<0.05) deviation from HW expectation, † Bonferroni adjusted values

Table 3.4. Summary of population-wise Hardy-Weinberg Equilibrium (HWE) tests and null allele frequencies at each locus. Individuals from Achanakmar (*n*=4) and Kanha-Pench corridor (*n*=5) were only included in the pooled Central Indian population.

	Melghat (*n*=15)		Satpura (*n*=11)		Tadoba (*n*=11)		Pench (*n*=51)		Kanha (*n*=50)		Bandhavgarh (*n*=22)		All (*n*=169)	
Locus	**HWE**	**Null**	**HWE**	**Null**	**HWE**	**Null**	**HWE**	**Null**	**HWE**	**Null**	**HWE**	**Null**	**HWE**	**Null**
Pati01	ns	0.11	ns	0.07	ns	-0.01	*	0.02	ns	-0.08	*	-0.07	ns	0.07
Pati09	*	-0.18	ns	-0.17	*	-0.23	*	-0.12	ns	-0.13	*	-0.19	*	-0.08
Fca304	ns	-0.03	ns	-0.13	ns	-0.12	ns	-0.03	ns	-0.04	*	0.20	ns	0.02
Fca441	ns	-0.17	*	0.14	ns	-0.21	ns	-0.06	ns	-0.05	*	-0.14	*	-0.08
6Hdz700	ns	-0.11	ns	-0.11	ns	-0.07	ns	0.00	ns	0.13	ns	-0.14	ns	0.05
F85	ns	-0.04	ns	-0.07	ns	0.03	ns	-0.01	ns	-0.04	*	0.05	ns	0.03
Fca954	*	0.19	ns	0.21	ns	0.02	ns	0.04	ns	0.07	*	-0.02	ns	0.06
F124	*	-0.18	*	-0.22	ns	ND	ns	-0.04	ns	0.00	ns	-0.13	ns	0.01
Pati15	ns	0.07	ns	0.17	*	ND	*	-0.07	ns	0.10	*	0.07	ns	0.07
F53	ns	0.17	*	0.27	ns	ND	ns	0.02	ns	-0.02	ns	0.11	ns	0.07
Pati18	ns	0.02	ns	-0.11	ns	-0.11	ns	-0.04	ns	0.06	ns	0.06	*	0.10

ns- not significant, * - $p<0.05$, ND – not done

Table 3.5. Overall loci summary statistics of population-wise mean genetic variation and bottleneck tests.

Population	Observed heterozygosity (St. Dev.)	Expected heterozygosity (St.Dev.)	Number of alleles (St.Dev.)	Allelic size range (St.Dev.)	*M* ratio (St.Dev.)	Wilcoxon's heterozygosity excess test	Mode-shift test
Melghat	0.654 (0.234)	0.674 (0.110)	4.7 (0.6)	17.5 (4.3)	0.75 (0.21)	ns	L-shaped
Satpura	0.673 (0.230)	0.639 (0.146)	4.2 (0.9)	16.7 (4.6)	0.70 (0.23)	ns	L-shaped
Tadoba	0.705 (0.195)	0.704 (0.094)	4.9 (1.5)	16.5 (5.9)	0.80 (0.15)	ns	L-shaped
Pench	0.750 (0.070)	0.734 (0.056)	7.6 (1.5)	26.8 (11.5)	0.83 (0.18)	ns	L-shaped
Kanha	0.685 (0.061)	0.674 (0.106)	7.2 (1.9)	22.5 (5.2)	0.86 (0.13)	ns	L-shaped
Bandhavgarh	0.641 (0.216)	0.607 (0.148)	4.5 (1.0)	18.6 (6.2)	0.66 (0.20)	ns	L-shaped
All	0.701 (0.059)	0.754 (0.039)	9.1 (2.2)	28.8 (10.5)	0.90 (0.14)	ns	L-shaped

ns- not significant

3.3.3. Genetic bottleneck

Summary statistics showed no indication of genetic bottleneck at either the long or short time scales in any of the six populations or in the pooled population. Except Bandhvagarh which had *M* ratio of 0.66, all other populations had above bottleneck threshold ($M >$ 0.68)values corresponding to stable populations (Table 3.5). All localities including the pooled population tested showed normal L-shaped allele distributions in the mode-shift test, indicative of stable non-bottlenecked populations (Table 3.5). None of the populations showed significant evidence of heterozygosity excess in the Wilcoxon's sign rank test for both the two-phased and stepwise microsatellite mutation models (Table 3.6). Only the Pench, Kanha and pooled populations showed significant ($p < 0.05$) evidence of heterozygosity deficiency (Table 3.6) suggestive of recent events of expansion in these populations.

Table 3.6. Results of population-wise bottleneck tests at each locus under the assumption of the two-phase (TPM) and stepwise (SMM) mutation models. *P* values < 0.05 are italicized.

Population	Mutation model	Pati01	Pati09	Fca304	Fca441	6Hdz700	F85	Fca954	F124	Pati15	F53	Pati18	Wilcoxon's test heterozygosity deficiency/ excess
	TPM	*0.015*	0.346	0.118	0.419	0.056	0.462	0.462	0.350	0.107	0.284	0.295	0.415/ 0.618
Melghat	SMM	*0.008*	0.389	0.100	0.464	0.078	0.517	0.428	0.398	0.092	0.247	0.249	0.206/ 0.817
	TPM	*0.032*	0.413	0.445	0.346	*0.035*	0.158	0.259	0.342	0.083	0.276	0.475	0.517/ 0.517
Satpura	SMM	*0.023*	0.454	0.462	0.385	*0.044*	0.140	0.288	0.370	0.068	0.248	0.434	0.449/ 0.584
	TPM	*0.008*	0.195	0.053	0.441	0.063	0.184	0.203	0.103	0.149	0.429	0.373	0.483/ 0.551
Tadoba	SMM	0.051	0.164	0.059	0.398	*0.049*	0.209	0.242	0.109	0.125	0.395	0.392	0.319/ 0.711
	TPM	0.914	0.129	0.262	0.497	0.085	0.081	0.060	0.277	0.342	0.103	0.186	*0.011/* 0.992
Pench	SMM	0.136	0.091	*0.010*	0.398	*0.044*	0.041	*0.028*	0.196	0.234	0.062	0.249	*0.008/* 0.994
	TPM	*0.045*	0.156	0.318	0.368	*0.015*	0.253	*0.008*	*0.018*	*0.010*	0.065	0.335	*0.003/* 0.998
Kanha	SMM	*0.023*	0.101	0.216	0.283	*0.007*	0.161	*0.003*	*0.026*	*0.003*	*0.032*	0.256	*0.003/* 0.998
	TPM	0.285	0.078	*0.042*	0.412	0.372	0.390	0.164	0.214	*0.036*	0.267	0.075	0.289/ 0.577
Bandhavgarh	SMM	0.315	0.082	*0.030*	0.362	0.304	0.319	0.206	0.203	0.231	0.296	0.058	0.207/ 0.413
	TPM	0.275	0.509	*0.049*	0.313	0.243	0.204	*0.037*	*0.033*	*0.034*	0.104	0.377	*0.008/* 0.994
All	SMM	0.402	0.393	*0.020*	0.188	0.124	0.104	*0.016*	*0.013*	*0.011*	*0.049*	0.507	*0.002/* 0.998

3.4. DISCUSSION

The low success in microsatellite loci amplification observed in this study is illustrative of the poor quality of scat samples obtained from the area and the technical difficulties of non-invasive genetic typing studies in tropical latitudes (Goossens and Salgado-Lynn 2013). Several studies have shown that PCR amplification success is contingent upon the age of the sample, the sample preservation and DNA extraction methods (Wasser *et al.* 1997; Frantzen *et al.* 1998; Murphy *et al.* 2000). While I used standardized methods of sample preservation and DNA extraction for my work, obtaining fresh scats was a challenge. Except for a few high density Tiger Reserves, majority of my sampled areas comprised low density tiger habitat where finding scats was difficult even with repeated sampling in the same area. Due to logistical constraints imposed by the size, terrain and remoteness of the study area, most scat samples obtained from the area hence were not fresh, ranging from several days to a few weeks in age. The harsh climate also enhanced degradation of genetic material present in scats. Despite these constraints, by primarily using noninvasively collected genetic data together with a few blood and tissue samples, a total of 169 individual tigers were identified in the area. Identified individuals displayed low overall $P_{ID\text{-}sib}$ value ($1.5\text{x}10^{-4}$), i.e. in other words the low $P_{ID\text{-}sib}$ value means that 1 individual in every 6,666 siblings carries the exact multilocus genetic signature at all eleven loci. The total number of individuals identified here represents about 49% of the estimated population in the area (Table 3.2; Jhala *et al.* 2011b). The relatively large sample size in relation to the estimated population enhances the significance of the results.

This study observed high levels of allelic diversity (9.1 ± 2.2) and heterozygosity (*Ho* = 0.70 ± 0.06, *He* = 0.75 ± 0.04), which is indicative of high genetic diversity of tigers in this region. Though not directly comparable with the present study because of the different markers used, previous studies on tigers from the Indian sub-continent and other range countries have reported low (Luo *et al.* 2004) to relatively high levels of genetic diversity (Mondol *et al.* 2009a; Reddy *et al.* 2012). A recent study in the Satpura-Maikal landscape in Central India (Sharma *et al.* 2012) which used seven microsatellite markers,

detected high heterozygosity (*Ho* = 0.65 ± 0.09, *He* = 0.80 ± 0.05), and allelic diversity (7.76 ± 1.96), with very low genetic subdivision (mean F_{ST} = 0.013 ± 0.006). Another study in Central India, which also included habitats just outside the scope of this study area (Joshi *et al.* 2013) observed high mean heterozygosity (*Ho* = 0.54, *He* = 0.81) and allelic diversity (11.71) levels. A range-wide study (Mondol *et al.* 2009a) conducted at five microsatellite loci showed that Indian tigers have higher heterozygosity (*Ho* = 0.70 ± 0.16) and allelic diversity (12.4 ± 3.6) compared to all other subspecies (*Ho* = 0.53 ± 0.07, mean number of alleles = 7.2 ± 1.2). Their study also reported low insignificant genetic structuring of the Central Indian tigers with the Northern (F_{ST} = 0.027, p=0.063) and Southern Indian populations (F_{ST} = 0.019, p=0.054). They attributed this to a historically large effective population size and inter-population connectivity in the region of Central and Peninsular India, explaining why despite centuries of immense trophy hunting and continued habitat fragmentation, extant tiger populations in the region currently retain close to 60% of the global genetic variation in the species (Mondol *etal.* 2009a).

As shown by the above findings, the high genetic diversity present in Indian tigers which is also witnessed among the contemporary populations in my study area, can be attributed to high ancestral effective population sizes due to historically abundant tiger numbers, population differentiation or past fragmentation in varied habitats and high rates of gene flow between regional tiger populations till *c.* two centuries ago (Mondol *et al.* 2009a). As a species, the tiger's evolutionary history is intricately linked with the massive climatic and habitat fluctuations of the Pleistocene, and the high genetic variation in the species likely arose from evolutionary and genetic adaptations which permitted colonization of diverse ecosystems in the region (Kitchener and Dugmore 2000). The most productive habitats which include the interspersion and inter-digitation of mixed forest types, forest edge, and grassland, abound in Central India and the Himalayan foothills. Here, the assemblage of ungulate prey reach their highest densities which in turn support high tiger numbers and permit small home range sizes compared to other habitats (Sunquist *et al.* 1999). The ensuing combination of high fecundity, good tiger densities, long-range dispersal capability and good habitat connectivity would have

facilitated high rates of gene flow between differentiated populations, thus contributing to a diverse gene pool in the Indian population (Kitchener and Dugmore 2000; Luo *et al.* 2004). Some measure of this historically high genetic variation is evidenced in the detection of unique genetic variants from museum samples (Mondol *et al.* 2013) and captive tigers (Luo *et al.* 2008; Sharma *et al.* 2008). These unique haplotypes and alleles are no longer detected in extant populations, either due to loss or sampling related (Sharma *et al.* 2008; Mondol *et al.* 2013).

The historic decline in tiger populations in the sub-continent started about 600 years ago during the Mughal period in the 14th and 15th centuries. This opened up vast tracts of Central Indian lowland forests and increased hunting of tigers and wildlife (Rangarajan 2001). Thereafter, tiger populations underwent accelerated decline, reaching their lowest ebb during the British colonial period in the 18th and 19th centuries (which continued till the early-mid 20th century), from commercial forestry and large-scale bounty hunting by the ruling classes (Rangarajan 2001). This juncture in the Indian tigers' history is imprinted with a massive reduction in effective population size and pronounced bottlenecking signature (Mondol *et al.* 2009a). Historical hunting records from the area reveal that upwards of 1,000 tigers and 2,000 leopards were killed from a single province, within just a few decades (Rangarajan 2001). Mondol *et al.* (2009a) estimated pre-decline tiger numbers in Peninsular India (comprising Central and Southern India) to be nearly 58,000 tigers. The actual numbers could potentially be much higher as hunted individuals far exceeded this number, with records of over 80,000 tigers being killed between 1875 and 1925 (Rangrajan 2001). Substantial population declines in the region continued well into the 1960s and 1970s due to unorganized large-scale hunting which nearly extirpated the species (Gee 1964). In recent decades, habitat fragmentation and relentless poaching since the early 1990s have extirpated tigers in adjacent reserves of Sariska and Panna (Gopal *et al.* 2010) and reduced small populations such as Achanakmar to only a handful of individuals (Jhala *et al.* 2011b).

By using Bayesian approaches, Mondol *et al.* (2009a) estimated a massive demographic decline of about 98% tiger numbers during the last 150 to 200 years in peninsular India.

However, my study and a recent work by Sharma *et al.* (2012) did not find significant genetic signature of past demographic collapse. Peery *et al.* (2012) found that such results are not unique, as several studies on well-documented population declines have not been able to detect genetic bottlenecks. Examples of species of conservation concern where well-known demographic declines have reduced population numbers to only tens of individuals, yet have not resulted in detection of bottlenecks include Scandinavian lynx (*Lynx lynx*, Spong and Hellborg 2002), California sea otters (*Enhydra lutris nereis*, Aguilar *et al.* 2008) and Amur tigers (*Panthera tigris altaica*, Henry *et al.* 2009). Simulation analyses showed that in many cases a demographic decline did not result in a detectable bottleneck in vertebrate populations because of the small number of samples and loci used in the analysis, and due to the sensitivity of the summary statistics to the assumed mutation models (Girod *et al.* 2011; Peery *et al.* 2012). Bottleneck detection is further compounded by several factors such as timing and duration of the population decline, pre-bottleneck genetic diversity, generation time, immigration and gene flow between populations (Cornuet and Luikart 1996; Garza and Williamson 2001; Williamson-Natesan 2005; Peery *et al.* 2012).

Compared to the threshold M ratio of 0.68 for bottlenecked populations (Garza and Williamson 2001), most tiger populations in this study had significantly higher M ratios (0.7 to 0.9) and showed no heterozygosity excess or mode-shift in allele frequencies. Only Bandhavgarh had a marginally lower ratio (M = 0.66) suggestive of genetic bottleneck. Simulations have shown a lag of about twenty post-bottleneck generations before the M ratio tests can detect a bottleneck, while the heterozygosity excess test was more suited to detecting bottlenecks within the first ten generations only (Peery *et al.* 2012). Tests of heterozygosity excess are expected to regain mutation-drift-equilibrium more rapidly than M ratios, as the latter rely on allele size variances and may not necessarily be influenced by new alleles (Garza and Williamson 2001; Williamson-Natesan 2005). While it is difficult to assign a time for bottleneck based on the summary statistics used here, the simulation results may imply that the Bandhavgarh population likely carry the signature of a bottlenecking episode more than ten to twenty generations ago. Non-observance of genetic bottleneck in the other populations is likely due to the

presence of gene flow in these areas (see gene flow section in Chapter 5) which can bias allelic distributions (Cornuet and Luikart 1996).

Non-detection of bottlenecks can also be interpreted in light of significant heterozygosity deficit (symptomatic of recent population expansion) detected in Pench and Kanha, and the entire Central Indian population (Table 3.6). Tests for heterozygosity excess or deficiency are suited for detecting recent population fluctuations within the last ten generations only (Peery *et al.* 2012). The signature of population expansion is congruent with the demographic increase of tigers in the area which recovered in the 1970s and 1980s (Panwar 1987), a period of about six to eight generations ago considering a generation time of five years in tigers (Smith and McDougal 1991). These two populations harbour the largest amount of genetic variation and are major sources of migrant individuals in the landscape (Table 3.4). The dual processes of population expansion and gene flow which are observed in these areas (see gene flow results in Chapter 5) could inadvertently have obscured the detection of population bottlenecks as the addition of new individuals could increase the number of alleles in a population, substantially biasing allelic and heterozygosity distributions (Cornuet and Luikart 1996).

CHAPTER 4

GENETIC STRUCTURE OF TIGER POPULATIONS IN CENTRAL INDIA

CHAPTER 4

GENETIC STRUCTURE OF TIGER POPULATIONS IN CENTRAL INDIA

4.1. INTRODUCTION

The assessment of genetic structure and gene flow among tiger populations in the Central Indian landscape is important as the entire area is heavily fragmented with possible metapopulations across many tiger habitats, protected areas and forest land. Understanding the genetic structure of populations is necessary for assessing metapopulation function and gene flow at a landscape or regional scale (Hanski and Gilpin 1997) and identifying units relevant for conservation (Moritz 1994; Moritz *et al.* 1995; Crandall *et al.* 2000). This chapter summarizes work related to investigating patterns of spatial genetic structuring among tiger source populations in the area.

Habitat fragmentation is the most serious threat to biological diversity, and is the root cause of most extinction events (Wilcox and Murphy 1985). Rapid fragmentation of natural ecosystems and wild populations is a result of ubiquitous human growth. The process of habitat fragmentation invariably leads to reduction in the total area and division of large contiguous natural landscape into several smaller discrete patches of natural ecosystems which are isolated from one another by human-dominated habitats (Saunders *et al.* 1991). This has resulted in range contraction as many species that historically were nearly continuously distributed across broad geographic areas are now confined to increasingly smaller patches of habitat (Young and Clarke 2000). Range contraction in turn has led to overall reductions in population size, reducing genetic diversity and dispersal (gene flow) among habitat patches, thus resulting in loss of evolutionary potential in countless species (MacArthur and Wilson 1967; Purvis *et al.* 2000; Young and Clarke 2000).

The underlying forces explaining the nature and effects of habitat fragmentation have strong theoretical foundation in a set of intriguing ideas and observations known as island

biogeography (MacArthur and Wilson 1967). The theory assumes that fragmentation of once contiguous natural ecosystems into ever smaller parcels of natural habitat have created fragmented habitat "islands" within a sea of inhospitable habitats modified by heavy human land-use activities, which is akin to the effects of isolation on the biota of oceanic islands. There are limitations in the applicability of the island model in understanding fragmentation, as terrestrial habitat "islands" are not as isolated as oceanic islands (Haila 2002). Nevertheless the essential ideas of island biogeography theory have provided a conceptual basis for understanding habitat fragmentation issues. The fundamental idea is that the number of species on an island (or habitat fragment) represents a balance between immigration and extinction. Small fragments (or islands) will have fewer species, due to higher extinction rates, compared to large fragments, and more isolated fragments will have lower immigration compared to less isolated habitats. Small and isolated populations which occur in fragmented ecosystems and with limited to no dispersal between local subpopulations, face heightened risk of extinction due to mortality from deterministic demographic (Lande 1988; Woodroffe and Ginsberg 1998), and stochastic genetic processes (Frankham *et al.* 2002). Hence, maintaining connectivity in fragmented ecosystems is important for long-term viability of species.

The genetic effects of population fragmentation may vary in severity depending on the extent of resulting demographic and genetic structuring, and pattern of gene flow among habitat fragments. Habitat and population fragmentation through mainly human intervention have created a network of patchily connected multiple local populations or metapopulations (Levins 1970; Hanski and Gilpin 1997). Local populations in the metapopulation differ in size and habitat quality, and have varying potential for long-term viability. Some local populations may go through local extirpations and then be recolonized by migrants from other source populations (Hanski and Gilpin 1991). These events will have complex and subtle effects on the genetic population structure and evolution of species. While totally isolated population fragments will have no opportunity for gene flow, local populations in the metapopulation which share connectivity with other source populations can experience occasional migration events. Though fragmented local populations will have lower and variable probability of persisting, population

connectivity maintained through gene flow by dispersing individuals from high density source populations to low density sink habitats will increase the long-term persistence of the overall metapopulation (Hanski and Gilpin 1991).

In order to understand metapopulation function and gene flow in fragmented habitats, it is important to investigate genetic structuring and identify population units for conservation. Detection of population units is necessary for testing hypothesis to understand historical biogeography (Small *et al.* 2003), demographic connectivity (Proctor *et al*. 2005), identifying conservation units (Mortiz 1994; Moritz *et al.* 1995; Crandall *et al*. 2000), and assessing metapopulation structure and function (Hanski and Gilpin 1997). Ecological studies on long-term abundance monitoring and investigation of survival and birth rates in the local population can benefit from knowledge pertaining to the spatial and genetic boundaries of population units (Lowe and Allendorf 2010). In addition, biologists and managers must be able to effectively identify population units and geographic boundaries between populations, to prioritize conservation among population units, because scarce financial resources preclude conservation of all units.

However, defining distinct population or management entities within species is far more vexing and controversial than defining species. The parameters which define management entities may not conform to a genetic perspective. This is largely because Management Units (MUs) only consider populations that are demographically independent and discrete; that is their population dynamics are governed by local birth and death rates rather than by immigration (Moritz 1994). As such, MUs usually represent subpopulations within a metapopulation that are important for the long-term persistence of the metapopulation and the species. MUs generally do not show long-term independent evolution or adaptive differentiation. On the other hand, the genetic histories of populations are scripted by both long-term and short-term forces. Genetic population size and structure is a result of much older evolutionary histories, but is also impacted by recent demographic changes such as migration (Wright 1951). The concept of Evolutionarily Significant Units (ESUs) was invoked in this context to designate populations (for separate management) which show significantly divergent allele

frequencies at nuclear or mtDNA loci (Moritz 1994; Moritz *et al.* 1995). However, a major criticism of this approach is that genetically defined ESUs ignore adaptive differences (Crandall *et al.* 2000). ESUs are unlikely to be detected in species with high gene flow, even with adaptive differences. Conversely, small bottlenecked populations which have low gene flow and require management intervention to increase genetic exchange could be falsely identified as ESUs though in reality the populations are not evolutionarily distinct. Crandall *et al.* (2000) have suggested that ESU and MU designation should be considered on the basis of both long-term reproductive isolation and adaptive differentiation among populations, and concordance across many different data types (e.g. genetic, morphological, behavioural, life history, and geographic). Knowledge of the genetic effects of habitat fragmentation is vital in prioritizing of population units for management.

Two common methods are used to delineate population boundaries in mammals, radio-telemetry and population genetics. Practical considerations limit telemetry studies at a large spatial scale as the effort required is extensive and long-term (Bethke *et al.* 1996; Taylor *et al.* 2001). Population genetics while offering a rapid insight into population structuring and landscape level processes affecting genetic structuring and gene flow between populations require *a priori* definitions of populations. Also traditional estimators such as F_{ST} have equilibrium assumptions of gene flow and drift which do not reflect the ecological reality of fragmented landscapes. Recent developments of individual based clustering techniques which use genotypic data to assign and cluster groups without *a priori* assumptions of population membership (Pritchard *et al.* 2000) have proven useful at investigating genetic structuring in fragmented ecosystems (Proctor *et al.* 2005; Bergl and Vigilant 2007).

Here, I used a Bayesian model based clustering technique (Pritchard *et al.* 2000) to delineate groups of individuals in clusters that may form the basis for a population using information obtained from multilocus genotypic data. The Central Indian landscape where my study is based contains highly fragmented and patchy tiger habitats. My objective was to quantify the extent of genetic structuring in the area as a consequence of

population fragmentation in the landscape. I hypothesized that populations which were connected by relatively intact forested habitats could still be exchanging migrants and thus have lower genetic divergence, compared to populations where connectivity was broken by agriculture and high density human settlements. To meet my objectives I employed individual clustering approaches which used information from multilocus microsatellite genotypic data to group individuals to genetic clusters (Pritchard *et al.* 2000). This test was suited for quantifying recent genetic structuring as it was free from the equilibrium assumptions associated in measures of classical pairwise genetic differentiation (Whitlock and McCauley 1999).

Determining population or management units for tigers in the Central Indian landscape is important for conservation, as the source populations are located in areas where further range contraction from habitat loss, and demographic fragmentation from poaching, is ongoing and a major threat to long-term survival (Jhala *et al.* 2011b; Fernandes 2012). Land-use policy and implementation would benefit from knowing population units for site specific conservation action and strategies for mitigating conflict with humans. Monitoring future trends in abundance, distribution and connectivity of vulnerable small habitats will allow adaptive management solutions for persistence of metapopulation structure if it exists.

4.2. METHODS

4.2.1. Sampling and laboratory work

A total of 169 individuals, which were genotyped at 11 microsatellite loci and collected throughout tiger reserves in the Central Indian landscape, were used for analysis. Details of sampling locations, DNA extraction, PCR and individual identification are described in Chapter 3 (*ibid*).

4.2.2. Data analyses

In order to detect patterns of population genetic structure in the area, I used two types of individual-based analyses, which do not assume hierarchical genetic structure in the data set or require *a priori* assumptions about population structure. First, I conducted an exploratory analysis of the data set through multidimensional representation of individual genotypes to detect broad patterns of spatial partitioning among populations (Peakall and Smouse 2006). Second, I used a rigorous Bayesian-based individual clustering method to identify populations by delineating genetically similarly clusters of individuals (Pritchard *et al.* 2000). Further, I compared the results of these two individual based analyses with traditional descriptive analyses based on F_{ST} (Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) statistics.

4.2.2.1. Multidimensional ordination of individuals

For exploratory data analysis to understand the populations relationships among individuals, I employed the multivariate statistical technique of principal coordinate analysis (PCoA), which allows a multidimensional representation of the major patterns of variation within a multivariate data set (e.g. multiple microsatellite loci with many alleles and many samples). In essence, PCoA is a procedure by which the major axes of variation are located within a multidimensional data set. Each successive axis explains proportionately less of the total variation, such that when there are distinct groups, the first two or three axes typically reveal most of the separation among them. PCoA was used to visualize the variability in the population data set using two-dimensional and three-dimensional plots constructed from a matrix of individual pair-wise genetic distances (Phi_{PT}, Excoffier *et al.* 1992). I used GENALEX 6.3 (Peakall and Smouse 2006) to carry out PCoA and the scatter of population-wise individual assignments was plotted on the first three PCo axes using NCSS 9 (www.ncss.com).

4.2.2.2. Bayesian clustering of individuals

Next, I used the Bayesian individual clustering approach in STRUCTURE 2.3.3 (Pritchard *et al.* 2000; Pritchard *et al.* 2009) to detect population structure among sampled localities in the area by assigning sampled individuals into a number of clusters (*K*) based on the multilocus genotype data alone. Individuals are given membership coefficients (*Q*) for each cluster so that the estimated membership coefficients of an individual sum to 1 across the *K* clusters. The clustering process ensures deviations from Hardy Weinberg and linkage equilibrium are minimized. Bayesian simulation methodology is based on a stochastic process known as a Markov Chain Monte Carlo (MCMC) to randomly generate probability distributions that are often difficult or impossible to obtain from standard analytical or likelihood equations (Beaumont and Rannala 2004). In Bayesian analysis, it is important for the MCMC chains to converge to allow the simulation algorithm to randomly sample the probability space. Convergence is typically achieved by discarding the first few thousands of MCMC steps called the 'burn-in' phase so that the starting parameters do not influence (bias) the final outcome.

I analyzed my data in STRUCTURE by using the admixed model and correlated allele frequencies option to carry out thirty independent simulations at each assumed population cluster (*K* = 1 to 10), with a burn-in length of 1 million MCMC steps and data collection phase of 5 million MCMC iterations. These run times, chosen after extensive exploratory runs, were sufficient to ensure convergence of the Markov chains. All runs were carried out both with (locprior=1) and without (locprior=0) using prior sampling locality information to assist the clustering procedure. The admixed model was chosen as most natural populations have some degree of admixture (Pritchard *et al.* 2009). I also assumed that the investigated tiger populations in the area had correlated allele frequencies as they historically belonged to one continuous population. The true *K* or most likely number of population clusters in the dataset was inferred from (i) the *ad hoc* parameter of log-likelihood change in probability of individual assignments to *K* clusters (*Ln P(K)*, Pritchard *et al.* 2000), and (ii) the second order rate of change in the likelihood of *K* values (*delta K*, Evanno *et al.* 2005). Both these values were computed from the

STRUCTURE output using the online resource STRUCTURE HARVESTER v0.6.91 (Earl and vonHoldt 2009). Individual *K* cluster membership coefficients (*Q*) obtained across different *K* runs were plotted using *distruct* (Rosenberg 2004) after matching the STRUCTURE outputted individual *Q* matrices in CLUMPP (Jakobsson and Rosenberg 2007).

4.2.2.3. Descriptive statistics of population subdivision

An Analysis of Molecular Variance (AMOVA, Excoffier *et al.* 1992) was carried out in GENALEX 6.3 (Peakall and Smouse 2006) to analyze genetic variation between the population clusters identified by the STRUCTURE analysis. Genetic variances were partitioned at two levels, viz. among all the STRUCTURE identified subpopulation groups and among populations within each group. Three different pairwise population estimators used for co-dominant microsatellite genetic distances, and which were implemented in the program GENALEX, were used in the analysis. I also estimated population pairwise F_{ST} (Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) statistics, and significance was calculated using 10,000 permutations in GENALEX. The performance of these estimators depends on the applicability of the underlying analytical models, and other factors such as microsatellite mutation rates (Balloux and Lugon-Moulin 2002). I used both estimators as it was not clear which of these estimators reflects the genetic structure of our genotype data.

4.2.2.4. Isolation-by-distance

As patterns of isolation-by-distance (IBD) are known to bias tests of hierarchical structuring and vice-versa (Meirmans 2012), I tested statistical correlation between genetic and geographical distances in a Mantel test (Mantel 1967). Pairwise F_{ST}, R_{ST} and Phi_{PT} genetic distance estimates, obtained from AMOVA analysis in GENALEX, were linearized using the formula $F_{ST}/(1 - F_{ST})$, as given by Rousset (1997). Matrices of linearized pairwise genetic distances were correlated against matrices representing inter-population geographic distances (GGD) and log-transformed geographic distances ($\log_{10}$

GGD). I also used the STRUCTURE identified population clusters as a covariate in a partial Mantel test (Smouse *et al.* 1986), to model the partial correlations between pairwise genetic distances and spatial distances, while controlling the effect of population clusters (following Meirmans 2012). Partial correlations between matrices representing pairwise genetic distances and spatial distance matrices were calculated with a third matrix describing whether population comparisons were made between (1) or within (0) the STRUCTURE identified clusters. A non-significant or negative partial correlation of genetic with geographic distance, after controlling for population clusters, would indicate that the observed genetic structure is not due to underlying IBD patterns. Mantel and partial Mantel tests were carried out with 10,000 randomizations in ZT (Bonnet and Van de Peer 2002) to evaluate the significance of the correlations.

4.3. RESULTS

4.3.1. Multidimensional ordination of individuals

According to the results of the PCoA based on Phi_{PT} genetic distance, individuals in the area were clustered into roughly four groups with varying degrees of population partitioning (Figures 4.1 and 4.2). The first three PC axes accounted for 60.2% variation of the overall variation in the dataset (Table 4.1). Tigers were observed to cluster in four major groups. Tigers from Kanha, Pench and Melghat formed three distinct clusters that partially overlapped each other, while Bandhavgarh tigers formed a discrete cluster with minimal overlap. Tigers from Satpura, Tadoba, and Achanakmar were scattered within the clusters formed by Kanha, Pench and Melghat.

Table 4.1. Principal coordinate (PCo) loadings of covariates relevant for inferring population structure, Eigen values of the coordinates, and the percent variation of the original data explained by the first six PCo axes.

	PCo1	PCo2	PCo3	PCo4	PCo5	PCo6
EigenValue	11.77	8.37	6.86	6.24	5.97	5.63
individual %	26.26	18.66	15.30	13.91	13.32	12.56
cumulative %	26.26	44.92	60.21	74.12	87.44	100.00

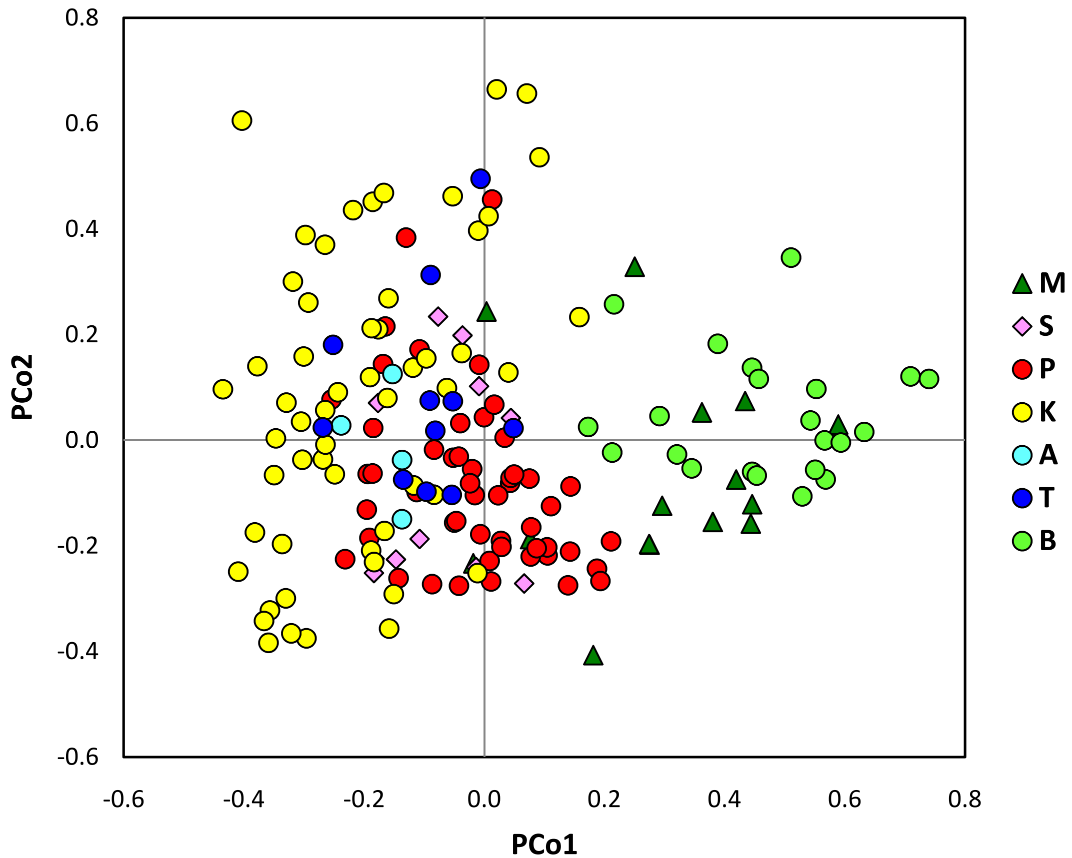

Figure 4.1. Two dimensional plot showing population relationships on the two most informative coordinate axes, which cumulatively account for ~ 45 % of the total variation in the data. Sampled populations are Achanakmar (A), Bandhavgarh (B), Kanha (K), Melghat (M), Pench (P), Satpura (S) and Tadoba (T).

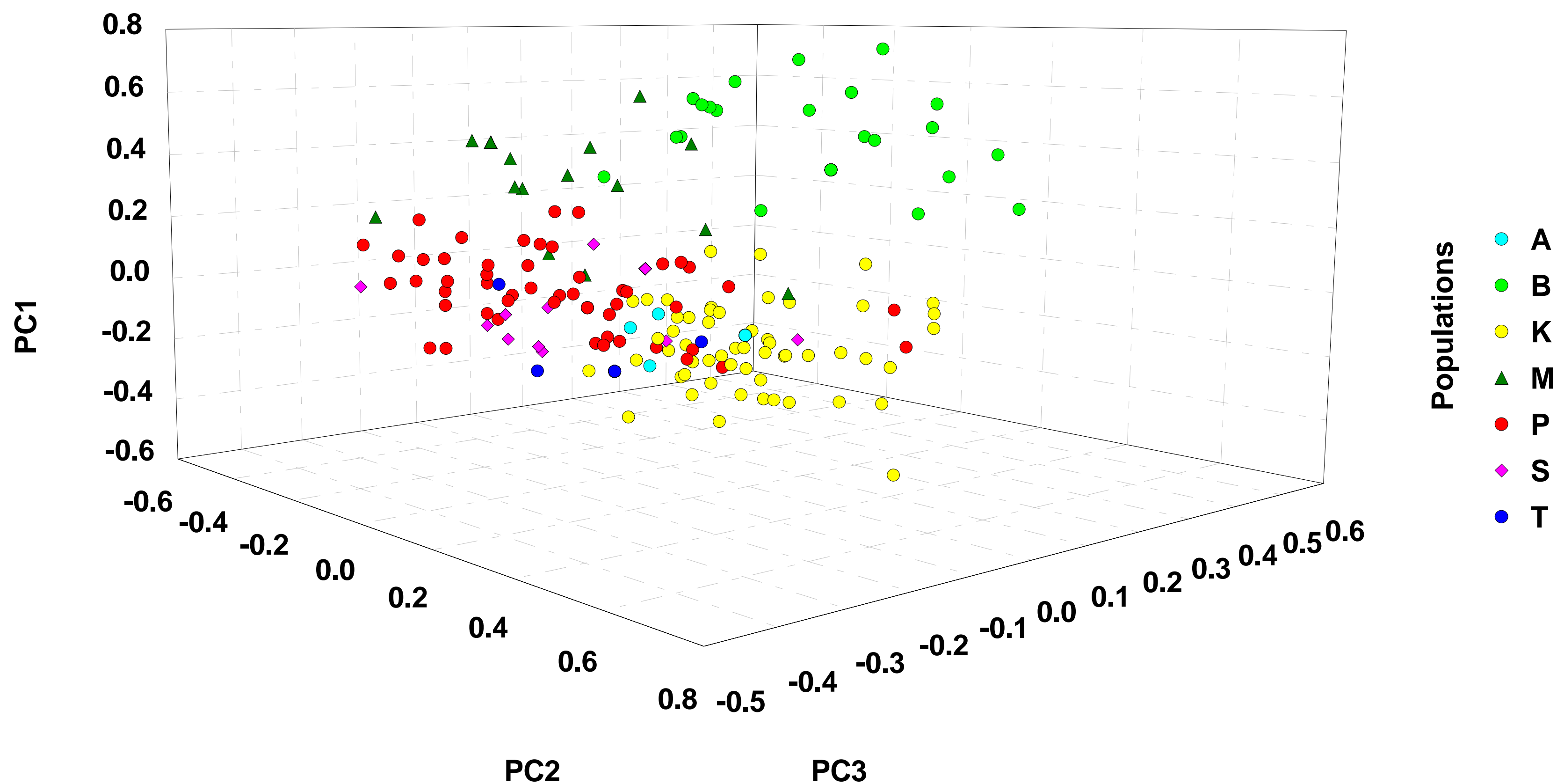

Figure 4.2. Three dimensional PCoA plot showing partitioning between different populations based on Phi_{PT} co-dominant genetic distance among individuals. The three coordinate axes collectively represent ~60 % of the total variation in the data. Sampled populations are Achanakmar (A), Bandhavgarh (B), Kanha (K), Melghat (M), Pench (P), Satpura (S) and Tadoba (T).

4.3.2. Bayesian clustering of individuals

Four clusters were obtained in the STRUCTURE analysis (Figures 4.3 and 4.4). Calculation of delta K from the output of the STRUCTURE runs using prior population information (locprior = 1), produced the largest modal value of the statistic at $K = 4$, suggesting pronounced population subdivision at $K = 4$ (Figure 4.3.a). On the other hand, the log-likelihood *Ln P(K)* value reached an inflection point at $K = 4$ before plateauing at $K = 6$ to 7 and leveling off at $K = 8$ (Figure 4.3.a). The variance in *Ln P(K)* increased at higher values of K, as reported previously with other studies (Rosenberg *et al.* 2001, Evanno *et al.* 2005). This disparity in population structuring patterns between delta K and log-likelihood values occasionally occurs in cases where the F_{ST} values are significant but STRUCTURE results are often inconsistent (Pritchard *et al.* 2009). Examination of individual Q summary barplots (Figure 4.3.b) yielded identical clustering patterns at all runs between assumed $K = 2$ to 4 (carried out both with and without prior sampling location information), and distinct population saturation, indicative of population subdivision was evident at $K = 4$ in conformance with the delta K approach. I therefore chose the four cluster solution ($K = 4$) as it best describes the levels of genetic subdivision in our sample of the Central Indian tiger population (Figure 4.4).

The four STRUCTURE identified population clusters (Figure 4.4) were largely in accord with the pattern of relationships suggested by the multidimensional PCoA plot (Figures 4.1 and 4.2), and the geography of the study area. Melghat represented a unique cluster in the westernmost periphery of the landscape, with a few individuals cross-assigned to the Pench cluster. Though individuals from Satpura and Tadoba showed mixed memberships to Pench and Kanha respectively, both populations constituted an allied cluster with the Melghat population in the STRUCTURE analysis. Pench formed a single large cluster in the west-central section. The tiger populations in Kanha, Achanakmar and the forested corridor between Pench and Kanha comprised the third cluster in the central and eastern sections of the landscape. Bandhavgarh, in the north-eastern part of the landscape, formed a distinct isolated population with all individuals assigned to their sampled locality.

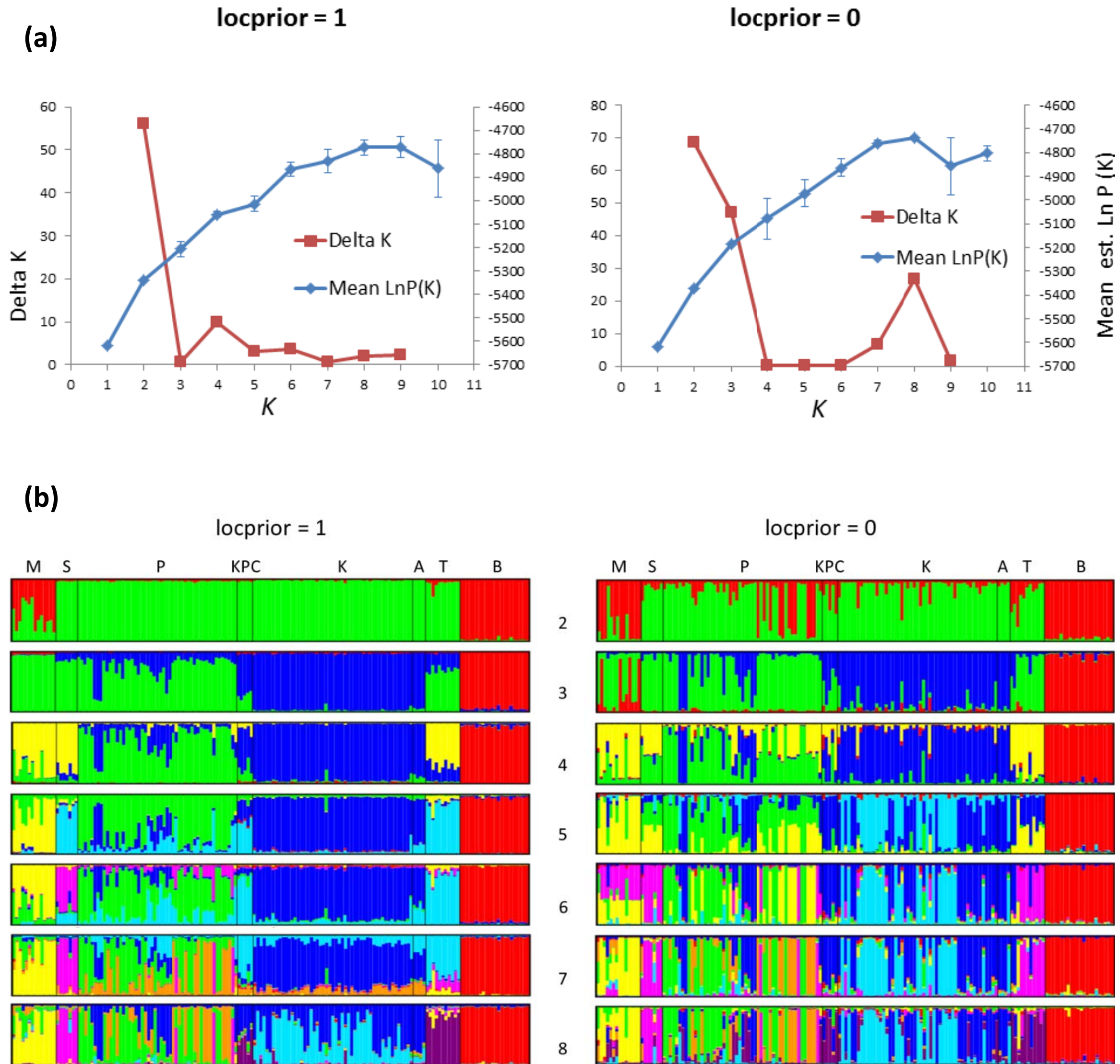

Figure 4.3. (a). STRUCTURE results showing the difference in delta *K* and mean *Ln P(K)* for an estimated number of *K* populations, in models run with (locprior = 1) and without (locprior = 0) prior sampling location information. **(b).** Summary barplots depicting results of sequential STRUCTURE runs (assumed *K* = 2 to 8), of sampled populations in central India clustered according to individual *Q* values. Cluster saturation at *K* = 4, indicative of four population clusters, is observed in runs carried out both with and without *apriori* location information. At *K* > 4, increased sub-structuring is detected, but there is no concordance in clustering between the prior (locprior = 1) and non-prior (locprior = 0) runs. Sampled localities are Melghat (M), Satpura (S), Pench (P), Kanha-Pench corridor (KPC), Kanha (K), Achanakmar (A), Tadoba (T) and Bandhavgarh (B).

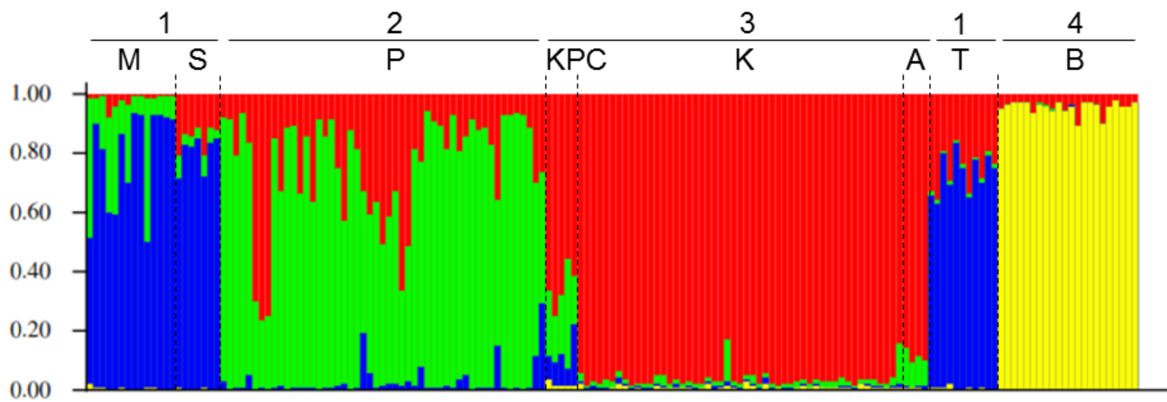

Figure 4.4. Summary of STRUCTURE Bayesian clustering run at K = 4 showing population assignments for each individual. Individuals are represented across the x-axis by a vertical line that may be divided into shaded segments that represent the individual's probability of originating (Q) from each of the four population clusters. Sampled populations are Melghat (M), Satpura (S), Pench (P), Kanha-Pench corridor (KPC), Kanha (K), Achanakmar (A), Tadoba (T) and Bandhavgarh (B).

4.3.3. Descriptive statistics of population subdivision

The AMOVA results indicated low but significant differentiation ($p < 0.01$) between the STRUCTURE identified population groupings, though variation between subpopulations within groups accounted for much of the genetic structuring observed in the area (Table 4.2). The major portion of genetic variance was found within populations (88%) with 7% among the population clusters and 5% among populations within clusters. Exact tests showed significant genetic variance on all three levels ($p < 0.01$). Both F_{ST} and Phi_{PT} values showed highly significant structuring ($p \leq 0.001$), and had relatively similar trends in magnitude with low sampling variance. In contrast, R_{ST} estimates showed no variation between groups, and had unreliably high sampling variances and mean square error estimates. Pair-wise F_{ST} and R_{ST} values indicated significant ($p < 0.05$) and varied (F_{ST} 0.049 to 0.241; R_{ST} 0.000 to 0.330) genetic structuring between all sampled populations in Central India (Table 4.3). The pattern of pairwise F_{ST} values was similar to the cluster patterns suggested by the STRUCTURE analysis. Within cluster F_{ST} estimates were mostly lower, ranging from moderately low (0.048 to 0.062) to high (0.079 to 0.102), compared to pairwise estimates between different clusters (0.127 to 0.217). In general, F_{ST} estimates were significantly higher and indicative of more pronounced structuring than R_{ST}.

Table 4.2. AMOVA results showing estimates of differentiation based on various genetic distance estimators.

Estimator	Source of variance	d.f.	SS	MS	Est. Var.	%	
	Among regions	5	146.4	29.3	0.3	7	
$\boldsymbol{F_{ST}}$	Among populations	1	7.2	7.2	0.2	5	F_{ST}= 0.115 (p=0.001)
	Within populations	331	1290.6	3.9	3.9	88	
	Among regions	5	995877.2	199175.4	0.0	0	
$\boldsymbol{R_{ST}}$	Among populations	1	82528.4	82528.4	3871.4	14	R_{ST}= 0.126 (p=0.001)
	Within populations	331	8203980.2	24785.4	24785.4	86	
	Among regions	5	292.9	58.6	1.2	11	
$\boldsymbol{Phi_{PT}}$	Among populations	1	14.4	14.4	0.7	8	Phi_{PT}= 0.191 (p=0.001)
	Within populations	162	1367.1	8.4	8.4	81	

d.f. - degrees of freedom; SS – sum of squares; MS – mean sum of squares; Est. Var. – estimated genetic variability; % - percentage variability

Table 4.3. Population pairwise F_{ST} (below diagonal) and R_{ST} (above diagonal) estimate of differentiation for Melghat (M), Satpura (S), Pench (P), Kanha (K), Achanakmar (A), Tadoba (T) and Bandhavgarh (B). *$p < 0.05$, **$p < 0.01$

	M	**S**	**P**	**K**	**A**	**T**	**B**
M	_	0.000	0.030	0.063**	0.000	0.171**	0.081**
S	0.166**	_	0.043*	0.049*	0.000	0.141**	0.148**
P	0.092**	0.110**	_	0.091**	0.001	0.257**	0.218**
K	0.136**	0.117**	0.055**	_	0.084*	0.164**	0.183**
A	0.180**	0.172**	0.049*	0.051**	_	0.150**	0.078
T	0.132**	0.134**	0.101**	0.102**	0.105**	_	0.330**
B	0.138**	0.241**	0.141**	0.168**	0.203**	0.226**	_

4.3.4. Isolation-by-distance

Mantel's r correlations between pairwise genetic distances and geographic distance metrics were non-significant except for significant relationship with linearized F_{ST} values (Table 4.4). Correlations in partial Mantel tests showed non-significant relationships across all three genetic distance estimators after controlling the effect of STRUCTURE identified population clusters. Though significant positive correlation was observed between F_{ST} and geographic distances in the standard Mantel tests, genetic and geographic distances were uncorrelated in the partial Mantel tests, after controlling the effect of population clusters, thus affirming that an isolation-by-distance pattern was not present in the data.

Table 4.4. Results of standard and partial Mantel tests for correlation between pairwise genetic and geographic distance metrics. The correlation coefficient (r)and probability (p) are shown using three different genetic distance estimators. Significant values (p < 0.05) are indicated by an asterisk (*). GGD-geographic distance, Log10 GGD – log transformed geographic distance, cluster – STRUCTURE identified population clusters.

			$F_{ST}/(1\text{-}F_{ST})$		$Phi_{PT}/(1\text{-}Phi_{PT})$		$R_{ST}/(1\text{-}R_{ST})$	
Matrix A	**Matrix B**	**Covariate**	***r***	***p***	***r***	***p***	***r***	***p***
Standard Mantel test								
Genetic	GGD		0.433	0.039*	0.335	0.098	0.125	0.314
Genetic	Log10 GGD		0.424	0.036*	0.316	0.121	0.164	0.244
Partial Mantel test								
Genetic	GGD	cluster	0.374	0.051	0.272	0.128	0.078	0.369
Genetic	Log10 GGD	cluster	0.361	0.053	0.252	0.145	0.117	0.331
Genetic	Log10 GGD	GGD	0.019	0.487	-0.029	0.446	0.170	0.257

4.4. DISCUSSION

By using a combination of classical population differentiation and individual based approaches, I was able to detect patterns of significant population sub-structuring in the region. Results from both the PCoA multidimensional plot and the model based individual clustering analysis in STRUCTURE (locprior = 1) suggest the presence of four genetic clusters in the area. Significant genetic structuring was also detected as seen in the population pairwise $F_{ST}$and R_{ST} values, which appeared to have biased parameter estimations in the STRUCTURE runs carried out without prior population information (locprior = 0) and resulting in higher hierarchical level of population subdivision at K = 8. In contrast, the runs carried out with population information (locprior = 1, K = 4) did not detect unnecessary genetic structuring, ignored the prior sampling location information if the ancestry of individuals was uncorrelated (Pritchard *et al.* 2009). Despite observing multiple delta K values, and weak evidence of population subdivision at K = 8 in the run carried out without prior information (locprior = 0), the cluster solution of K = 4 appeared optimal because of the following reasons. In cases where STRUCTURE detects multiple clustering options with similar probabilities, typically the least value of K which captures much of the biological complexity in the sample is usually correct (Pritchard *et al.* 2009). Additionally, the presence of related individuals in our sample and the model of correlated allele frequencies used for analysis can lead to overestimation of the true value of K (Pritchard *et al.* 2009). I therefore chose the four cluster solution as it represents a conservative estimate of the amount of population genetic structuring in the area.

Support for the four STRUCTURE identified clusters was not strong in AMOVA, as the major portion of genetic variance was attributed to within population variation. Though the AMOVA results and the F_{ST} statistics indicated significant pairwise structuring across all populations in the area, the pattern obtained was similar to the individual clusters identified by STRUCTURE and the ordination results in PCoA. F_{ST} values were generally lower for localities within the same cluster compared to pairwise estimates between different clusters. I therefore treated the genetic distance estimators including

F_{ST} and its analogues as relative measures of population differentiation. The estimation of these parameters requires prior identification of populations and unless the population units are clearly known, such *a priori* designation may not reflect realistic biological patterns as they would only be representing *ad hoc* division of populations (Pearse and Crandall 2004). Importantly, the assumptions of demographic and genetic equilibrium along with long time scales under which F_{ST} and its analogues are based may not be suited for estimating genetic differences between populations which have undergone fragmentation or demographic fluctuation events only recently. On the other hand, the results of the model based clustering approach which partitioned individuals into relatively distinct clusters based on iterative assignments, made much more sense of the biological realities of the area as the localities observed here are likely artifacts of recent population fragmentation.

Additionally, the differences observed between F_{ST} and R_{ST} values can provide valuable insights into the balance between genetic drift and mutation events in the studied populations. In this study, pairwise genetic differences between tiger populations in the landscape showed higher F_{ST} values compared to R_{ST} values. The F_{ST} statistic is based on identity of alleles by descent. It thus accounts for gene flow between populations as the basic premise under which it estimates pairwise genetic differences (Hardy *et al.* 2003). In contrast, R_{ST} relies on allele size and single stepwise mutations are the primary contributors of genetic variation for this statistic (Hardy *et al.* 2003). R_{ST} estimations produced non-significant results across many pairwise comparisons, and had higher sampling variances compared to $F_{ST.}$ This suggests that populations in the area were not long-isolated for mutations to cause genetic differences between populations and the primary cause of genetic structuring in the area is due to genetic drift. The apparent isolation-by-distance (IBD) pattern observed due to the significant correlation of F_{ST} (but not Phi_{PT}or R_{ST}) with geographic distance is an artifact of metapopulation structure and discrete population clusters present in the area (Allendorf *et al.* 2013). This observation was affirmed by non-significant partial correlations between genetic vs. geographic distances using the STRUCTURE identified population clusters as a control.Apart from

geography, genetic structure is also a result of population history, which is not accounted in IBD tests (Meirmans 2012).

The Bayesian based clustering algorithm in STRUCTURE detects population subdivision by using allele frequency differences and discontinuities in Hardy-Weinberg equilibrium among populations to guide the population assignment procedure (Pritchard *et al.* 2000). The ability to distinguish historic from more recent divisions, i.e. the chronology of fragmentation events is not well understood. Therefore, it is only through an understanding of the specific study are that interpretation is possible and should be done with caution. However, some insights into deep population divisions can be inferred from the results of the sequential K analysis (Figure 4.3.b). The first split that occurred in the data when only two populations were assumed (K = 2), effectively divided the Bandhavgarh population (and Melghat to some extent) from all other populations in the study area. The next higher level of population structuring at K = 3, isolated the Bandhavgarh population, and split the Kanha-Achanakmar localities from the large cluster composed of Melghat, Satpura, Pench and Tadoba. Further population split which occurred at assumed K = 4, separated the latter cluster into Melghat, Satpura and Tadoba into a unit cluster, from Pench. Though the sequential pattern of genetic structuring is difficult to correlate with the timeline of actual population fragmentation events, definitive patterns do emerge. The early divergence of Bandhavgarh from other localities at assumed K = 2 and 3, was repeatedly detected across successively higher values of assumed K = 4 to 8, which suggests that this split could be historically significant for Bandhavgarh. On the other hand, population divergence in the other localities seems more recent by comparison. Kanha-Achanakmar clustered distinctly from the other localities in the next split. This was followed by Pench, and the populations of Melghat-Satpura-Tadoba rounding up the final cluster. While there may be evidence of higher hierarchical structuring, e.g. in the pairwise F_{ST} comparisons and STRUCTURE non-prior runs, I limited my analyses of the highest hierarchical population division at K = 4, as corroborated by delta K = 4, and due to inconsistencies between prior and non-prior clustering results at higher values of assumed K (> 4).

The four population clusters identified in the landscape are composed of two large clusters and two smaller peripheral populations. The two central clusters are represented by the populations of Pench and Kanha-Achanakmar. These two population clusters along with the intervening corridors comprise the majority of tiger occupied habitat in the area. The two peripheral populations of Melghat and Bandhavgarh show greater fragmentation with respect to the two central clusters. Melghat-Satpura-Tadoba represented populations with patchy connectivity and formed a unique cluster in the western and southern limits of the landscape, with few individuals being cross-assigned to the Pench and Kanha clusters. To the east of Melghat and Satpura, Pench formed a unique cluster. A few individuals in Pench had cross-assignments with Kanha suggesting gene flow between these two population clusters substantiated by a functional habitat corridor between Pench and Kanha. The next cluster in the eastern part of the landscape was represented by tiger populations in Kanha and Achanakmar. Individuals from the forest corridor between Pench and Kanha were also assigned to this cluster, but most had mixed assignments to both populations indicating that this was not a distinct but rather an admixed population. The last cluster was represented by Bandhavgarh, in the northern part of the landscape, which formed a distinct isolated population where all individuals were assigned to the sampled locality. The overall pattern of genetic structuring observed in the landscape is concordant with existing habitat connectivity and indicative of the role of habitat fragmentation in partitioning allele frequencies between populations.

The results of my study imply that population subdivision and genetic structure across most localities in the area was strongly associated with habitat features that offer resistance to dispersal at different intensities such as agricultural land, high density human settlements and urban infrastructure and not only by geographical distance between populations. The tiger habitats in the region are patchy with some populations still having varying degrees of connectivity, while being conspicuously absent in others (Jhala *et al.* 2011b). The best patches of contiguous forested habitat are present in the corridor between Pench and Kanha, which extends eastward to Achanakmar. Likewise, the Satpura and Melghat populations are also connected through swathes of degraded forests, which are interspersed with agricultural land and medium density human

settlements. Connectivity between Pench and Satpura is fragile as parts of the linkage are disrupted by mining activities, and broken up in places by agriculture, habitations, major highways and railway lines. The population in Tadoba is linked with Kanha in a stepping stone connectivity through patchily distributed forests. Though the intervening matrix between Tadoba, Pench and Melghat is heavily human dominated, the populations are tenuously linked by degraded forest patches and tiger occupied habitats such as Bor Wildlife Sanctuary. Bandhavgarh has linkages with forest habitat and tiger reserves further east (Jhala *et al.* 2011b), but seems isolated from tiger populations in the study area by human settlements and agricultural land. Though Bandhavgarh is patchily connected to Achanakmar and subsequently to Kanha, no cross-assigned individuals or traces of residual gene flow were detected between these populations. It appears there could be some barriers or historical sinks in this population. However, the population density in Bandhavgarh (14 tigers/ 100 km^2, Jhala *et al.* 2011b) is high with tigers regularly dispersing out of the area and entering into conflict with people. It is therefore likely that movement barriers in the form of human-caused mortality are a primary contributor to reduced gene flow in this population.

Though results from the individual based analyses suggest subdivision at four genetic clusters for the Central Indian tiger populations, the significant F_{ST} structuring observed between all populations in the area could also be indicative of ongoing fine-scale genetic differentiation in the area. Existing patterns of population structuring are a result of past fragmentation effects and fragmentation is not a static process. The rate at which gene flow occurs in the area is small and likely to become ever weaker, due to continued habitat loss and burgeoning anthropogenic activity in the area. Many localities still retain marginal inter-population connectivity, as evidenced by the presence of individuals having immigrant ancestry and further substantiated by camera trapping and radio-telemetry. However, genetic isolation of almost all populations in the foreseeable future is likely if current patterns of habitat fragmentation persist. In the case of Bandhavgarh the extent of fragmentation appears to be so great that it may have already become genetically isolated for some time now from other populations located towards the south (Achanakmar and Kanha) and west (Satpura). Efforts should be made to revitalize the

corridor connecting Bandhavgarh with Achanakmar and subsequently to the gene pool of the main Central Indian landscape complex a combination of restorative ecology and legal instruments (see Chapters5 and 6).

One perspective for exploring the significance of biological boundaries in these tiger populations is to examine the potential existence of Evolutionarily Significant Units (ESUs, Moritz 1994) and Management Units (MUs, Moritz *et al.* 1995). ESUs have been defined as populations that have been isolated sufficiently long enough to have evolved mtDNA-based reciprocally monophyletic status, i.e. mtDNA lineages within each group are more related than between groups. This definition was criticized as too stringent (Paetkau *et al.* 1999) and more holistic definitions incorporating adaptive and molecular genetic data (Crandall *et al.* 2000) have been considered for ESU determination. Though it appears that the Bandhavgarh population may be genetically isolated from other localities, there is evidence of tigers moving out from this population (Jhala *et al.* 2011b), and presumably subject to retributive killing and poaching. While it may be isolated there is no compelling evidence to declare this population as an ESU; mtDNA haplotypes from Bandhavgarh are not unique (Mondol *et al.* 2009a) and there is no proof of adaptive traits for this population. In such a system, where the local population dynamics are dominated by birth and death rates and not by recruitment from other populations (Moritz *et al.* 1995), the criterion of Management Unit (MU) as a conservation status may be more appropriate. The Bandhavgarh population would easily qualify as a management unit at any rate, allele frequencies are extremely divergent, and it appears to have minimal movement between adjacent populations. For the rest of the tiger populations in the area, there is evidence of some exchange of genes between the population units identified from my analysis. Though these systems do not fit the classic MU model, it would be prudent to also consider these local populations that maintain metapopulation connectivity, as management units. This is because tigers are vulnerable to excessive human-caused mortality, and the mortality risks are much higher for dispersing individuals, especially crucial for maintaining long-term viability of fragmented local populations (Kenney *et al.* 1995).

CHAPTER 5

GENE FLOW AMONG TIGER POPULATIONS IN THE LANDSCAPE

CHAPTER 5

GENE FLOW AMONG TIGER POPULATIONS IN THE LANDSCAPE

5.1. INTRODUCTION

Gene flow, which is mediated by natal dispersal of individuals, is an important process underpinning population dynamics and metapopulation ecology (Hanski and Gilpin 1997). Gene flow is the cohesive force that binds together geographically separated populations into a single evolutionary unit – the species. The interruption of gene flow through habitat and population fragmentation is considered to be the root cause behind the current extinction crisis (WCMC 1992). Disruption of gene flow in small and isolated population fragments can lead to reduced genetic diversity and inbreeding resulting in decreased fitness, loss of adaptive potential, and vulnerability to diseases, making some local populations susceptible to extinctions (Frankham *et al.* 2002). Rates of gene flow in animals are positively correlated with rates of dispersal. It is precisely because of this fact that knowledge of inter-population dispersal and gene flow rates will improve understanding of colonization and recolonization dynamics, and our ability to manage complex systems containing multiple populations, particularly metapopulations which have been created by increasing human influence (Hanski and Gilpin 1997; Young and Clarke 2000). An important element of my thesis is detecting inter-population dispersal events and estimating rates of gene flow between local populations, so as to understand the effect that disruption of these processes would have on metapopulation structure and viability in a highly vagile large carnivore species.

Natal dispersal or the movement of individuals from their natal area to their eventual breeding area acts as a mechanism to minimize inbreeding by reducing competition for mates and resources among related individuals (Greenwood 1980; Pusey 1987; Pusey and Wolf 1996). At a regional or landscape scale, gene flow acts to increase genetic diversity (Paetkau *et al.* 1998), decrease population structure (Chessier *et al.* 1993), influence inter-population source-sink dynamics (Pulliam 1998), facilitate population colonization

and recolonization (Hanski and Gilpin 1997), and mediate abundance, distribution and range expansion in species (MacArthur and Wilson 1967). The rise in prominence of the metapopulation paradigm has generated interest in obtaining dispersal data on immigration and emigration and incorporating them into more realistic models of population dynamics (Hanski and Gilpin 1997). However, reliable data on dispersal and gene flow has been difficult to obtain due to intrinsic practical and logistical constraints posed by the particular target species and the study system being investigated.

Measuring dispersal is challenging especially for species which have long life spans, large distributions, wide ranging behavior, and in multi-population systems (Proctor 2003; Proctor *et al.* 2005). Yet this is often the information that is required to understand the processes mediating population fragmentation. Three methods have been frequently attempted to measure dispersal in large mammals, (i) radio-telemetry, (ii) capture-mark-recapture and (iii) indirect population genetic measures of gene flow. Traditionally, the direct estimation of dispersal and inter-population movement would have entailed the collection of radio-telemetry or capture-mark-recapture data from many individuals representing a large slice of the study population, and following the fates of individual animals over a regional scale and over several generations. The method would be logistically challenging, cost-prohibitive and inherently impractical, especially for species with long life spans and large ranges (Koenig *et al.* 1996). Similarly classical genetic methods provide indirect and unreliable estimates of inter-population migration in recently disturbed systems (Whitlock and McCauley 1999). Extrapolations of migration rate from measurements of genetic differentiation (F_{ST} and N_m) require equilibrium between the long-term forces driving genetic differentiation and are thus difficult to base in contemporary time scales (Neigel 2002). Recent methodological developments in non-invasive genetic sampling and robust statistic analytical tools based on likelihood and Bayesian assignment tests and individual clustering techniques enable identification of contemporary migrants within the last one or two generations (Pritchard *et al.* 2000; Piry *et al.* 2004; Wilson and Rannala 2003). These techniques have paved the way for conducting large-scale population genetic studies among species living in fragmented natural habitats (Proctor *et al.* 2005; Bergl and Vigilant 2007).

Aided by technological advances in non-invasive genotyping and analytical methods used to detect gene flow, researchers are increasingly resorting to genetic methods in the study of population structure and dispersal. Individual based Bayesian analyses allow inference of population structure, gene flow and demographic history with greater precision than previous approaches which relied upon idealized population models and summary statistics (e.g.F_{ST}, Pearse and Crandall 2004). By employing multilocus microsatellite genotypic data obtained from noninvasively collected samples, researchers have been able to infer population structure and gene flow in species as diverse as grizzly bear (*Ursus arctos*, Paetkau *et al.* 1998; Proctor *et al.* 2005), lowland gorilla (*Gorilla gorilla diehli*, Bergl and Vigilant 2007) and tiger salamander (*Ambystoma californiense*, Wang *et al.* 2009), to name just a few.

Measuring and monitoring gene flow can lead to a better understanding of the processes required to maintain viable populations and metapopulations in the face of increasing habitat fragmentation and ecosystem change. Historically the tiger populations in the Central Indian landscape were part of a large panmictic population and habitat connectivity which permitted unrestricted movement to tigers and other wildlife (Rangarajan 2001). Although habitat connectivity is currently present in varying degrees of patchiness to permit dispersal and maintain metapopulation structure in the area (Jhala *et al.* 2011b), it is rapidly being lost to development activities (Fernandes 2012). The patchy habitats in the region have been speculated to limit movement of tigers, and thus restrict population-level gene flow. Recent studies have found evidence of fragmentary population connectivity in the area (Sharma *et al.*2013; Joshi *et al.* 2013). Sharma *et al.* (2013) detected first generation migrants and found contemporary levels of gene flow to be drastically reduced among population pairs which have lost forest connectivity, while structurally intact corridors still maintained high historical pre-disturbance levels of gene flow among tiger populations. Using landscape connectivity models and migrant assignment tests, Joshi *et al.* (2013) reported long-range dispersal across fragmented populations, and observed that habitat quality of connecting corridors as governed by anthropogenic disturbance levels, and not distance between reserves, affected tiger

movement. These results indicate that tigers may be more resilient at negotiating movement across human-dominated landscapes.

In this chapter, I present work related to identification of migrants from multilocus microsatellite genotypic data using Bayesian individual clustering methods and assignment tests. By identifying recent migrants, an assessment on the functionality of present corridors in the area would be obtained which is important to prioritize conservation. Furthermore, I wanted to check patterns of migration between populations and estimate rates of gene flow across different temporal scales. This would provide information on source-sink dynamics across the populations needed to maintain metapopulation level processes.

5.2. METHODS

5.2.1. Samples and microsatellite loci genotyping

I used microsatellite genotypic data from 169 individual tigers, which were genotyped at eleven loci. Details of samples, laboratory procedures and genotype data used in the analysis are described in Chapter 3 (*ibid*).

5.2.2. Detection of migrants

I used three different approaches implemented in GENECLASS 2.0 (Piry *et al.* 2004), STRUCTURE 2.3.3 (Pritchard *et al.* 2009) and BAYESASS 2.3.3 (Wilson and Rannala 2003), to detect migrants and individuals with migrant or mixed ancestry. First, I selected the ‘detect migrants’ function in GENECLASS as it is explicitly designed to identify first generation migrants (Paetkau *et al*. 2004; Piry *et al*. 2004), i.e. individuals born in a population other than the one in which they were sampled. GENECLASS employs a suite of likelihood-based statistics, in combination with re-sampling methods, to calculate probabilities that individuals are first generation migrants. The probability of individual

genotypes originating from each locality was calculated by comparing the genotypes of cross-assigned individuals with a simulated set of 10,000 genotypes that were generated using area-specific allele frequencies. Though several simulation methods are implemented in GENECLASS, I chose the Paetkau *et al.* (2004) routine as it demonstrates accurate type I error rates, as a result of their improved simulation scheme which is more representative of natural population processes compared to other methods (Rannala and Mountain 1997, Cornuet *et al*. 1999). I used the likelihood-based estimator L_h/L_{max}, which is the ratio of the likelihood of an individual being assigned to its sampled population (L_h) with respect to all sampled populations (L_{max}). This statistic was used since I had sampled all potential source populations in the Central Indian Landscape. The Bayesian criterion of Rannala and Mountain (1997) was employed in combination with the re-sampling algorithm of Paetkau *et al.* (2004) to determine the critical value of the test statistic (L_h/L_{max}) beyond which individuals were assumed to be migrants. Both type I error α levels of 0.01 and 0.05 were investigated to determine threshold values for migrants. The assignment test assumes that all loci in each population are in genotypic equilibrium, which was verified using GENEPOP and FSTAT.

Next, I used STRUCTURE, by incorporating prior population information and *a priori* designation of the migration rate (MIGPRIOR) to assist the clustering process. This allows the program to calculate the posterior probability of whether individuals are residents of their sampled population/ cluster or migrants from other areas. STRUCTURE was run this way with the previously inferred cluster memberships (k = 4) used as prior population information. In order to check for possible biases in *a priori* ad hoc assignment of the migration rate (MIGPRIOR), I ran a range of MIGPRIOR values (0.001, 0.005, 0.01, 0.02, 0.05, 0.07, and 0.1) for exploratory analysis as suggested by Pritchard *et al.* (2009). These values were selected as they encompassed a wide range of assumed migration rate in tiger population from extremely low (0.001, i.e. one migrant among 1,000 tigers) to high gene flow (0.1, i.e. ten migrants among 100 tigers). Choice of MIGPRIOR did not substantially affect the output, therefore results for MIGPRIOR = 0.05 are presented here. The data was also run without using sampling locations as prior to determine whether the pre-defined population clusters is in rough concurrence with the

genotype data. Burn-in length and number of MCMC iterations were kept the same as for previous clustering runs without prior population information (provided in Chapter 4 section 4.2.2.2).

Third, the non-equilibrium Bayesian assignment test of BAYESASS was used to provide estimates of the posterior probability of recent migration rates between populations, by tracing each individual's immigrant ancestry within the last two to three generations. The method is robust to violations from Hardy-Weinberg equilibrium as it measures contemporary gene flow within the last few generations based only on multilocus allele sharing among individuals. To determine convergence of the MCMC chains, BAYESASS was run with five independent replicates, exploring different burn-in and run times. After initial trials, a total run length of 8 millionMCMC iterations was selected, of which the first 2 million runs comprising the burn-in phase was discarded. The remaining 6 million runs composed the data collection phase with the MCMC chain being sampled every 2000 steps. Individual assignments and immigrant ancestries were calculated at a migration rate prior set at 5%. Varying the prior migration rate (1 to 15%) did not affect the results.

Finally, to corroborate the results of the above assignment based migrant decisions, a likelihood based parentage analysis was carried out in CERVUS 3.0 (Kalinowski *et al.* 2007; Slate *et al.* 2000) to identify likely parent-offspring relationships between putative migrants and an individual in the cross-assigned source population. CERVUS calculates likelihood ratios for each candidate parent from population allele frequencies. It simulates parents and offspring developing a distribution of relative log-likelihood ratios to determine the significance for any given potential parent-offspring relationship. The simulation routine incorporates estimates of the rate of genotyping error, the number and proportion of potential parents sampled, and the proportion of genotyping success. The procedure assumes loci are unlinked which was verified using GENEPOP. The overall likelihood for each candidate parent is calculated by multiplying together the likelihood ratios at each locus, which is expressed as a log of the odds (LOD) ratio. The LOD score is the natural logarithm of the overall likelihood ratio. A positive LOD score means that

the candidate parent is likely to be the true parent, whereas a negative value means that the candidate parent is less likely to be the true parent. LOD scores for strict (95%) and relaxed (80%) confidence limits were estimated as 7.0 and 4.9 respectively. Critical LOD scores were calculated from a simulated set of 10,000 offspring and 300 candidate parent genotypes, assuming that 25% of candidate parents were sampled, 93% of loci were typed, with a typing error of 0.01 to 0.10. The existence of full sibs of the offspring (i.e. migrant individuals whose parentage is being tested) in the population may reduce the power of parentage assignment, as siblings may share marker alleles obtained from the parents at each locus. Non-excluded full sibs are likely to have an equal or slightly higher LOD score than the true parent (Marshall *et al.* 1998). In practice, it is difficult to tease apart sibling from parent relationships without accurate field observational data on the individual tigers being tested. Hence, all candidate parents identified from the analysis were also considered as likely siblings with equal likelihood.

5.2.3. Estimation of contemporary and historical migration rates

In order to estimate gene flow, I used the programs BAYESASS 2.3.3 (Wilson and Rannala 2003) and MIGRATE 3.3.2 (Beerli and Felsenstein 2001; Beerli 2008) to compare migration rates over contemporary and historical timescales respectively. Though the two programs use different approaches to derive estimates of gene flow, both programs generate parameters from which a comparative estimate of the proportion of genetic migrants in the population per generation (m) can be inferred. In BAYESASS, a Bayesian approach incorporating an MCMC sampling scheme is used to estimate migration rates between pairs of populations approximately two to three generations back. With an estimated generation time of 5 years in tigers (Smith and McDougal 1991), this period corresponds to a timescale of nearly 10-15 years ago. MIGRATE on the other hand, uses the coalescent to estimate the relative mutation-scaled effective population size, theta, Θ_{Ne} ($4N_e\mu$; where N_e is the effective population size and μ is the mutation rate) and asymmetric mutation-scaled immigration rate M (m/μ). The mutation-scaled immigration rate M, which is the immigration rate m divided by the mutation rate μ, is a measure of the importance of immigration events over mutation in contributing to

variation in the population (Beerli and Felsenstein 2001). The relative effective population size, theta, is the amount of individuals representing an idealized (Wright-Fisher) population that will result in the same amount of genetic drift as in the actual population. The number of migrants per generation, $4N_em$, is the product of theta and *M*. MIGRATE assumes mutation-migration-drift equilibrium with values of *M* and theta constant over time. Parameter estimates in MIGRATE data back nearly *4Ne* generations into the past (approximately thousands of years ago), estimated by using a conservative mutation rate statistic of 10^{-3} per generation (Ellegren 2000) and a 5 year generation time in tigers on the obtained theta values. Hence, these migration rates provide estimates of gene flow that post and pre-date the estimated time (approximately 600 years ago) when humans began to significantly alter the habitats in which these tigers currently live.

For both the BAYESASS and MIGRATE runs, I used the STRUCTURE defined population clusters to estimate pairwise migration rates. BAYESASS runs were performed as described in the preceding section on detecting migrants (see Section 5.2.2 above). A total of 8 millionMCMC iterations were carried out, discarding the first 2 millionsteps as burn-in and the remaining 6 million steps were sampled at every 2,000 iteration intervals of the MCMC chain. Runs were carried out with a migration rate prior of 0.05 while other parameters were kept at default settings. The average result from three independent BAYESASS runs is presented.

Estimations of mutation-scaled historic gene flow and relative effective population size were carried out in MIGRATE by using the Bayesian procedure and the Brownian motion model as an approximation for the step-wise microsatellite mutation model. The parameters of mutation rate were allowed to vary across loci by letting the sampler estimate it from the data. A slice sampling scheme was adopted with the uniform prior boundaries for theta and *M* both set to 0 (lower) and 100 (upper) with an increment at every 10 steps. Following initial trial runs, the Bayesian search criteria for the MCMC sampler in the final run was set at 10 replicates of 1 long chain of 50,000 steps with every 100 steps of the chain being recorded. This final run recorded a total length of 5×10^7 visited parameter values (i.e. 10 replicates x 1 chain x 50,000 steps x 100 increments).

The first $1x10^7$ steps of the run which comprised the burn-in were discarded, and the remaining $4x10^7$ runs were sampled. To increase efficiency of the sampler in exploring the genealogy space, I used the thermodynamic option using four different chain temperatures of 1, 1.5, 3 and 10,000. Convergence of the run was assessed through changes in the profile of posterior distributions across different start parameters and run lengths. Parameter estimates from initial runs were used to calculate starting values of theta, for use as new parameters during subsequent runs. The posterior estimates of *M* and theta obtained in the final run were similar to the results of the initial trial runs. Locus-wise mutation rates also produced similar estimates across runs. All runs were carried out on MIGRATE-n 3.3.2, complied for a parallel computing environment, and available at the online Bioportal cluster computing facility at the University of Oslo, Norway (http://www.bioportal.uio.no/).

5.3. RESULTS

5.3.1. Migrants

The detection of migrants in STRUCTURE, GENECLASS and BAYESASS yielded a total of seventeen individuals as having putative immigrant ancestry (Table 5.1). Identical migrant assignment across all three programs was observed in seven individuals (D954, D955, D958, D1297, D1399, D1843 and D1892), while assignment was equivocal in the remaining Gender identification revealed 12 out of 75 males (16 %) and 5 out of 84 females (6%) as individuals with immigrant ancestry in the entire area.

(i) STRUCTURE results - Eight putative migrants were identified in the STRUCTURE analysis. Of these, four individuals (D954, D955, D958 and D1399) were identified with >80% migrant and cross-assignment probability to a single non-home cluster in the STRUCTURE analysis, carried out without prior population information. The remaining four samples represented individuals (D1843, D1892, D1297, D2058) which had weaker

migrant probability (P>0.5 to <0.7) and showed variable Q (0.289 to 0.758), and with the majority of samples having cluster memberships to more than one non-home locality.

(ii) GENECLASS results - GENECLASS also identified eight individuals as putative migrants (P<0.01), with high log likelihood of cross-assignment (L_h/L_{max}> 2.0). Lowering the likelihood threshold (L_h/L_{max}< 2.0) yielded further six putative migrants (P<0.05).

(iii) BAYESASS results - A total of fifteen individuals with likely immigrant or admixed ancestry were detected in the BAYESASS analysis. Of the fifteen total migrants, eleven individuals had high migrant cross-assignment probabilities (P> 0.8), and four individuals had intermediate migrant assignments (P = 0.509 to 0.617). In general, the posterior probabilities of migrant assignment were higher in BAYESASS compared to the STRUCTURE analysis. Additionally, BAYESASS identified six more individuals (D1075, D1381, D1383, D1393, D1400 and D1987) as potential migrants (P=0.509 to 0.926) which were not assigned as migrants by either STRUCTURE or GENECLASS.

Figure 5.1 shows the posterior distributions of individuals assigned to nonimmigrant (gen0), first (gen1) or second generation immigrant (gen2) ancestry states in GENECLASS, STRUCTURE and BAYESASS. All GENECLASS migrants with L_h/L_{max}> 2.0 were classified as 100% first generation migrants. Two individuals (D955, D958) with >90% gen1 assignment and three individuals (D954, D1399 and D1843) with relatively high gen1 assignment probability (>0.5 to 0.7) were considered as migrants. Five individuals (D525, D1892, D2058, D1297 and D1987) showed moderate levels of migrant assignment and immigrant ancestry patterns are indicative of admixed status. The assignment status of seven more individuals (D1075, D1381, D1383, D1393, D1400, D1140 and D2154) was equivocal. While STRUCTURE could not assign them as migrants, they were identified as potential second generation migrants or admixed individuals in BAYESASS.

Identification of offspring-candidate parent pairs in CERVUS yielded parentage relationships in thirteen out of seventeen putative migrants. No evidence of likely

parentage (or sibling) relationships in the offspring population was observed. The cross-assigned population in ten of the thirteen individuals matched the parentage assignment in CERVUS, which serves to further corroborate the results of the migrant assignments. Only three mismatches (D1075, D1393 and D1987) were observed between the source populations identified by CERVUS and the population assignments depicted by the migrant analysis but this could be due to low information in the data as opposed to incorrect migrant assignment. Except for negative LOD value in two pairs (D955-D1182, LOD = -0.36; D1400-D1168, LOD = -2.15), LOD scores were positive in the remaining eleven putative parent-offspring pairs. The relationship between a potential migrant sampled in Pench (D958) with a candidate parent from Kanha (D1205) was identified with > 80% confidence in assignment (LOD = 5.3). LOD scores in remaining offspring-parent pairs were below the 80% confidence limit (< 4.9).

Table 5.1. Results of migrant assignments based on STRUCTURE, GENECLASS (migrants based on $**\alpha_{0.01}$ and $*\alpha_{0.05}$ type I error levels) and BAYESASS analyses. *Q* assignments depict individual membership to each of the four STRUCTURE identified population clusters of Melghat-Satpura-Tadoba (MST), Pench (P), Kanha (K), and Bandhavgarh (B). Localities depicted include Melghat (MTR), Satpura (STR), Pench (PTR), Kanha-Pench corridor (KPC), Kanha (KTR) and Tadoba (TATR).

tiger ID	sex	sampled locality	GENECLASS assigned population	GENECLASS *Lh/Lmax*	STRUCTURE *Q* assignment clusters (MST/P/K/B; no prior population information, *K*=4)	STRUCTURE migrant probability (gen1, gen2)	BAYESASS assigned population	BAYESASS migrant probability (gen1, gen2)	CERVUS assigned parent/ population/ pair LOD score	Final migrant status
D955	♂	*PTR*	*KTR*	*3.234***	*0.035/ 0.009/ 0.889/ 0.067*	*0.996 (0.978, 0.018)*	*KTR*	*0.999 (0.990, 0.009)*	*D1182/ KTR/ -0.360*	Migrant
D958	♀	*PTR*	*KTR*	*2.959***	*0.033/ 0.016/ 0.936/ 0.015*	*0.982 (0.964, 0.018)*	*KTR*	*0.997 (0.987, 0.010)*	*D1205/ KTR/ 5.320*	Migrant
D954	♂	*PTR*	*KTR*	*3.883***	*0.009/ 0.019/ 0.963/ 0.009*	*0.863 (0.774, 0.089)*	*KTR*	*0.962 (0.825, 0.137)*	*D402/ KTR/ 0.760*	Migrant
D1399	♂	*PTR*	*KTR*	*3.222***	*0.087/ 0.022/ 0.885/ 0.007*	*0.868 (0.552, 0.316)*	*KTR*	*0.998 (0.702, 0.296)*	*NE*	Migrant
D1843	♂	*MTR*	*PTR*	*2.343***	*0.275/ 0.527/ 0.186/ 0.013*	*0.590 (0.502, 0.088)*	*PTR*	*0.986 (0.842, 0.144)*	*D1381/ PTR / 4.210*	Migrant
D1297	♀	*KPC*	*PTR*	*6.444***	*0.075/ 0.683/ 0.215/ 0.026*	*0.610 (0.130, 0.480)*	*PTR*	*0.559 (0.124, 0.436)*	*D1043/ PTR/ 2.490*	Admixed
D1987†	♂	STR	PTR	2.407**	0.481/ 0.325/ 0.180/ 0.014	0.311 (0.005, 0.306)	PTR	0.550 (0.006, 0.544)	D2057/ MTR/ 0.300	Admixed
D525	♂	TATR	KTR	2.562**	0.664/ 0.023/ 0.300/ 0.013	0.170 (0.031, 0.139)	KTR	0.617 (0.086, 0.531)	NE	Admixed
D2154	♀	TATR	PTR	<2.0*	0.460/ 0.158/ 0.358/ 0.024	0.007 (0.001, 0.006)	TATR	0.188 (0.056, 0.132)	NE	Admixed
D1892	♂	MTR	PTR	<2.0*	0.658/ 0.289/ 0.042/ 0.011	0.584 (0.113, 0.471)	PTR	0.996 (0.011, 0.985)	D578/ PTR/ 0.070	Admixed
D2058	♀	MTR	MTR	0	0.147/ 0.758/ 0.019/ 0.076	0.508 (0.173, 0.335)	PTR	0.895 (0.036, 0.859)	NE	Admixed
D1140	♀	KTR	PTR	<2.0*	0.070/ 0.556/ 0.344/ 0.030	0.124 (0.016, 0.108)	PTR	0.241 (0.035, 0.206)	D1043/ PTR/ 3.540	Admixed
D1075	♂	PTR	KTR	<2.0*	0.065/ 0.237/ 0.663/ 0.034	0.096 (0.012, 0.084)	KTR	0.509 (0.161, 0.348)	D1926/ STR/ 2.473	Admixed
D1381	♂	PTR	KTR	<2.0*	0.213/ 0.466/ 0.316/ 0.004	0.190 (0.000, 0.190)	KTR	0.832 (0.012, 0.820)	D1185/ KTR/ 0.351	Admixed
D1383	♂	PTR	PTR	0	0.068/ 0.510/ 0.420/ 0.003	0.159 (0.001, 0.158)	KTR	0.926 (0.009, 0.917)	D1114/ KTR/ 4.471	Admixed
D1393	♂	PTR	KTR	<2.0*	0.089/ 0.083/ 0.807/ 0.021	0.188 (0.013, 0.175)	KTR	0.872 (0.264, 0.608)	D578/ PTR/ 1.066	Admixed
D1400	♂	PTR	PTR	0	0.146/ 0.110/ 0.738/ 0.006	0.229 (0.015, 0.214)	KTR	0.829 (0.201, 0.628)	D1168/ KTR/ -2.155	Admixed

† Tigers reported dead during the study period, M-Male, F-Female, NE- not established

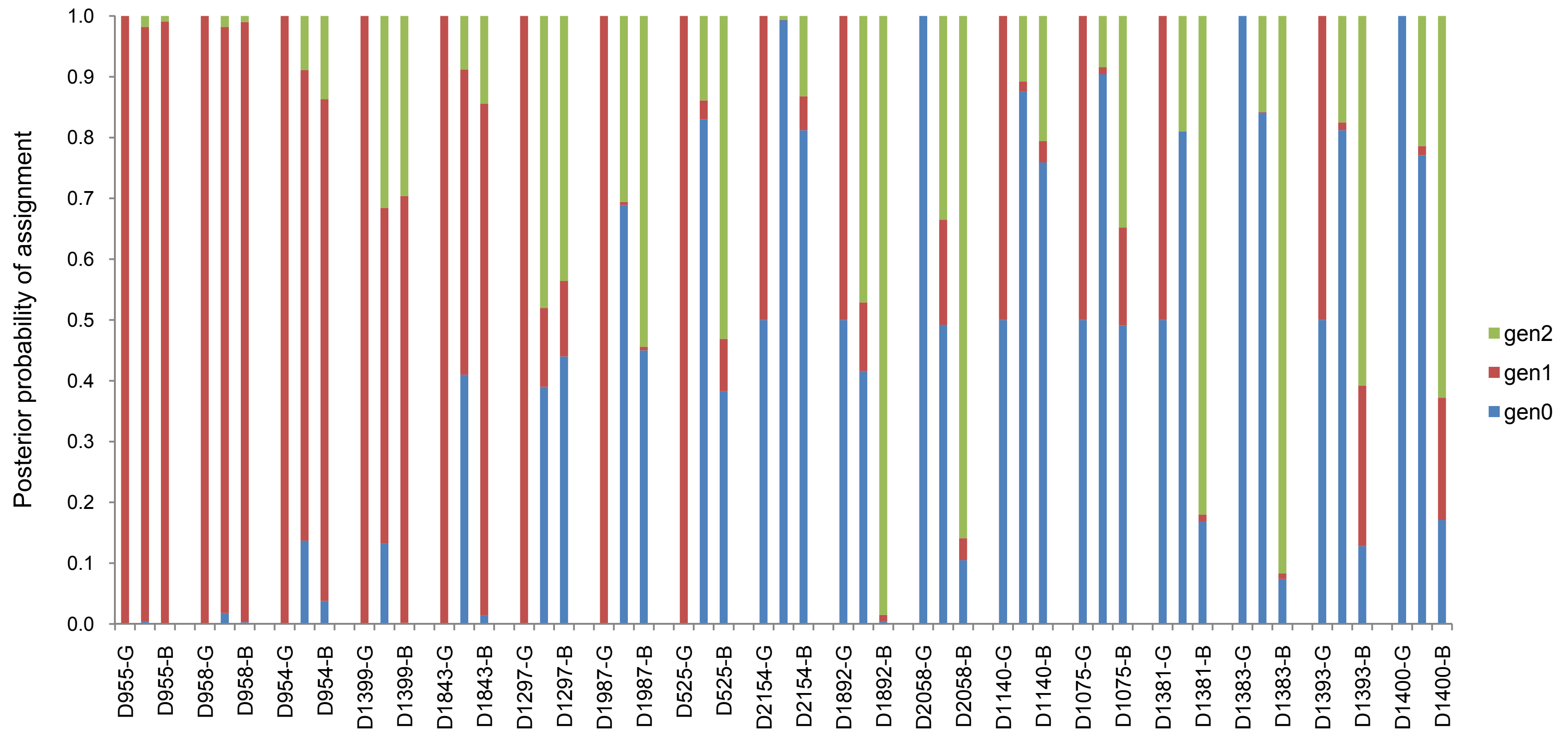

Figure 5.1. Posterior distributions of individual assignment to nonimmigrant (gen0), and first (gen1) and second generation immigrant (gen2) ancestry states. Suffixes after indvidual tiger IDs indicate assignment probabilties as obtained in GENECLASS (G), STRUCTURE (S) and BAYESASS (B).

5.3.2. Contemporary and historical gene flow rates

The mean posterior distributions of pairwise immigration rates depicting contemporary gene flow estimates in BAYESASS are shown in Table 5.2. Most populations have low migrant proportions with the exception of migration from Pench to Melghat (m = 0.09) and Kanha to Pench (m = 0.07) where migration rates exceeded 5% (Table 5.2). Gene flow between Melghat and Pench was asymmetric and there appears to be a possible source-sink relationship because the expected proportion of migrants into the Pench population from Melghat is much smaller (m = 0.015). Asymmetric migration was also visible between the Pench and Kanha population clusters, as the proportion of migrants from Pench to Kanha was negligible (m = 0.006). The Bandhavgarh population was devoid of migrants as suggested by the lack of gene flow between other populations (m< 0.01). As in the locality-wise analysis, similar asymmetry and rates of migration were obtained between the various STRUCTURE defined population clusters (Table 5.3).

Results from the MIGRATE analysis showed low estimates of relative effective population size (theta, Θ) and historical mutation scaled immigration rate (M) suggesting low overall migrant proportions in the area (Table 5.4). Theta estimates were low to moderate and ranged from 0.57 (Bandhavgarh), 0.77 (Kanha-Achanakmar, and Melghat-Satpura-Tadoba) to 1.5 (Pench). Estimates of M which ranged from a high of 6.17 (Pench to Bandhavgarh) to a low of 0.9 (Bandhavgarh to Melghat-Satpura-Tadoba) revealed limited to no migration among populations in the landscape (Table 5.4). Of the twelve pairwise population comparisons, ten pairs showed asymmetric migration patterns, with the higher value of M representing immigration from the population with the larger theta value (Kanha and Pench clusters), to the population with the smaller theta value (Melghat-Satpura-Tadoba and Bandhavgarh clusters). The number of migrants per generation ranged from almost zero (Bandhavgarh to Melghat-Satpura-Tadoba cluster) to nine (Pench to Bandhavgarh). The Pench and Kanha clusters represented the largest source populations for immigrants in the area. Though marginally higher migration from Pench to Kanha was visible compared to migration in the other direction, both population clusters had overall symmetric gene flow.

Table 5.2. Locality-wise contemporary migration rates, *m*, estimated using BAYESASS showing means (± standard deviation) of the posterior distributions along with the 95% confidence intervals in parenthesis. The populations into which individuals are migrating are listed in the rows, while the sources of the migrants are listed in the columns. Values along the diagonal are proportions of individuals derived from the source populations each generation. Migration rates ≥ 0.05 are in bold. Individuals from Achanakmar and the Kanha-Pench corridor were not included in this analysis due to low sample sizes.

	Source					
	Melghat	**Satpura**	**Pench**	**Kanha**	**Tadoba**	**Bandhavgarh**
Destination						
Melghat	**0.856 ± 0.042 (0.773, 0.937)**	0.014 ± 0.015 (0.000, 0.054)	**0.092 ± 0.038 (0.026, 0.173)**	0.015 ± 0.016 (0.000, 0.057)	0.011 ± 0.013 (0.000, 0.046)	0.012 ± 0.014 (0.000, 0.051)
Satpura	0.014 ± 0.017 (0.000, 0.059)	**0.899 ± 0.045 (0.805, 0.975)**	0.037 ± 0.031 (0.000, 0.115)	0.026 ± 0.026 (0.000, 0.099)	0.012 ± 0.015 (0.000, 0.049)	0.012 ± 0.015 (0.000, 0.054)
Pench	0.004 ± 0.006 (0.000, 0.020)	0.025 ± 0.014 (0.003, 0.059)	**0.897 ± 0.029 (0.838, 0.952)**	**0.065 ± 0.025 (0.022, 0.120)**	0.005 ± 0.006 (0.000, 0.024)	0.003 ± 0.004 (0.000, 0.016)
Kanha	0.008 ± 0.007 (0.000, 0.026)	0.003 ± 0.004 (0.000, 0.014)	0.005 ± 0.007 (0.000, 0.024)	**0.976 ± 0.015 (0.940, 0.996)**	0.007 ± 0.008 (0.000, 0.030)	0.002 ± 0.004 (0.000, 0.014)
Tadoba	0.025 ± 0.032 (0.000, 0.114)	0.012 ± 0.015 (0.000, 0.054)	0.015 ± 0.021 (0.000, 0.068)	0.021 ± 0.029 (0.000, 0.102)	**0.917 ± 0.068 (0.756, 0.998)**	0.010 ± 0.014 (0.000, 0.048)
Bandhavgarh	0.003 ± 0.006 (0.000, 0.017)	0.003 ± 0.006 (0.000, 0.020)	0.003 ± 0.006 (0.000, 0.020)	0.003 ± 0.006 (0.000, 0.019)	0.003 ± 0.006 (0.000, 0.019)	**0.986 ± 0.014 (0.952, 0.999)**

Table 5.3. Contemporary migration rates, *m*, among STRUCTURE identified population clusters estimated using BAYESASS showing means (± standard deviation) of the posterior distributions along with the 95% confidence intervals (CI) in parenthesis. The population clusters into which individuals are migrating are listed in the rows, while the sources of the migrants are listed in the columns. Values along the diagonal are proportions of individuals derived from the source populations each generation. Migration rates ≥ 0.05 are in bold. Depicted population clusters are Melghat-Satpura-Tadoba (MST), Pench (P), Kanha (K) and Bandhavgarh (B). Individuals from Achanakmar and the Kanha-Pench corridor are included in the Kanha cluster.

	Source			
	MST	**P**	**K**	**B**
Destination				
MST	**0.915 ± 0.027 (0.860, 0.962)**	**0.064 ± 0.025 (0.020, 0.117)**	0.015 ± 0.016 (0.000, 0.058)	0.006 ± 0.007 (0.000, 0.026)
P	0.015 ± 0.016 (0.000, 0.054)	**0.905 ± 0.029 (0.846, 0.958)**	**0.076 ± 0.026 (0.029, 0.129)**	0.004 ± 0.005 (0.000, 0.019)
K	0.009 ± 0.009 (0.000, 0.033)	0.006 ± 0.008 (0.000, 0.029)	**0.981 ± 0.014 (0.950, 0.999)**	0.003 ± 0.004 (0.000, 0.017)
B	0.005 ± 0.007 (0.000, 0.028)	0.005 ± 0.007 (0.000, 0.027)	0.005 ± 0.008 (0.000, 0.028)	**0.986 ± 0.014 (0.950, 0.999)**

Table 5.4. Means of posterior distributions ofmutation scaled immigration rate, *M* (*m/μ*), along with the 95% CI limits (before comma) and mean number of migrants ($4N_em$, i.e. product of theta and *M*) per generation (after comma) estimated from MIGRATE analysis. The values along the diagonal are estimates of the relative effective population size, theta. Population clusters listed in rows depict the populations into which individuals migrate, while the source populations of individuals are shown in the columns. Depicted population clusters are Melghat-Satpura-Tadoba (MST), Pench (P), Kanha-Achanakmar (KA) and Bandhavgarh (B). Individuals from Achanakmar and the Kanha-Pench corridor are included in the Kanha cluster.

	Source			
	MST	**P**	**K**	**B**
Destination				
MST	0.77 [0 - 1.93]	4.43 [0 - 7.2], 6.6	1.83 [0 - 3.87], 1.4	0.9 [0 - 2.67], 0.5
P	1.43 [0 - 3.53], 1.1	1.5 [0.533 - 3.0]	1.83 [0 - 4.4], 1.4	2.83 [0 - 5.4], 1.6
K	2.97 [0 - 6.13], 2.3	2.17 [0 - 5.4], 3.3	0.77 [0 -2.0]	2.43 [0 - 4.8], 1.4
B	3.9 [0 - 19.4], 3	6.17 [0 - 25], 9.3	4.03 [0 - 21.8], 3.1	0.57 [0 - 1.53]

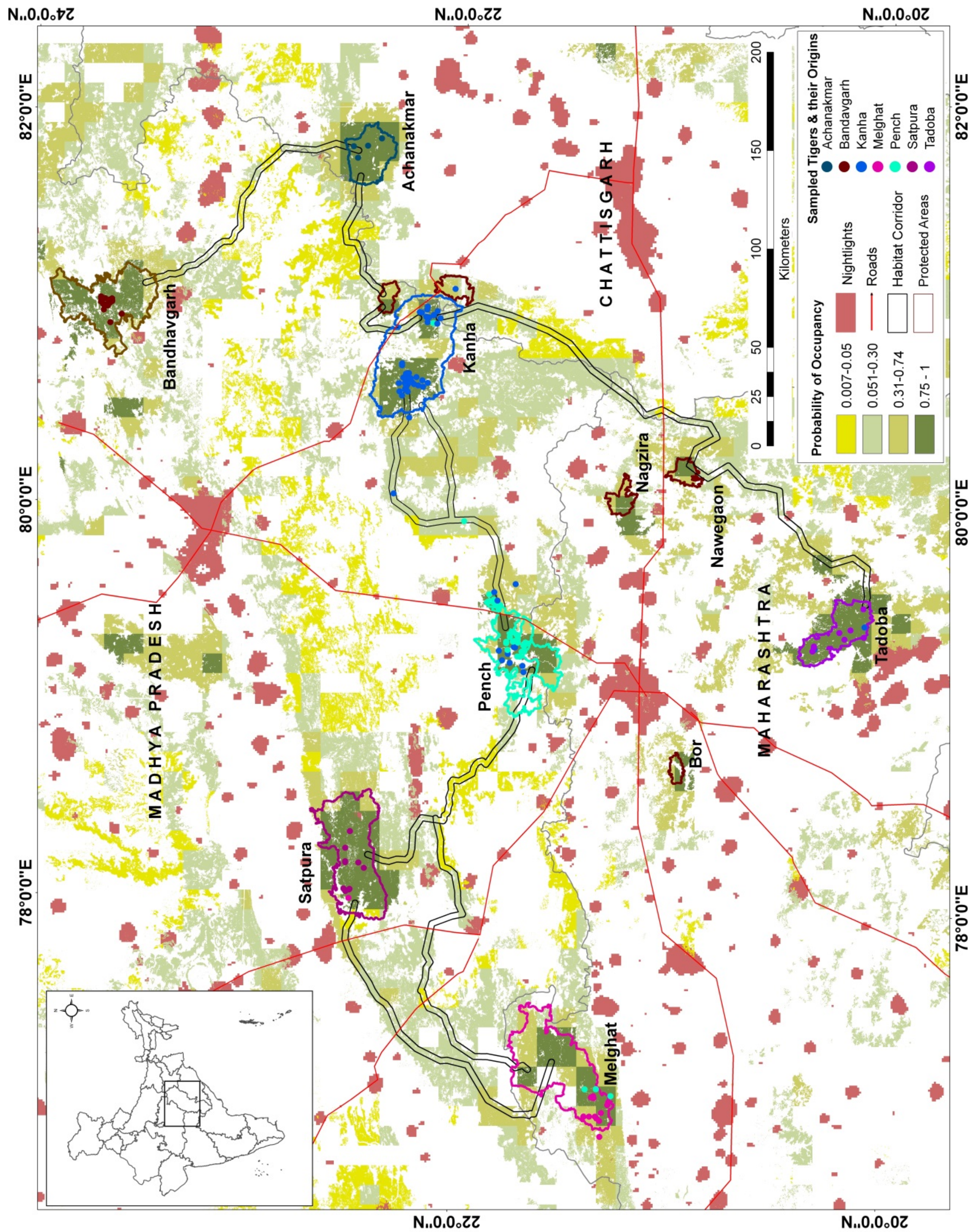

Figure 5.2. The study area of Central India showing tiger habitat (forest cover) coded with tiger occupancy probability, protected areas, human habitation (night lights), major roads and least-cost habitat corridors connecting tiger reserves. Individually genotyped tigers (n=169) are shown as colour coded dots at their sampled locations with their colours matching their genetically assigned population. Occupancy probability and least-cost corridors were obtained from the country-wide tiger estimation exercise (Jhala *et al.* 2011b and Yumnam *et al*. unpublished).

5.4. DISCUSSION

The Central Indian landscape is a mosaic of habitats where tiger densities ranging from high (Bandhavgarh, 14 tigers/100 km^2, Jhala *et al*. 2011b) to low (Achanakmar, 0.1 tigers/100 km^2, Jhala *et al*. 2011b) tiger are juxtaposed in a matrix encompassing a range of land-use regimes from undisturbed natural forest to intensively modified agricultural land and high density human settlements (Figure 5.2). Though this area is one of the most fragmented tiger habitats in India, some potential for gene flow between populations within the landscape existed since tigers like most highly vagile large carnivores, have the capability to disperse across great distances where habitat connectivity is present, ~150 km linear distance between two protected areas in the Nepal Terai (Sunquist *et al.* 1999). However, in the same fragmented human-dominated Terai Arc landscape of Nepal, tigers were not found to disperse across expanses of agricultural land (10-20 km wide) though they did traverse through stretches of degraded forest (Smith 1993). So far, the only published estimates on tiger dispersal in the Central Indian Landscape are from recent spatial genetic studies which observed movement across protected areas located more than 200 km (Sharma *et al.* 2013) and 600 km (Joshi *et al.* 2013) apart, suggesting that tigers may be more resilient in traversing fragmented habitats than previously reported. Unpublished field studies based on camera-traps have recorded tigers to disperse between Pench and Kanha, a geographic distance of more than 100 km (Jhala and Qureshi unpublished data).

Identification of migrants by the methods used here requires that the populations being investigated have sufficient genetic separation and low levels of migration. I found in my study that the power to detect migrant individuals varied across localities in the region. There was no power to detect migration between localities in the same cluster (such as Kanha and Achanakmar). In these situations it was difficult to tease apart actual migration events from similar patterns that arose from shared allelic frequencies and low genetic separation. On the other hand, migrant identification between localities in different population clusters produced robust assignments because of distinct genetic differences. Migrant detection was highest between localities which had discernible

genetic separation and had relatively intact habitat corridors with confirmed tiger presence, such as between Kanha and Pench populations where four migrants and seven likely admixed individuals were detected. Other studies by Joshi *et al.* (2013) and Sharma *et al.* (2013) respectively detected one and two first generation migrants between Pench and Kanha. The intervening forest patch between Kanha and Pench not only served as a movement corridor but also had some resident tigers as evidenced from camera trap data (WWF-India 2012). A noninvasive genetics study by Sharma *et al.* (2012) detected seventeen individuals from this corridor. At the extreme end in Bandhavgarh, no migrants were detected even though distinct genetic separation of Bandhavgarh from other populations provided sufficient power to distinguish migrants. Between the Melghat and Pench populations where three individuals with migrant ancestry were obtained, there was limited but adequate power to detect migrants as genetic separation was distinct but at a lower level than Bandhavgarh. One individual sampled in Satpura was cross-assigned to Pench. The corridor between Pench and Satpura has fragmented forest connectivity and tigers were reported from this area within the last ten years (Jhala *et al.* 2008). Two individuals with admixed ancestry were also detected between Kanha and Tadoba, though one of these had mixed assignments with Pench as well suggesting gene flow in the last few generations. Areas of degraded forest and low density tiger occupancy such as Nagzira and Nawegaon Wildlife Sanctuaries located strategically between Tadoba and Kanha, and Bor located between Tadoba, Pench and Melghat may serve to provide stepping stone type corridor connectivity (Figure 5.2). These areas have resident tigers, and it seems likely that the populations are sustained by sporadic dispersing individuals from larger source populations. A recent study by Joshi *et al.* (2013) observed that Tadoba, Nagzira and Melghat form a unit genetic cluster, and based on asymmetric migration ratesit seems likely that the tiger population in Nagzira acts as a 'sink' for the high densityTadoba population.

Designation of migrant status to an individual was contingent upon - (i) significant assignment of first generation migrant status in GENECLASS (P<0.01, $Lh/Lmax \geq 2.0$); (ii) observance of >50% migrant or cross-assignment probability in STRUCTURE and BAYESASS; (iii) high assignment probability to first generation immigrant ancestry state

(gen1>50%) in both STRUCTURE and BAYESASS; and (iv) high membership (Q>0.8) to a single non-home cluster in the STRUCTURE analysis without prior population information. Further, in most cases of migrants, the successful parentage assignment corroborated the migrant status of the individual tiger. I considered a conservative approach by identifying individuals as putative migrants only if all three programs suggested evidence of immigrant ancestry. Hence, I designated four individuals, i.e. three males (D954, D955 and D1399) and one female (D958), which strictly fulfilled all of the above criteria, as first generation migrants. Further, a fourth male individual (D1853) which had high cross-assignments but had marginally lower Q value (0.527) compared to the other migrants was also designated as a first generation migrant. The immigrant ancestry status in the remaining ten putative migrant individuals was classified as admixed based on - (i) intermediate levels of migrant probability in STRUCTURE; (ii) membership to more than one cluster in the individual Q assignments; and (iii) high probability of assignment to second and not to firstgeneration immigrant ancestry state in STRUCTURE and BAYESASS.

It is important to note that when looking for individual migrants using population assignments, results will yield a "most likely" population of origin. Hence, to guard against the potential bias of missing some assignments due to unsampled true populations, my study area included all potential tiger source populations and habitats, well within the dispersal distance for my study species. Determination of individual migrants based on genetic data is particularly challenging in recently disturbed systems as the genetic signals lag behind fragmentation events. Therefore it was prudent to base my results on a combination of methodologies. In this work I used the frequency based assignment (Paetkau *et al.* 1995; Rannala and Mountain 1997) and a Bayesian cluster-based method (Pritchard *et al.* 2000) to corroborate my results. The results of my ecological conclusions are bolstered by the fact that even though there were some disagreements between the assignment techniques in selecting individual migrants, the ecological implications did not change – migration is limited and sex-biased (very little female movement). Though one of the first generation migrants was a female, an overwhelming proportion of individuals with immigrant ancestry were males (80%; 4

males out of 5 total migrants), confirming that the dispersal is male-biased in tigers (Smith 1993). The low number of strict first generation migrants is evidence that contemporary migration events are small in proportion to the amount of individuals with likely admixed ancestry. The identification of samples with admixed ancestry suggests that most migration events in this landscape have occurred within a few generations and is proof that migrant tigers are able to reproduce in the new locality. This genetic evidence is further supported by field observations where a sub-adult male tiger photo-captured in Pench tiger reserve in 2006 was observed to be territorial breeding male in Kanha tiger reserve in 2010 (Jhala and Qureshi unpublished data). The data and analyses show that tigers likely disperse between Kanha-Pench, Pench-Satpura-Melghat, Kanha-Tadoba, Melghat-Tadoba and Tadoba-Pench.

My results which suggest dispersal across fragmented habitats are similar to results from recent studies in the Satpura-Maikal area (Sharma *et al.* 2013) and other southern populations (Joshi *et al.* 2013) of the Central Indian Landscape. Sharma *et al.* (2013) detected first generation migrants between Kanha-Pench, Kanha-Satpura, Kanha-Melghat, Pench-Satpura and Satpura-Melghat, and none between Pench-Melghat. Another study by Joshi *et al.* (2013) identified contemporary migrants between Nagarjunasagar-Kanha, Nagarjunasagar-Pench, Nagarjunasagar-Nagzira, Melghat-Nagzira, Kanha-Melghat and Kanha-Pench, but none in Tadoba. The level of migration in the landscape was low but interestingly they detected long-range dispersal events between Kanha-Nagarjunasagar in the order of 600 km. Joshi *et al.* (2013) observed that though tigers were found to disperse long distances, the effects of increasing human activity and associated infrastructural development in the region was negatively impacting tiger movement and reducing gene flow rates among populations.

In my study, both the contemporary and historical analyses observed low estimates of migration rates between populations in the landscape. While this result is superficially similar to the findings of Joshi *et al.* (2013), it is in sharp contrast to Sharma *et al.* (2013) where they found high historical and contemporary gene flow although historical rates were much higher than contemporary rates. As observed by Joshi *et al.* (2013), low levels

of contemporary gene flow are expected given that the area is highly fragmented and extant populations occupy habitats that are completely surrounded by heavily modified landscapes, altered by agriculture and high density human settlements, thus making it difficult for tigers to disperse between populations. The highest estimate of contemporary migration was seen from Pench to Melghat, but there appears to be an asymmetric source-sink relationship between the two populations with very low migration from Melghat to Pench. Such a pattern is expected given that Pench has high tiger density (4 tigers/ 100 km^2) serving as a source population, while the population density in Melghat is low (2 tigers/ 100 km^2). Asymmetric contemporary rates of gene flow were also visible between Pench and Kanha, as migration from Kanha (6 tigers/ 100 km^2) to Pench was much higher compared to movement in the opposite direction from Pench to Kanha. This is similar to the result of Joshi *et al.* (2013) where there was high migration (>5%) in both directions between Kanha and Pench, and Kanha acted as the biggest contemporary source for immigrants. In contrast, Sharma *et al.* (2013) state that only Pench is acting as a contemporary source population and contemporary gene flow from Pench to Kanha and Satpura is very high, and has remained relatively stable since historic pre-disturbance times. A comparative evaluation at historical and contemporary time scales in their study showed that gene flow between the Pench–Satpura and Melghat–Satpura pairs remains similar, whereas there has been a 47–70% reduction in gene flow between Kanha–Satpura, Pench–Melghat and Kanha–Melghat. My results showed that historical patterns of migration between the two major population clusters of Kanha and Pench were of equal proportion in both directions, and that both Kanha and Pench acted as source populations in contemporary times as well. Although Sharma *et al.* (2013) reported higher historic and contemporary gene flow rates compared to my study, both studies similarly observed that Kanha and Pench were important historic source populations and were the main drivers of gene flow in the area.

Contemporary density estimation studies using photographic capture-recapture techniques in the region (Jhala *et al.* 2011b) help explain some of the patterns observed in the gene flow analysis. During 2006, the Kanha population experienced a phase of relative expansion compared to a decline observed later in 2010. The situation was

reversed in Pench where the population was relatively low during 2006-2009 compared to a later phase of expansion in 2010 (Jhala *et al.* 2008, 2011b). From the genetic sampling done in Pench during both 2007 and 2010, which pre and post-dated this period of population expansion in the locality, a range of individuals representing likely migrant or admixed ancestry to Kanha was obtained. All first generation migrants from Kanha to Pench were detected during 2007 only, when the population in Kanha was in expansion phase. In 2010, seven individuals showing admixed ancestry to Kanha were obtained in Pench. Dispersal and subsequent breeding by immigrants from Kanha to Pench during periods of population expansion in the former explains the patterns of immigrant ancestry detected in the Pench population.

The pattern of historic migration observed among populations in the area suggests some form of source-sink relationship between the larger central clusters of Pench and Kanha on one end and the other peripheral localities at the other end. The cluster represented by Melghat-Satpura-Tadoba is subject to high amount of gene flow from Pench, indicative of the fact that these localities were well connected till recent times. Bayesian sequential clustering runs in STRUCTURE showed mixed memberships suggesting admixed origin and recent separation among these localities (Chapter 4). Contemporary gene flow analyses also reveal high migration between these two clusters, and tigers were present in the degraded corridor connecting Pench and Satpura till recent history. Bandhavgarh represented the most fragmented population in contemporary times, and also appears as a historical sink population. Gene flow from most localities in the area to Bandhavgarh was high, but was extremely low in the opposite direction. It may also be likely that Bandhavgarh could have connectivity and share gene flow with other localities further north and east, outside the main populations of this study area.

While longstanding evolutionary forces likely affect dispersal behaviour of species, it is also important to consider the role of demographic fragmentation and anthropogenic factors in imposing limits on gene flow (Allendorf *et al.* 2013). Estimates of migration rate and relative effective population size in MIGRATE span at least 4*Ne* generations back, or several thousand years ago in relative terms (Beerli 2009), estimated about 2,000

to 8,000 years before present (BP) in this study. This period of time is well before the well-known population collapses that began about 700 to 600 years BP in tigers (Rangarajan 2001; Mondol *et al.* 2009a). Though ancient population fragmentation is perceived to have occurred in response to the forest clearing activity of agro-pastoralist Neolithic people during the mid-Holocene about 5,000 - 3,000 years ago (Chandran 1997), the effect is difficult to evaluate as early farmers likely did not clear forests at a scale comparable to recent centuries (Misra 2001). However, it is conceivable that the shift from hunter-gatherer to agro-pastoralist lifestyle and successful colonization of new areas would have negatively impacted large mammal populations including tigers and prey species through hunting, as seen during human colonization events in other parts of the world (Alroy 2001; Surovel *et al.* 2005). While the effect is difficult to evaluate, the low long-term gene flow estimates observed in this study may suggest that populations may have been subject to historic fragmentation and genetic drift as suggested by other phylogenetic (Luo *et al.* 2004) and biogeographic (Kitchener and Dugmore 2000) studies. On the other hand, evidences from a range-wide study of tigers have observed a massive decline of about 98% tiger numbers since the last 200 years in peninsular India (Mondol *et al.* 2009a). Historical hunting records dating at least 600-700 years ago provide evidence that tigers in the region were massively hunted to extinction from areas outside current reserve boundaries (Rangarajan 2001). The population decline continues apace today, as omnipresent fragmentation and rampant poaching of tigers, other carnivores and prey species have reduced populations such as Achanakmar to only a few individuals (Jhala *et al.* 2011b), and resulted in local extirpation in adjacent reserves of Sariska and Panna (Gopal *et al.* 2010).

Alternatively, the low estimates of historic migration rate raise concerns whether these results are artifacts of sampling or population related. MIGRATE parameter estimations assume coalescent-based models of constant population sizes and mutation-migration-drift equilibrium (Beerli 2009). Hence, departures from these assumptions such as recent and sudden declines in population sizes can negatively bias the posterior parameter distributions of N_e and hence M estimations (Beerli 2009). However, this study and a recent work by Sharma *et al.* (2012) did not find significant evidence of past

demographic contraction (Chapter 3) which would have caused a downward bias in MIGRATE posterior distributions. Only the population of Bandhavgarh had below par *M* ratios (<0.68) suggestive of bottleneck, but the evidence was equivocal since no significant heterozygosity excess or a mode-shift in allele frequencies was detected. Furthermore, the significant heterozygosity deficit (symptomatic of recent population expansion) observed in the Pench and Kanha populations could mask signatures of population bottleneck as the addition of new individuals could increase the number of rare alleles which can bias allelic and heterozygosity distributions (Cornuet and Luikart 1996). Though bottlenecks were not observed in this study, the analyses may be undermined in a few populations having low sample sizes (<20 individuals), as tests such as the mode-shift in allele frequencies is known to be affected by sample size variances (Cornuet and Luikart 1996). However, a demographic decline may not necessarily result in a bottleneck as several factors such as duration of decline, pre-bottleneck diversity, and gene flow between populations can affect the probability of detecting a bottleneck (Cornuet and Luikart 1996; Garza and Williamson 2001; Girod *et al.* 2011; Peery *et al.* 2012). Based on both the theta estimates and absence of bottlenecks, our results suggest that the bigger source populations in the study area have had a relatively stable population history, compared to the smaller populations, as also observed in Sharma *et al.* (2013).

A source of bias in long-term gene flow estimations in MIGRATE is the reliance on mutation rates for estimating parameters, as depending on the particular mutation rate used, the historical migration rates may change by several orders of magnitude (Beerli and Felsenstein 2001; Abdo *et al.* 2004). Estimations of mutation rate are not available in felid species, and studies have instead mostly relied on mutation rate derived from human data to understand phylogeography, population history and divergence times in felid species (Driscoll *et al.* 2002; Anderson *et al.* 2004; Charruau *et al.* 2011; Sharma *et al.* 2013). However, mutation rates at microsatellite locus are complex, and have different rates of evolution, depending on repeat type and length (Chakraborty *et al.* 1997). Sharma *et al.* (2013) used a dinucleotide microsatellite mutation rate of 1×10^{-2} per generation in their estimation of historical migration rates from mutation-scaled estimates of

immigration rate (*M*). My study used three different classes of microsatellites comprising di-, tri- and tetranucleotide motiffs which complicated reliance on a single mutation rate. MIGRATE allows mutation rates to be modeled from the data in recognition of the subjectivity involved in assigning a single realistic rate for multiple classes of loci. I used this option in MIGRATE by letting the program calculate the specific locus-wise mutation rates from the data, as suggested by Beerli (2010). I found estimates of locus-wise mutation rates to be similar and consistent across all runs. Barring short run times (burn-in $<10^5$ iterations), the posterior distributions of *M* and theta were largely consistent across many different run lengths (see below).

Finally, a difficulty researchers routinely face is obtaining reliable estimates of Bayesian posterior distributions and convergence issues as there are no clear rules as to how long to run MIGRATE. Exploring optimal parameters and run length is particularly cumbersome on computationally intensive and slow programs such as MIGRATE (Beerli 2009). Even with access to cluster computing facilities which sped up the analyses eight to ten-fold compared to analysis on a single processor, my runs typically lasted anywhere from days to weeks to finish. This precluded a thorough analysis of the longest run lengths (≥50 million iterations) to assess the reliability of posterior distributions. MCMC runs below 10^5 burn-in iterations did not produce reliable convergence in my data set, even with sub-sampling a portion of the population. Typical runs where posteriors more or less converged were obtained around 10^6 burn-in and 5×10^6 total iterations. Hence for my final production run, I set the sampler at ten times this length, at 5×10^7 iterations of which the initial 1×10^7comprised the burn-in. Runs were investigated both with a random subset of the population as well as the entire data set. While randomly analysing a subset of *c*. ten individuals per population drastically reduced run time, it frequently inflated the posterior distributions of *M* but theta estimates were similarly consistent to runs with the full data set. Similar inconsistencies in the estimation of *M* have been reported in extensive simulation work which used earlier versions of the program (Abdo *et al.* 2004).

The overall findings of this chapter on gene flow study broadly reiterate the results of recent spatial genetic studies on tiger populations in the same area (Sharma *et al.* 2012, 2013; Joshi *et al.* 2013). Similarities, contrastsand limitations are inherent in all three studies which I discuss below. A possible concern with the migrant detection approaches used in my study is the low sample size in some populations. Except for Tadoba, this was not a consequence of less sampling effort or a lower ratio of sampled individuals to actual population size (Chapter 3), but likely reflects a truly smaller population. Simulation studies by Paetkau *et al.* (2004) caution the use of MCMC resampling methods implemented in Rannala and Mountain (1997) as they tend to over-estimate migrants from a limited data set. In my case, this translates to the fact that there may be less migration than I report in some of the smaller populations in my study system. Due to low sample sizes, individuals from Achanakmar (*n*=4) and the Pench-Kanha corridor (*n*=5) were not analyzed separately in my study, as doing that would have overestimated migration rates. Instead these localities were clubbed to the Kanha cluster, following the results of STRUCTURE assignments. Such a conservative approach was also adopted by Joshi *et al.* (2013) to detect migrants based on STRUCTURE clusters and migrant sensitivity tests.

Joshi *et al.* (2013) detected high pairwise genetic distances and low gene flow between most localities in the area, which is similar to my results and in contrast to the study by Sharma *et al.* (2013). However, sampling related biases may be of concern in their study as sites such as Kanha, Pench and Melghat were clearly under-sampled. STRUCTURE has a problem identifying a cluster that is represented by a low number of samples (Pritchard *et al.* 2000). Hence, though they relied on conservative methods (performed STRUCTURE migration prior sensitivity tests, and generated confidence limits on spatially correlated allele frequency maps) of migrant identification, the proportion of putative migrants with respect to sampled individuals in each population, was abnormally high ranging from 20% in Kanha (3 out of 15) and 28% in Pench (2 out of 7), to 40% in Melghat (2 out of 5). Particularly noteworthy in their study is the high proportion of emigrants (43%, i.e. 3 out of 7 total migrants) from Nagarjunasagar observed in distant areas such as Kanha and Pench. On the other hand, there is no immigration from other

localities into Nagarjunasagar as observed in the results of conservative (STRUCTURE and SCAT) analyses. Properly validated with more representative sampling and long-term monitoring, and with movement data from field based methods, these results may shed new light on density dependent processes of migration in large mammals (Carr *et al.* 2007), and dispersal behaviour in the tiger (Sunquist 1981; Smith 1993).

Long-distance dispersal in relatively undisturbed systems is not uncommon in large carnivores including tigers (Sunquist 1981; Heptner and Sludski 1992). However, in fragmented human-dominated habitats, it has hitherto been reported only in a few large carnivore species such as cougar (*Puma concolor*, Thompson *et al*. 2005; Stoner *et al.* 2008) and wolf (*Canis lupus*, Ciucci *et al.* 2010) which are resilient, with lesser energetic requirements and wider prey preferences (Thompson *et al*. 2009) compared to the tiger. Joshi *et al.* (2013) observed long-range dispersal across a human-dominated landscape between Nagarjunasagar and Kanha in the order of >600 km Euclidean distance, and state that tiger dispersal in Central India is not affected by distance but by human associated developmental features such as high density settlements. While fragmentary connectivity between Kanha and Nagarjunasagar is modeled in their study byusing landscape resistance surfaces, it is not clear how conducive the connecting habitat matrix will be for dispersing tigers, in terms of prey availability and mortality threats.

Another finding by Joshi *et al.* (2013) is the non-detection of migrants from Tadoba, which is in sharp contrast to the very high migration rates (>10%) obtained from this population to Nagzira (12%) and Melghat (19%) in their study. These patterns likely represent source-sink relationships as migration to Tadoba was less than 1% from both areas, and low power to identify migrants since all three populations belonged to the same cluster. Their study also observed symmetric migration between Kanha and Pench with both areas experiencing a high amount of migrants in both directions (>5%), though emigration from Kanha to Pench was slightly higher (9%). Individual population assignments indicate these two localities are part of a single population cluster. These results are in broad concurrence with the results of my study which show that Kanha and Pench have high migration rates and low genetic distances due to a large number of

immigrants. Similarly I also found Tadoba and Melghat are part of the same population cluster, with Melghat receiving migrants from Pench.

Unlike the results of the study by Joshi *et al.* (2013) and in a few populations in my study, low sample sizes do not seem to be an issue in the study by Sharma *et al.* (2013) as more tiger numbers were detected compared to current population size estimates (Jhala *et al.* 2011b) from the sampled areas. While stochastic laboratory errors in genotyping could bias population estimates (Creel *et al.* 2003), the higher tiger numbers may also be related to broader area coverage and non-differentiation of cubs from adults in the genetic tests, compared to field-based population estimation (Jhala *et al.* 2011b). Sharma *et al.* (2012) found high genetic diversity, and low genetic structuring indicative of admixture among the four tiger populations of Kanha, Pench, Melghat and Satpura, using microsatellite genotypic data from seven loci. In contrast the results of my study and Joshi *et al.* (2013) which used more microsatellite loci, detected visible hierarchical structuring between populations and low levels of gene flow, as evidenced from pairwise F_{ST} genetic distances and distinct population clustering in STRUCTURE. Sharma *et al.* (2013) attribute the observed low level of genetic structuring to high levels of contemporary and historic gene flow among these populations, which they subsequently demonstrated by a combination of migrant assignment tests and coalescent based modeling of gene flow and historical population divergence (Sharma *et al.* 2013).

While Sharma *et al.* (2013) were apparently not restricted by low sample numbers; non-detection of distinct genetic clusters in their data precluded them from using the conservative Bayesian clustering algorithm to identify migrants. Accordingly, they based their migrant decisions and gene flow estimations on indirect measures of genetic distance (F_{ST} and N_m) and assignment tests (Rannala and Mountain 1997; Paetkau *et al.* 2004). A major handicap in using genetic methods to identify migrants among undifferentiated or admixed populations as done in their study is the uncertainty of assigning likely population of origin to individuals. In such cases, the individual migrant assignments and migration rate estimates tend to get overestimated as these populations likely constitute a single interbreeding population (Wilson and Rannala 2003; Kuhner

2006; Beerli 2010). Though, the analytical approaches used in their study appear robust, it is unclear whether the indistinct population structuring and high gene flow reflects ground ecological reality or low resolution due to the fewer number of markers used.

Dispersal and gene flow are important processes promoting long-term viability in threatened and endangered species (Hanski and Gilpin 1997; Frankham *et al.* 2002). Measuring inter-population connectivity is important to document gene flow, and is a challenging parameter to measure in recently fragmented systems. Beyond the ecological question being investigated, my results show how you measure connectivity is important. Had I measured it strictly using measures of genetic differentiation (F_{ST} and N_m) or genetic distance as a surrogate for relative amounts of gene flow as done by Sharma *et al.* (2013), I would have missed and a major factor affecting tiger ecology and population dynamics the diminished female movement (i.e. demographic connectivity) across the fragmented human-dominated corridors. The use of robust statistical tools to assist clustering among highly variable genetic markers in my study demonstrate the ability and value of the genetic method to provide insights into demographic processes of immigration and emigration in recently disturbed systems, traditionally an intractable yet increasingly important ecological question worldwide.

As the human environment interrupts the dispersal process for tigers, population dynamic processes will be affected on several levels. My data provide evidence that diminishing connectivity is further fragmenting the patchily connected tiger populations in Central India into demographically and genetically isolated small populations. This has wide-ranging implications for long-term viability as the potential for the tiger populations in the region to operate as a metapopulation is dependent on demographic and genetic connectivity (male and female movement) between the local populations. Inter-population dispersal is necessary to facilitate population augmentation, rescue, or recolonization of small isolated populations. The paucity of female movement in particular and migration events in general leaves the population entirely dependent on local birth and death rates to remain functional, more so in species with sex-biased dispersal such as tigers and large mammals. The smaller populations such as

Achanakmar, Melghat and Satpura will hence be subject to increased risk of local extirpation over the long-term, due to deterministic demographic and genetic processes (Lande 1988; Frankham *et al.* 2002).

CHAPTER 6

SYNTHESIS

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The assessment of metapopulation structure and gene flow among tiger populations in the Central Indian landscape is the running theme of my thesis. Assessing gene flow in species across populations in complex fragmented habitats is critical to understand how landscapes structure genetic variation and maintain population connectivity (Manel *et al.* 2003). Historically, while some areas may have been naturally fragmented, tiger populations in Central India were connected by contiguous habitat and dispersing tigers (Forsyth 1919). It is perceived that the habitat and population contiguity would have resulted in a large panmictic population numbering upwards of 50,000 tiger individuals till the onset of large-scale anthropogenic induced fragmentation, about 200 years ago in the area (Mondol *et al.* 2009a). Starting from the Mughal and later the British periods, extensive logging for timber resources, coupled with bounty driven extermination and trophy hunting of tigers and other wildlife have fragmented and isolated most tiger populations in the area (Rangarajan 2001). The relentless pressure on the tiger and its habitat which continues unabated till today has fragmented the distribution of the once large contiguous population into patchily distributed metapopulations of varying long-term viability potential. Surviving tiger populations in the area are confined to remote pockets of forested habitat in the Central Indian highlands which have remained relatively undisturbed due to their inaccessible terrain. The surrounding plains and valleys have all been long colonized and natural areas converted to agriculture and settlements since ancient times (Rangarajan 2001). In recent years, rapid urbanization and proliferation of high speed road and rail networks, and large-scale mining activities threaten to further fragment tiger habitats (Jhala *et al.* 2011b; Fernandes 2012). It is in this context that I seek to understand if the existing tiger populations in the landscape still possess relictual connectivity through functional habitat corridors or whether they have been effectively fragmented into small isolated populations with reduced chances of long-term survival.

Tigers are a conservation-reliant species. Their survival is contingent upon the availability of undisturbed forested habitat having good ungulate prey base, with access to cover and water (Seidensticker *et al.*1999). Where human persecution has been effectively eradicated, and habitat and prey secured through legal protection and active enforcement, tiger populations have been found to rebound and thrive even in isolated habitats such as Bandhavgarh and Ranthambor. However history is replete with many cases of extinction events, which inform us time and again that increasingly isolated small populations cannot survive for long in the face of unrelenting human disturbance, as in the extinction of oceanic island populations of many species after human colonization (Frankham *et al.* 2002). The need for dispersal mediated connectivity is vital for the continued survival of small tiger populations in the area by linking them with larger populations which are relatively tolerant to the stochastic effects of environmental, demographic and genetic drift (Hanski and Gilpin 1997). At least two tiger reserves, Sariska and Panna, which suffered local extirpation events were entirely due to rampant poaching activities and further exacerbated by isolation from other tiger source populations (Gopal *et al.* 2010). Both areas have now been repopulated through translocation of individuals from nearby tiger reserves (Sankar *et al.* 2010; Gopal *et al.* 2010). While active translocation may be the last ditch effort to rescuing the demographic and genetic heath of isolated small populations, securing and maintaining linkages with important source populations through habitat corridors is important in regional conservation planning (Beier and Noss 1998; Crooks and Sanjayan 2006). Though the larger source populations which contain about 20 – 25 breeding pairs are relatively secure (Chapron *et al.* 2008; Gopal *et al.* 2010; Walston *et al.* 2010), the long-term metapopulation persistence of tigers is dependent on habitat connectivity and the dispersal capabilities of the species across highly patchy human-dominated landscapes (Smith 1993; Chapron *et al.* 2008).

Understanding the effects of habitat fragmentation and ecosystem change on the genetic structure of tiger populations is an important element of this study. From time immemorial, human populations have always been dependent on forests and their resources, and by their activity have fragmented natural ecosystems and the distribution

of species (Woodroffe *et al.* 2005). Human land-uses such as hunting, agriculture, cattle grazing, collection of timer and non-timber forest resources, and deliberately or accidentally caused fires, which result in fragmentation of forest ecosystems and their native species, conflict with conservation objectives. Fragmentation is the most serious form of habitat loss because very small losses can divide larger tiger populations into much smaller units that have a lower probability of persisting (Walston *et al.* 2010).

An important question that arises in context to the long line of spatial and temporal transition from historically connected tiger habitat to extant clumped tiger distribution is whether the process of fragmentation has slowed or stopped in the area? In particular, what are the drivers of short and long-term ecosystem change in the landscape? It seems unlikely that present trends of fragmentation will cease, as the tiger populations in the Indian subcontinent are located in one of the densest human populated areas in the world (Jhala *et al*. 2011b). While high human density in the vicinity of reserves puts significant strain on forest resources located inside protected areas, many urban centres located far away from tiger reserves also put adverse pressure on forested habitats (DeFries *et al.* 2010). Besides having several global priority tiger conservation units (TCU) of long-term conservation significance, the region of Central India is one of the most mineral rich areas in the country. Rising demand for energy resources by an exploding population and increasing urbanization has led to indiscriminate and unregulated mining in the region, conflicting with conservation interests as the mineral resources are mostly located within forest areas (Fernandes 2012). Also, the rapid increase in traffic volume, due to the proliferation in multi-lane highways and high speed rail networks, within swathes of vital habitat corridors linking tiger reserves need urgent conservation action (Vattakaven 2010). Conservation has low priority in the inexorable development schemes of a growing economy fuelled by consumption of high energy resources and better accessibility between urban centres. While development is unavoidable, it is essential that development activities incorporate a strong conservation ethic to balance needs. Halting and reversing these gargantuan forces that fragment habitats is a challenging proposition. All habitats cannot be saved but few representative priority areas need to be identified,

conserved and restored for maintaining landscape level gene flow (Noss 1987; Beier and Noss 1998).

While the protected area boundaries have somewhat stabilized due to effective legal protection and a blanket ban on timber extraction from forest areas, many forested habitats located outside reserves which have good conservation potential continue to be exploited, thus necessitating urgent conservation planning and restoration (Vattakaven 2010; WWF-India 2012). Some of these habitats already constitute or have potential to serve as vital corridors linking source populations, but are often lost to development projects. Conservation biology has often been referred to as the "science of scarcity", mostly because critical information is not readily available for most species as they are rare and endangered, so managers and biologists often need to plan conservation activity based on scarce empirical evidence (Soule 1985). There are several factors that hinder a landscape-based approach to tiger and overall biodiversity conservation such as lack of detailed information on species occurrence, the overly protected area centric conservation focus and undocumented evidence of corridor functionality (Sanderson *et al.* 2006; Tilson and Nyhus 2010). Since the last decade, the country-wide tiger monitoring exercise conducted every five years and intensive annual monitoring at priority source populations such as Kanha and Pench have yielded a wealth of information on the current state of habitats including reliable tiger and prey occupancy and population trends across major landscapes (Jhala *et al.* 2008, 2011b). However, the emphasis is almost entirely protected area centric, and solid scientific evidence is still needed to validate the functionality of corridors.

In this study, I have used non-invasive methods of sample collection together with genetic analyses of individual tigers to detect patterns of genetic structure among populations and identify recent tiger movement between source populations. The probability of detecting a migrant by the likelihood and Bayesian statistical analytic methods used in my study is contingent upon the investigated populations having sufficient genetic structure to unambiguously assign individuals to populations. Genetic structure is a consequence of genetic distance between populations, and genetic distances

are in turn determined primarily by genetic drift (Wright 1951). The process of genetic drift operates at variable rates in different populations, as it is subject to the variances of local effective population sizes, migration between population pairs and time since disturbance (Wright 1951). As such, the time scale that genetic distances reflect is variable and difficult to quantify. Traditional genetic methods invariably reveal processes that have occurred in the past, and as such may not reflect current state of habitat fragmentation in the landscape which is a more recent phenomenon (Whitlock and McCauley 1999). In this respect, the Bayesian individual clustering methods and assignment tests I used in my analyses are more suited to investigating recent population fragmentation and gene flow as they are free from the equilibrium based assumptions in classical population genetic approaches. Also, unlike traditional population based measures, these methods (Pritchard *et al.* 2000) do not require prior designation of population which can be arbitrary and difficult to delineate in recently disturbed systems.

Using individual assignment tests, I detected movement between population pairs, when I captured an animal in a local population other than the one it was born into. The assignment test avoids issues related with long-term genetic equilibrium by measuring gene flow within the last two generations only (Wilson and Rannala 2003; Piry *et al.* 2004). However, I sampled only a portion of animals living in my study area as my methods of field sampling were limited between one to sometimes two seasons in each reserve due to logistic constraints. Hence, I may have missed detecting more individuals moving between corridors or individuals which dispersed outside of my sampled season. Because the power to detect migrants using assignment tests decreases with low genetic separation between populations, this may imply that I may have missed migrants which may be moving between areas of low genetic distance.

While my study looks at a short temporal scale by only identifying recent tiger movement, the actual time frame in which the particular migrant dispersed from its natal area is impossible to tell with genetic data (except with long-term and large-scale radio-telemetry data). This is because dispersal and gene flow can have varying time frames from the perspective of individual tiger survival, reproductive success and overall life

history traits. The generation time in tigers is approximately 5 years and they are relatively long-lived animals with life spans of more than 10 years in the wild (Smith 1993). Philopatry entails that female offspring usually reside and establish territories adjacent to or overlapping their maternal home ranges, while male offspring usually start dispersing at a sub-adult stage. Considering their life spans and life history traits, this would mean that individual dispersal as observed in this study could reflect events that have occurred much earlier than observed. For example, an individual may have dispersed a decade ago, and I only detected it this year as a migrant. Due to their territorial nature and high intra-specific conflict, it is more likely that dispersing individuals have had limited and varying reproductive success compared to already established individuals in the new area (Smith 1993). Hence, actual dispersal and admixture events may have occurred much earlier than the approximate time in generations as observed from my analysis.

Movement and dispersal have different connotations from both an ecological and genetic perspective. The population assignment tests I used only identify an individual cross-assigned to another local population rather than its sampled population as a likely migrant. It is unable to distinguish whether the putative migrants identified in my study reflect actual dispersal events or ranging behavior of individuals. However, given that the geographic distance between sampled areas located in my study far exceed the regular home range sizes in the species my results are likely skewed towards the detection of individuals that have moved permanently from their natal area. I found movement rates to be low, with extremely few individuals showing first generation migrant status. Four out of the five identified first generation migrants were males. Despite uncertainty over the exact number of individuals with migrant ancestry, strong sex-biased dispersal and fragmentation are apparent. The identification of individuals with admixed ancestry signify that most gene flow events have occurred in the last few generations with extremely limited contemporary movement of tiger individuals of either gender. Furthermore, the large genetic distances between reserves suggest that there has been a reduction in the amount of tiger movement and gene flow between the areas. This has relevance for landscape based conservation efforts in the area, as apart from a few

functional corridors, much of the surrounding habitat outside reserves is heavily modified through human consumptive uses.

The process of dispersal in tigers begins once cubs reach about nineteen to twenty eight months in age, and requires several years with dispersing individuals establishing new home ranges often adjacent to the natal area in case of females (Smith 1993). Males generally disperse farther than females and often settle in poorer habitat since the better habitats are already occupied by resident tigers (Sunquist 1981; Smith 1993). When this process entails moving through risky human dominated patches, successful dispersal is greatly diminished as tigers spend extended periods of time in proximity to human habitations. Since many of these areas are depauperate of natural wild prey, often tigers predate on livestock and are killed as a result of threat to human and animal safety. Besides, a significant number of tigers and other wildlife are poached in these marginal habitats (Kumar and Wright 1999). Even tigers living inside reserves are not completely immune from the detrimental effects of poaching despite the best efforts at safeguarding them. Often these reserves have porous perimeters and are located in areas which are surrounded by high density human settlements. Many reserves also have a sizeable number of villages and people living inside their boundaries. There is often strong resentment or grudging acceptance of tiger conservation, on account of being denied access to forest resources in these areas (Karanth and Gopal 2005).

The conclusions from my work are threefold. First, there are relatively large genetic distances across tiger reserves as attested by the presence of well-defined genetic structure between most populations located in the study area. Second, dispersal and gene flow between tiger populations is limited due to heavy disturbance across the human-dominated habitat corridors in the landscape. Third, the sizes of genetic population clusters are clearly beyond the boundaries of protected areas, and have to be managed in a metapopulation framework. Genetic structuring was lowest among the Pench, Kanha and Achanakmar populations, evident in the low genetic distances, and the detection of large numbers of immigrant individualsandsome first generation migrants. All other populations had comparatively higher genetic distances and reduced gene flow. Though

Satpura, Melghat and Tadoba represented populations with fragmented connectivity, recent exchange of individuals was observed from the central clusters of Pench and Kanha. In terms of population division, the Bandhavgarh cluster is the most intense as evinced from distinctive allelic distributions, large genetic distances, and complete lack of gene flow, together with evidence of consistent and early split from other populations in the study area.

The genetic population structure that I detected among Central Indian tigers has important implications for conservation and management of this critically endangered and fragmented species. Conservation efforts must focus on the maintenance and if possible, expansion of forest habitat between tiger populations. Such actions remain a challenge to both managers and biologists alike, but should be attempted in all areas where substantial habitat is still present (Quinteiro *et al.* 2010). The best patches include the relatively intact forested corridors linking Kanha with Pench and Achanakmar. Most of the other areas require substantial restoration of corridors. The tiger populations linking Taboba with Melghat and Pench, and Pench with Satpura are separated by habitat discontinuities in the form of human settlements, agricultural land and heavy vehicular traffic. Much of the forest that constitute tiger habitat are already legally protected. However, portions of some key corridors such as those linking Pench with Kanha, Kanha with Achanakmar, Tadoba with Kanha, Melghat with Satpura, and Satpura with Pench have no legal status, or served by commercial forestry interests and developmental projects (Vattakaven 2010). Revision of status is necessary to prevent further fragmentation as these corridors are biologically functional and have evidence of resident tigers (Sharma *et al.* 2012; WWF-India 2012). Apart from habitat loss, human activities such as retributive killing for livestock depredation or perceived threat and commercial poaching have steadily increased mortality and decreased migration between key areas. The recent extirpations of tigers in Sariska and Panna and the few tigers which currently persist in Achanakmar are cases in point. Strict implementation of anti-poaching strategies (as done in Kaziranga, India), includinglocal communities as stakeholders in forest resources (as in Chitwan, Nepal), prompt compensation and lastly preventive actions such as voluntary relocation of people from areas where large carnivores

currently persist (as in Chilla area of Rajaji National Park, India) have met some success in reducing conflict and increasing tiger numbers in recent times (Seidensticker *et al.* 2010).

Tigers have the hallmarks of resilient species as they have high reproductive rates and dispersal capability (Sunquist *et al.* 1999). They have been known to recover quickly from heavy hunting in well protected, highly productive and prey rich habitats such as in Chitwan, Nepal, and disperse across long geographic distances in forested landscapes (Smith 1993). However, it remains to be seen how the current rate of fragmentation and deterioration in habitat quality, will impact the resilience of the overall metapopulation to stochastic and deterministic increases in mortality such as poaching (Kenny *et al.* 1995). Tigers are apex predators and have large home ranges in response to their high energy requirements. Like the tiger, its natural prey is also affected by the adverse effects of habitat fragmentation and poaching. Reduction in prey availability and access to resources like cover and water through habitat deterioration will decrease the survival of some small populations. With increased fragmentation, the amount of human-tiger interface at the perimeters of reserves also increases. Tigers that stray out of protected areas will be subject to high mortality. The low number of migrants seen in the area is probably due to a combination of avoidance and increased mortality in human-dominated habitats.

The eventual loss of connectivity between populations in the near future, as observed currently in Bandhavgarh, is likely for most populations in the area if present trends of fragmentation continue unabated. Already the connecting patches linking Pench and Satpura are getting fragmented though there was evidence of tiger occupancy in thisareaduring this decade (Jhala *et al.* 2008). In such situations, alternative strategies such as translocation need to be considered. Recent translocation efforts have successfully reverted local extinctions of tigers in Sariska and Panna (Gopal *et al.* 2010). While a discussion on the feasibility of translocation is beyond the scope of this thesis, the results of this study could help assist future translocation projects. Translocation effects could easily enrich genetic diversity by moving animals among each of the four

distinct genetic population clusters (Crandall *et al.* 2000). Translocation between localities would restore natural patterns of gene flow (Goossens *et al.* 2002), and avoid concerns associated with admixture of genetically distinct populations (Moritz *et al.* 1995; Luo *et al.* 2008). Translocations could be particularly effective in the localities of Melghat, Satpura and Achanakmar given their small population sizes, low contemporary migration and susceptibility to drift.

The work contained in my study has been exploratory in nature, as I did not design experimental procedures to test specific hypothesis. Rather, it was purposeful and organized at measuring variation around an existing state. By investigating genetic patterns arising from habitat and population fragmentation, I wanted to see if there were any links between observed genetic structure and population connectivity. Through this exploratory process, I obtained several interesting insights as to how differential dispersal and gene flow rates between populations were affecting metapopulation and landscape connectivity in tigers.While the genetic data offer important insights for the management of the Central Indian tigers, they only represent one aspect of information on the effects of habitat and population fragmentation facing the species. Many other factors including poaching pressure, prey depletion, population dynamics, spatial ecology, movement behaviour, habitat loss and fragmentation, corridor functionality, along with politics, logistics and economics need to be considered in developing a comprehensive landscape-based conservation plan for the species.

In my study I have only used genetic data to detect variation and pattern, and did not consider several other factors which can affect metapopulation function and gene flow. Habitat integrity, prey density, tiger occupancy and density in and around protected areas, are important ecological parameters and can all influence inter-population movement and dispersal (Sunquist *et al.* 1981, 1999; Smith *et al.* 1993; Miquelle *et al.* 1999; Karanth and Sunquist 2000; Jhala *et al.* 2011b). Prey distribution is governed by availability of suitable habitat and hence they are not uniformly distributed across the landscape. The patchy distribution of prey in the area (Jhala *et al.* 2011b) could in turn structure tiger distribution and influence inter-population dispersal. But beyond these correlative

patterns, the question still remains as to whether the patchiness is due to recent alterations in habitat, prey availability or past mortality patterns in the population? Of immediate conservation priority, one needs to know where and in which types of habitat can tigers disperse. Answers to these questions can reveal a better understanding of how tigers move between areas and the landscape elements that facilitate dispersal and promote metapopulation function. Due to the visible genetic structuring observed between most populations in the study area, it would be possible to apply the methods used in the study to monitor future changes in inter-population movement and connectivity strategies.

Investigating metapopulation structure and gene flow among species in patchy habitats has been a popular research question for many years now. As is the case with most research in ecology, interest and theory have preceded the tools available to study these aspects. These aspects were traditionally investigated and the theoretical foundations were conceptualized in abundant species with short life spans, which disperse small distances, form visible metapopulations and show patch extinction-colonization dynamics such as in many Lepidopteran species (Hanski 1994). Since then, the metapopulation models have been extended to include fragmented populations which are vulnerable to extirpation (Hanski and Gilpin 1997), but may not show classic patch extinction-recolonization dynamics such as in many large mammal species (Proctor *et al.* 2005). Large carnivores such as tigers are notoriously difficult to study, as they are elusive and occur at low densities over large areas. Studies based on radio-telemetry and camera-trapping have traditionally provided insights into dispersal behavior and spatial ecology of tiger populations (Karanth and Nichols 1998; Miquelle *et al.* 1999; Smith *et al.* 1999; Sunquist *et al.* 1999). The recent advent of non-invasive genetic sampling and individual assignment techniques has enabled landscape-level studies of population genetic structure and gene flow. This shift from traditional equilibrium based genetic measures to more realistic models incorporating how alleles actually segregate in natural systems, forms the basis of the genetic structuring and assignment tests used to investigate population structuring and detect migrants.

Range contraction and local extinctions across many species irrefutably affirm the negative impacts of fragmentation on other communities of the ecosystem. Elephant (*Elephas maximus*) and wild buffalo (*Bubalus arnee*) have long been extinct from the area, and they currently survive in few isolated pockets in the south-eastern periphery of the landscape. Seasonally migratory gaur (*Bos gaurus*) herds though relatively secure in some protected areas have suffered huge range contraction and went locally extinct in Bandhavgarh nearly two decades ago. Though recently reintroduced three years ago with individuals sourced from Kanha, the extinction of gaur herds in Bandhavgarh is symptomatic of larger habitat fragmentation events or a result of silent mortality from livestock borne diseases that have impaired movement and migratory contact with other source populations in the landscape (Pabla *et al.* 2011). Even sympatric large carnivores such as the leopard (*Panthera pardus*) though somewhat tolerant of disturbed habitats compared to the tiger, are also facing a reduction in gene flow and survive as a metapopulation (Dutta *et al.* 2012). The status of sloth bear (*Melursus ursinus*) and dhole (*Cuon alpinus*) is unknown, though the region is one of the last global strongholds for both species (Hunter 2009; Jhala *et al.* 2011b). Though reasonably secure inside protected areas, the sloth bear suffers heavily from anthropogenic killing, either retributively or preventively in conflict situations, as well as for commercial trade in gall bladders (Yoganand *et al.* 2006). Dhole populations are subject to episodic declines caused by infectious diseases, and this may be acting in concert with habitat and prey depletion to further fragment populations of this species (Venkataraman and Johnsingh 2004; Iyengar *et al.* 2005). These observations together with my results affirm the need to investigate the state of population fragmentation and metapopulation structure in a wider range of species across the Central Indian landscape. Further regional-scale fragmentation studies across the major tiger occupied landscapes in the subcontinent need to be conducted on a suite of species encompassing different guilds ofboth carnivore and prey species. Such studies are necessary to understand and improve the resolution of how communities are being structured and impacted by ecosystem-level changes.

APPENDIX

APPENDIX

Table A1. PCR in reference species, showing amplified (+) and not amplified (-) samples; cyt *b* 187 bp (this study), cyt *b* 309 bp (Kocher *et al.* 1989).

Sl No	Species	Sample Code	Lab ID	Description, Locality	Sample type	cyt *b* 187	cyt *b* 307
1	Tiger	Pti1	D254	Kanha, Madhya Pradesh	Blood	+	+
2	Tiger	Pti2	D528	Kanha, Madhya Pradesh	Blood	+	+
3	Tiger	Pti3	D398	Kanha, Madhya Pradesh	Blood	+	+
4	Tiger	Pti4	D404	Kanha, Madhya Pradesh	Blood	+	+
5	Tiger	Pti5	D426	Kanha, Madhya Pradesh	Blood	+	+
6	Tiger	Pti6	D442	Kanha, Madhya Pradesh	Blood	+	+
7	Tiger	Pti7	D430	Kanha, Madhya Pradesh	Blood	+	+
8	Tiger	Pti8	D401	Kanha, Madhya Pradesh	Blood	+	+
9	Tiger	Pti9	D407	Kanha, Madhya Pradesh	Blood	+	+
10	Tiger	Pti10	D399	Kanha, Madhya Pradesh	Blood	+	+
11	Tiger	Pti11	D402	Kanha, Madhya Pradesh	Blood	+	+
12	Tiger	Pti12	D253	Kanha, Madhya Pradesh	Blood	+	+
13	Tiger	Pti13	D532	Pench., Madhya Pradesh	Blood	+	+
14	Tiger	Pti14	D431	Pench., Madhya Pradesh	Blood	+	+
15	Tiger	Pti15	D1122	Pench., Madhya Pradesh	Blood	+	+
16	Tiger	Pti16	D530	Ranthambhore, Rajasthan	Blood	+	+
17	Tiger	Pti17	D531	Ranthambhore, Rajasthan	Blood	+	+
18	Tiger	Pti18	D432	Ranthambhore, Rajasthan	Blood	+	+
19	Leopard	Ppa1	D680	Ranthambhore, Rajasthan	Tissue	+	+
20	Leopard	Ppa2	D801	Kanha, Madhya Pradesh	Scat	+	+
21	Leopard	Ppa3	D223	Gir National Park, Gujarat	Tissue	+	+
22	Leopard	Ppa4		Uttarakhand	Tissue	+	+
23	Leopard	Ppa5		Uttarakhand	Tissue	+	+
24	Leopard	Ppa6		Uttarakhand	Tissue	+	+
25	Leopard	Ppa7		Uttarakhand	Tissue	+	+
26	Leopard	Ppa8		Uttarakhand	Tissue	+	+
27	Leopard	Ppa9		Uttarakhand	Tissue	+	+
28	Striped Hyena	Hy1	D537	Kutch, Gujarat	Blood	-	+
29	Striped Hyena	Hy2	D557	Kutch, Gujarat	Blood	-	+
30	Striped Hyena	Hy3	D926	Rajaji N. P., Uttarakhand	Tissue	-	+
31	Striped Hyena	Hy4	D943	Ranthambhore, Rajasthan	Tissue	-	+
32	Striped Hyena	Hy5	D944	Ranthambhore, Rajasthan	Tissue	-	+
33	Striped Hyena	Hy6	D945	Sawai Mansingh WLS, Rajasthan	Tissue	-	+
34	Wolf	Cl1	D1	Velavadar N.P., Gujarat	Blood	-	+
35	Wolf	Cl2	D50	Velavadar N.P., Gujarat	Blood	-	+
36	Wolf	Cl3	D163	Velavadar N.P, Gujarat	Blood	-	+
37	Domestic Dog	Clf1	D177	Local breed, Assam	Hair	-	+
38	Domestic Dog	Clf2	D190	Ghaddi Dog, Palampur, Himachal	Hair	-	+
39	Domestic Dog	Clf3	D231	Gir N.P., Gujarat	Blood	-	+
40	Golden Jackal	Ca1	D156	Velavadar N.P., Gujarat	Tissue	-	+
41	Golden Jackal	Ca2	D172	Velavadar N.P., Gujarat	Blood	-	+

42	Golden Jackal	Ca3	D246	Haryana	Tissue	-	+
43	Sloth Bear	Mu1	D1351	Kanha-Pench Corridor, M.P.	Scat	-	+
44	Domestic Goat	Ch1		Dehradun, Uttarakhand	Tissue	-	+
45	Wild Pig	Ssc1	D174	Velavadar N.P., Gujarat	Tissue	-	+
46	Human	Hs1		B Yumnam (experimenter)	Blood	-	+

Table A2. Information on thepilot test carried out on scats (*n*=65) for species identification of tiger samplesby PCR and *Bam*HI restriction enzyme digestion.

Sl No	Sample ID	Locality	Band 1 (bp)	Band 2 (bp)	Species ID, Remarks
1	D495	Kanha Tiger Reserve	67	120	Tiger
2	D496	Kanha Tiger Reserve	67	120	Tiger
3	D499	Kanha Tiger Reserve	67	120	Tiger
4	D500	Kanha Tiger Reserve	67	120	Tiger
5	D501	Kanha Tiger Reserve	67	120	Tiger
6	D502	Kanha Tiger Reserve	67	120	Tiger
7	D503	Kanha Tiger Reserve	67	120	Tiger
8	D504	Kanha Tiger Reserve	67	120	Tiger
9	D505	Kanha Tiger Reserve	67	120	Tiger
10	D506	Kanha Tiger Reserve	67	120	Tiger
11	D507	Kanha Tiger Reserve	--	--	not established, PCR failed
12	D510	Kanha Tiger Reserve	67	120	Tiger
13	D512	Kanha Tiger Reserve	67	120	Tiger
14	D516	Kanha Tiger Reserve	67	120	Tiger
15	D517	Kanha Tiger Reserve	67	120	Tiger
16	D518	Kanha Tiger Reserve	67	120	Tiger
17	D569	Pench Tiger Reserve	67	120	Tiger
18	D570	Pench Tiger Reserve	67	120	Tiger
19	D571	Pench Tiger Reserve	67	120	Tiger
20	D572	Pench Tiger Reserve	67	120	Tiger
21	D574	Pench Tiger Reserve	67	120	Tiger
22	D576	Pench Tiger Reserve	67	120	Tiger
23	D577	Pench Tiger Reserve	67	120	Tiger
24	D578	Pench Tiger Reserve	67	120	Tiger
25	D579	Pench Tiger Reserve	67	120	Tiger
26	D580	Pench Tiger Reserve	67	120	Tiger
27	D581	Pench Tiger Reserve	67	120	Tiger
28	D582	Pench Tiger Reserve	67	120	Tiger
29	D792	KanhaTiger Reserve	67	120	Tiger
30	D793	KanhaTiger Reserve	67	120	Tiger
31	D794	KanhaTiger Reserve	67	120	Tiger
32	D795	KanhaTiger Reserve	67	120	Tiger
33	D796	KanhaTiger Reserve	67	120	Tiger
34	D797	KanhaTiger Reserve	67	120	Tiger
35	D798	KanhaTiger Reserve	67	120	Tiger
36	D800	KanhaTiger Reserve	67	120	Tiger
37	D1371	Pench Tiger Reserve	67	120	Tiger
38	D1373	Pench Tiger Reserve	67	120	Tiger

39	D1374	Pench Tiger Reserve	67	120	Tiger
40	D1376	Pench Tiger Reserve	--	--	not established, PCR failed
41	D1377	Pench Tiger Reserve	67	120	Tiger
42	D1378	Pench Tiger Reserve	67	120	Tiger
43	D1379	Pench Tiger Reserve	67	120	Tiger
44	D1380	Pench Tiger Reserve	67	120	Tiger
45	D1381	Pench Tiger Reserve	67	120	Tiger
46	D1382	Pench Tiger Reserve	67	120	Tiger
47	D1383	Pench Tiger Reserve	67	120	Tiger
48	D1384	Pench Tiger Reserve	67	120	Tiger
49	D1385	Pench Tiger Reserve	67	120	Tiger
50	D1390	Pench Tiger Reserve	67	120	Tiger
51	D1391	Pench Tiger Reserve	187	--	leopard
52	D1392	Pench Tiger Reserve	187	--	leopard
53	D1393	Pench Tiger Reserve	67	120	Tiger
54	D1394	Pench Tiger Reserve	--	--	not established, PCR failed
55	D1395	Pench Tiger Reserve	--	--	not established, PCR failed
56	D1396	Pench Tiger Reserve	187	--	leopard
57	D1397	Pench Tiger Reserve	187	--	leopard
58	D1398	Pench Tiger Reserve	187	--	leopard
59	D1404	Pench Tiger Reserve	187	--	leopard
60	D1184	KanhaTiger Reserve	67	120	Tiger
61	D1185	KanhaTiger Reserve	67	120	Tiger
62	D1288	Kanha- Pench corridor	187	--	leopard
63	D1291	Kanha- Pench corridor	187	--	leopard
64	D1292	Kanha- Pench corridor	187	--	leopard
65	D1296	Kanha- Pench corridor	187	--	leopard

Table A3. Microsatellite loci genotype data of individual tigers, including information on gender (M-male; F-female; U – unknown), sampling site and GPS coordinates

No.	ID/ sample type	Gender	Site	Latitude/ Longitude	Pati01	Pati09	Fca304	Fca441	6HDz700	F85	Fca954	F124	Pati15	F53	Pati18
1	D1598	M	MTR	21.32408	200	123	129	149	137	176	180	262	211	144	217
	scat			77.09781	203	129	129	157	144	176	192	270	214	149	217
2	D1599	F	MTR	21.31753	188	114	127	149	137	0	171	266	211	144	225
	scat			76.95233	188	123	129	157	139	0	171	270	211	144	225
3	D1622	U	MTR	21.34586	188	120	129	149	144	155	171	266	211	0	213
	scat			77.05544	209	123	129	157	146	164	180	266	214	0	225
4	D1843	M	MTR	21.30333	194	114	125	140	141	164	180	262	0	0	213
	scat			76.94000	206	123	127	149	146	176	180	270	0	0	213
5	D1862	F	MTR	21.29656	188	114	129	140	137	155	169	266	211	0	225
	scat			76.97986	188	123	143	149	139	164	169	270	211	0	225
6	D1892	M	MTR	21.37644	188	126	129	149	141	164	180	262	205	149	213
	scat			77.09533	203	129	129	153	144	164	192	270	217	161	221
7	D1900	M	MTR	21.35133	188	120	129	157	144	164	171	250	211	149	221
	scat			76.95997	200	123	143	157	144	164	171	266	211	153	225
8	D1911	M	MTR	21.26425	203	123	129	153	139	164	180	262	214	149	225
	scat			77.03481	206	129	129	157	141	164	192	270	214	149	229
9	D2056	F	MTR	21.33550	188	114	129	149	137	155	171	266	211	144	213
	scat			77.06281	188	123	143	153	139	164	171	270	211	144	225
10	D2057	F	MTR	21.32414	188	114	129	149	137	159	180	262	211	144	213
	scat			76.96075	188	120	129	157	144	164	192	270	214	149	225
11	D2058	F	MTR	21.29964	188	114	129	140	139	168	171	0	211	140	0
	scat			76.86083	203	123	129	149	141	176	171	0	211	140	0
12	D2060	F	MTR	21.33336	188	114	129	149	137	159	171	266	211	144	0
	scat			77.05286	188	123	143	153	139	164	171	270	211	144	0
13	D1837	M	MTR	21.25453	188	114	129	140	137	155	180	262	211	0	213
	scat			77.06614	188	120	129	149	144	159	192	270	214	0	225
14	D2073	F	MTR	21.37792	188	114	129	140	137	159	180	262	211	144	213
	scat			76.95742	188	120	129	149	144	164	192	270	214	149	225
15	D2075	F	MTR	22.46708	188	114	129	144	137	155	171	266	211	144	213
	scat			78.19664	188	123	143	149	139	164	171	270	211	144	213

16	D1476	F	STR	22.52881	200	114	127	149	139	0	171	250	208	140	221
	scat			78.19681	200	120	129	157	144	0	184	258	208	149	221
17	D1480	M	STR	22.52872	200	120	127	149	144	159	180	258	208	144	221
	scat			78.20200	206	126	143	149	146	172	184	270	211	149	225
18	D1926	F	STR	22.50828	200	114	125	149	141	164	171	0	208	149	0
	scat			78.05969	206	123	129	149	146	164	178	0	211	149	0
19	D1962	M	STR	22.50533	206	120	125	149	144	155	171	262	208	0	221
	scat			78.06272	206	129	129	149	146	164	184	262	208	0	225
20	D1987	M	STR	22.50908	200	120	129	149	139	159	180	262	211	144	221
	tissue			78.01744	203	129	129	157	144	168	184	270	235	149	225
21	D2002	F	STR	22.44067	200	120	125	149	146	164	171	262	208	149	221
	scat			78.17089	206	129	129	149	146	164	184	266	211	149	225
22	D2003	F	STR	22.52464	206	120	125	149	144	155	173	262	208	0	221
	scat			78.05142	206	129	129	149	146	164	184	262	211	0	221
23	D2022	U	STR	22.53375	206	114	125	149	144	0	0	262	208	144	221
	scat			78.06433	206	120	129	149	146	0	0	262	208	149	225
24	D1963	M	STR	22.51211	206	114	125	140	144	155	171	262	208	144	0
	scat			78.02303	206	120	129	149	146	164	184	262	211	149	0
25	D2020	M	STR	22.51178	206	114	125	149	144	155	171	262	208	149	221
	scat			78.09867	206	120	129	157	146	164	184	262	211	149	221
26	D2030	F	STR	21.76322	206	120	125	149	144	155	171	262	208	149	221
	scat			79.29550	206	129	129	157	146	164	184	262	211	149	221
27	D532	F	PTR	21.74444	200	120	127	149	141	164	180	262	211	140	209
	blood			79.31867	203	126	129	157	146	168	186	262	214	140	225
28	D431	M	PTR	21.79069	209	123	129	149	141	168	180	262	208	140	221
	blood			79.26103	209	129	129	157	141	176	186	270	211	140	225
29	D1122	M	PTR	21.75722	200	123	125	149	139	164	171	262	211	140	209
	blood			79.29422	209	123	127	157	141	164	186	270	235	149	225
30	PSG01	F	PTR	21.79903	200	123	127	140	141	164	180	262	211	140	209
	scat			79.31525	203	129	129	149	146	168	186	262	214	140	209
31	PSG02	M	PTR	21.74458	194	114	129	140	141	155	180	262	208	140	225
	scat			79.35256	209	123	129	149	144	164	196	270	211	149	225
32	D954	M	PTR	21.69647	194	123	125	149	139	164	178	262	235	0	221
	scat			79.31489	203	123	127	157	141	164	190	274	235	0	221
33	D955	M	PTR	21.72622	191	117	119	140	137	159	178	270	208	153	0
	scat			79.27375	203	123	129	140	141	168	178	274	235	153	0

34	D958	F	PTR	21.74661	194	123	123	140	139	168	175	0	0	153	205
	scat			79.24936	206	123	129	149	141	168	178	0	0	153	221
35	D970	M	PTR	21.73458	194	123	125	140	144	164	180	270	208	140	221
	scat			79.24794	206	123	129	149	146	176	186	270	211	140	225
36	D1024	F	PTR	21.74739	200	123	125	153	141	164	171	262	211	144	205
	scat			79.35492	209	126	125	157	141	168	171	270	235	149	221
37	D1026	F	PTR	21.75042	203	123	127	144	141	133	169	270	208	140	221
	scat			79.35692	209	126	129	149	141	133	178	270	211	149	221
38	D1029	F	PTR	21.78269	203	123	129	149	144	164	180	266	208	140	221
	scat			79.34628	209	129	129	157	146	168	180	270	211	161	225
39	D1039	M	PTR	21.74575	200	123	125	149	139	123	171	270	208	149	221
	scat			79.33617	209	123	129	157	141	164	180	270	235	149	225
40	D1043	M	PTR	21.71328	203	123	127	149	141	168	180	262	208	140	209
	scat			79.27497	203	129	129	157	146	176	186	266	214	161	221
41	D1051	F	PTR	21.71544	203	114	125	149	141	168	175	262	196	161	209
	scat			79.17119	209	123	127	149	141	176	186	262	217	161	209
42	D1052	M	PTR	21.78131	203	123	125	140	141	164	180	262	211	140	209
	scat			79.21617	209	129	127	144	144	168	180	270	217	140	225
43	D1064	M	PTR	21.81711	203	123	125	153	144	164	178	262	211	140	0
	scat			79.22858	209	129	127	157	144	168	180	270	217	149	0
44	D1066	F	PTR	21.83278	200	123	115	149	141	133	178	266	196	140	0
	scat			79.27617	203	129	129	149	144	164	180	266	211	149	0
45	D1073	F	PTR	21.76800	203	123	125	149	141	159	171	262	196	140	225
	scat			79.32292	206	129	127	157	141	168	180	266	211	149	225
46	D1075	M	PTR	21.74444	203	114	125	149	139	164	171	270	0	149	0
	scat			79.31867	206	123	125	157	141	168	180	270	0	149	0
47	D1076	M	PTR	21.71900	203	123	127	140	141	164	180	262	211	140	209
	scat			79.30625	209	129	129	140	141	168	180	270	217	149	225
48	D1079	F	PTR	21.79903	200	123	127	149	141	164	171	262	211	149	209
	scat			79.32133	206	126	129	157	141	168	180	262	214	149	217
49	D1381	M	PTR	21.76011	191	114	125	140	141	159	180	262	214	144	213
	scat			79.32558	206	123	127	144	144	164	184	270	226	149	221
50	D1383	M	PTR	21.79206	191	123	125	149	137	159	171	270	205	144	213
	scat			79.28067	200	129	127	157	141	133	180	274	208	149	221

51	D1390	M	PTR	21.74936	200	129	129	140	141	164	180	266	208	140	209
	scat			79.35797	203	129	143	157	144	164	186	274	217	149	221
52	D1393	M	PTR	21.70983	191	123	125	149	137	159	171	262	208	140	205
	scat			79.31989	197	123	129	149	144	164	180	270	208	149	205
53	D1394	F	PTR	21.71575	191	114	127	144	139	155	186	266	208	128	0
	scat			79.30100	197	123	129	144	144	176	186	274	217	149	0
54	D1395	F	PTR	21.72125	191	114	127	140	139	155	171	258	208	140	221
	scat			79.20231	197	123	129	144	141	168	180	270	211	149	225
55	D1399	M	PTR	21.76542	191	123	125	140	131	159	180	258	0	149	221
	scat			79.36997	206	129	129	149	141	164	196	258	0	153	225
56	D1400	M	PTR	21.85100	191	123	123	140	139	159	196	266	0	140	213
	scat			79.50272	206	129	125	149	141	164	196	270	0	149	221
57	D571	F	PTR	21.83478	200	123	125	149	133	164	171	258	205	144	217
	scat			79.49561	200	126	129	157	135	168	171	270	214	149	221
58	D573	F	PTR	21.85978	206	129	127	144	141	164	171	266	208	140	209
	scat			79.52422	206	129	145	144	144	164	184	278	211	149	221
59	D574	M	PTR	21.85808	200	123	127	140	144	164	171	266	214	140	217
	scat			79.52594	203	129	129	149	146	176	180	270	214	149	225
60	D576	M	PTR	21.85742	191	123	127	140	144	164	180	262	208	140	221
	scat			79.52681	203	129	127	149	146	176	186	270	211	149	225
61	D577	F	PTR	21.85394	200	123	125	136	0	164	169	262	205	149	217
	scat			79.52803	209	123	125	149	0	164	180	270	211	149	225
62	D578	F	PTR	21.84903	191	123	129	149	144	164	180	266	0	140	0
	scat			79.52322	203	129	143	157	146	176	180	270	0	149	0
63	D580	M	PTR	21.84764	203	123	129	140	144	164	180	266	208	140	221
	scat			79.52472	203	129	129	149	146	176	180	270	211	140	225
64	D581	M	PTR	21.83078	191	123	127	149	144	164	180	258	205	149	217
	scat			79.50497	200	126	127	157	146	172	192	270	211	149	225
65	D1109	F	PTR	21.83467	200	123	125	153	144	164	171	258	202	144	217
	scat			79.49767	200	126	129	153	144	164	171	270	214	149	217
66	D1111	M	PTR	21.83608	203	123	129	149	141	168	180	262	208	140	221
	scat			79.48286	209	129	129	157	141	176	186	270	211	140	225
67	D1114	F	PTR	21.86408	200	123	125	140	141	123	171	258	205	144	217
	scat			79.51922	200	126	129	149	144	133	178	270	214	149	221
68	D1580	F	PTR	21.88186	191	123	127	149	144	164	171	270	205	144	217
	scat			79.52214	200	126	129	149	144	164	171	282	208	149	221

69	D1581	F	PTR	21.83736	200	123	123	140	141	164	171	258	205	144	217
	scat			79.49303	200	126	127	149	144	164	171	270	214	149	221
70	D1582	F	PTR	21.88306	206	129	125	153	144	164	171	266	208	140	209
	scat			79.55747	206	129	141	157	146	164	184	278	208	149	221
71	D1584	M	PTR	21.86053	200	123	125	149	144	164	180	266	208	140	221
	scat			79.53108	203	129	127	149	146	176	180	270	211	140	225
72	D1585	U	PTR	21.85833	191	123	127	144	144	164	171	266	208	140	221
	scat			79.52603	203	129	127	149	144	168	180	270	211	140	225
73	D1587	F	PTR	21.85961	200	123	121	149	144	164	0	270	205	149	217
	scat			79.52503	209	123	127	157	144	164	0	270	211	149	225
74	D1588	F	PTR	21.86147	200	123	121	149	141	164	0	270	205	149	217
	scat			79.52567	209	123	127	157	144	168	0	270	211	149	225
75	D1591	F	PTR	21.86147	200	123	125	149	141	164	171	258	205	144	217
	scat			79.52567	200	126	129	149	144	164	171	270	214	149	221
76	D1592	M	PTR	21.86086	206	129	125	153	0	164	171	266	208	140	209
	scat			79.51644	206	129	129	157	0	164	184	270	211	149	221
77	D1594	U	PTR	21.85956	191	117	129	149	144	155	0	0	208	136	213
	scat			79.56908	209	123	129	157	144	155	0	0	208	149	213
78	D1330	F	KTR	21.75944	206	123	129	140	131	164	0	0	196	0	0
	scat			79.61094	206	126	141	149	141	168	0	0	208	0	0
79	D1332	M	KTR	21.84369	191	120	119	140	139	168	178	0	208	161	221
	scat			79.52700	200	123	129	149	139	168	188	0	220	161	225
80	D1337	M	KTR	22.33792	191	120	127	140	131	151	0	0	202	0	0
	scat			80.05689	203	126	129	149	141	159	0	0	202	0	0
81	D1297	F	KTR	22.00536	203	123	125	144	141	164	186	262	205	0	0
	scat			79.92267	206	123	127	149	144	168	190	262	208	0	0
82	D1336	U	KTR	22.26864	188	120	127	140	131	140	0	266	0	0	0
	scat			80.43906	191	126	127	149	135	164	0	274	0	0	0
83	D1299	M	KTR	22.27989	203	123	123	149	139	159	169	258	205	0	0
	scat			80.66178	206	126	127	157	141	168	184	270	208	0	0
84	D254	F	KTR	22.31608	206	123	129	153	139	159	171	266	217	149	221
	blood			80.57050	206	129	143	157	139	164	180	274	235	149	221
85	D528	M	KTR	22.27106	206	120	125	153	141	159	171	258	208	149	221
	blood			80.61983	206	120	129	157	141	176	180	266	217	153	225

86	D398	M	KTR	22.20917	206	123	127	149	141	159	171	262	208	149	221
	blood			80.62706	206	129	129	149	144	159	180	270	217	149	221
87	D403	M	KTR	22.28986	200	123	123	149	141	164	180	266	208	149	221
	blood			80.57964	206	129	123	149	141	176	186	270	208	153	225
88	D426	M	KTR	22.27989	206	123	129	153	141	168	171	258	208	149	221
	blood			80.66178	206	123	129	157	141	176	186	262	208	153	225
89	D441	M	KTR	22.28592	203	120	127	149	153	159	180	266	211	149	221
	blood			80.65869	206	126	143	157	153	159	180	270	220	149	225
90	D430	M	KTR	22.28561	203	123	141	153	139	164	171	270	217	149	221
	blood			80.63789	206	126	143	157	146	176	180	274	235	149	221
91	D401	F	KTR	22.26444	203	114	127	157	141	159	180	258	235	144	205
	blood			80.63683	206	123	143	157	141	172	186	262	235	161	221
92	D407	F	KTR	22.28261	200	114	127	157	141	159	180	258	217	149	221
	blood			80.64853	206	123	143	157	146	172	180	270	235	161	221
93	D399	F	KTR	22.26425	200	114	127	157	141	159	180	258	217	149	221
	blood			80.63631	206	123	143	157	144	172	180	270	235	161	221
94	D402	F	KTR	22.31894	203	114	127	157	141	164	180	258	235	144	205
	blood			80.61136	206	123	143	157	141	172	186	262	235	161	221
95	D406	F	KTR	22.29806	206	123	129	157	139	159	171	262	217	140	217
	blood			80.63969	206	129	129	157	139	164	180	270	217	149	217
96	D272	F	KTR	22.27231	206	123	129	136	139	159	171	266	217	140	205
	scat			80.66253	209	129	143	140	141	164	180	274	235	149	221
97	D273	M	KTR	22.21461	194	123	129	149	139	159	171	270	0	149	205
	scat			80.96689	206	129	143	157	139	164	180	274	0	149	221
98	D288	M	KTR	22.28400	188	123	129	140	139	159	171	262	208	0	221
	scat			80.59156	206	126	129	149	141	172	171	262	217	0	221
99	D290	M	KTR	22.18694	206	123	125	149	135	151	171	0	208	149	0
	scat			80.91950	206	129	129	149	141	164	180	0	208	153	0
100	D296	F	KTR	22.23658	206	123	125	149	141	159	171	262	0	144	0
	scat			80.64244	206	123	125	149	144	176	180	270	0	153	0
101	D489	M	KTR	22.27678	200	123	127	149	139	159	180	266	217	149	221
	scat			80.56936	206	123	143	157	144	159	180	270	235	149	221
102	D503	F	KTR	22.20594	206	117	119	149	141	159	171	258	0	140	221
	scat			80.59414	206	123	129	157	141	172	180	258	0	144	221
103	D504	F	KTR	22.22839	194	114	127	149	141	159	180	0	0	144	217
	scat			80.60114	194	123	129	157	150	164	180	0	0	149	217

104	D507	M	KTR	22.25808	206	114	127	153	141	155	171	258	208	149	205
	scat			80.61797	206	123	129	157	141	155	196	262	208	153	221
105	D510	F	KTR	22.28564	194	114	143	149	141	164	175	270	205	149	205
	scat			80.58906	206	120	143	157	144	168	190	270	208	153	221
106	D586	F	KTR	22.18386	188	117	127	149	141	159	169	262	214	149	0
	scat			80.61461	194	123	129	157	141	164	184	270	214	149	0
107	D789	M	KTR	22.26756	206	123	129	149	141	164	178	258	208	140	221
	scat			80.63139	206	123	129	157	141	176	180	262	208	149	225
108	D795	F	KTR	22.28972	206	123	125	149	139	164	180	258	208	153	217
	scat			80.70136	206	126	125	153	141	176	180	266	208	153	225
109	D1131	M	KTR	22.27739	200	123	127	140	139	159	180	274	217	149	209
	scat			80.60581	206	123	129	149	144	159	180	274	217	149	221
110	D1137	F	KTR	22.26425	203	123	125	149	137	159	171	262	208	149	217
	scat			80.63333	206	123	129	157	139	159	180	262	217	149	217
111	D1140	F	KTR	22.29061	203	123	125	140	141	164	171	0	208	144	0
	scat			80.61247	206	123	127	149	146	168	180	0	208	161	0
112	D1144	F	KTR	22.24764	200	114	127	149	141	159	180	262	208	149	0
	scat			80.60750	206	123	143	157	141	172	180	270	217	161	0
113	D1162	M	KTR	22.30344	188	117	125	149	137	159	171	266	208	149	0
	scat			80.54950	206	126	141	153	139	168	180	270	235	161	0
114	D1164	F	KTR	22.28561	200	123	127	149	141	151	180	258	208	149	221
	scat			80.60456	206	129	143	157	144	159	180	270	217	161	221
115	D1165	F	KTR	22.27989	206	123	125	149	141	151	180	0	208	153	0
	scat			80.66178	206	123	127	149	141	151	190	0	208	153	0
116	D1168	M	KTR	22.28564	203	123	125	149	139	151	180	0	208	149	209
	scat			80.58906	206	129	129	149	139	159	180	0	208	149	221
117	D1170	M	KTR	22.28819	200	123	127	149	139	151	180	266	208	149	221
	scat			80.58481	206	123	143	157	144	159	180	270	217	149	221
118	D1172	F	KTR	22.28944	200	117	127	149	139	151	180	0	208	149	0
	scat			80.71119	203	123	129	157	144	159	180	0	208	149	0
119	D1174	F	KTR	22.15939	203	123	127	140	139	159	169	0	208	149	0
	scat			80.91458	206	129	129	149	141	159	180	0	217	149	0
120	D1176	F	KTR	22.13969	203	123	127	157	139	159	180	266	208	149	221
	scat			80.97469	206	129	129	157	139	159	180	270	217	149	221

121	D1179	M	KTR	22.15811	206	123	129	153	137	159	171	262	208	149	217
	scat			80.95394	206	129	143	157	139	172	171	266	217	149	221
122	D1180	F	KTR	22.19367	191	123	127	140	141	159	171	262	0	149	209
	scat			80.94622	206	123	129	149	144	164	186	270	0	153	221
123	D1182	M	KTR	22.12658	191	123	127	140	141	159	171	258	0	136	209
	scat			80.93914	206	123	129	149	141	164	178	270	0	149	221
124	D1183	F	KTR	22.14142	191	123	125	140	141	159	171	258	0	149	209
	scat			80.91206	206	123	129	140	141	164	186	270	0	153	209
125	D1185	M	KTR	22.15711	191	114	127	140	141	159	184	266	0	140	0
	scat			80.95581	200	123	129	157	141	164	190	274	0	149	0
126	D1193	F	KTR	22.18664	206	123	129	140	141	159	171	262	0	144	0
	scat			80.99719	206	129	129	149	141	168	171	262	0	153	0
127	D1194	M	KTR	22.18903	206	123	127	140	141	159	180	258	0	149	221
	scat			80.98256	206	129	129	149	141	159	188	258	0	153	221
128	D1196	M	KTR	22.14153	206	123	125	140	141	159	178	0	226	149	205
	scat			80.90147	206	129	127	149	141	164	190	0	226	153	225
129	D1198	F	KTR	22.14414	191	123	127	149	141	151	190	0	0	149	217
	scat			80.99528	206	129	129	157	141	164	190	0	0	153	221
130	D1200	U	KTR	22.22394	188	114	129	140	137	164	186	270	0	144	0
	scat			80.96911	206	123	129	153	141	164	192	270	0	149	0
131	D1605	F	KTR	22.18703	206	123	125	140	141	151	171	262	208	0	0
	scat			80.94092	209	123	129	149	144	159	178	270	217	0	0
132	D1298	F	KTR	22.05717	203	117	125	144	135	0	0	262	208	132	0
	scat			81.08717	206	123	127	149	144	0	0	262	208	153	0
133	D1217	M	ATR	22.46922	191	123	127	149	139	151	178	270	205	149	221
	scat			81.80492	197	126	129	157	141	151	180	270	208	149	225
134	D1284	F	ATR	22.51572	191	123	125	149	139	164	180	258	208	149	205
	scat			81.74408	191	126	127	157	141	172	186	270	208	153	209
135	D1285	M	ATR	22.53528	191	123	127	149	135	164	175	270	208	149	209
	scat			81.80217	206	126	129	153	141	168	178	270	208	149	221
136	D1637	F	ATR	22.40417	200	114	125	149	139	164	180	258	208	0	209
	scat			81.84158	206	123	127	157	141	172	186	262	217	0	209
137	D524	M	TATR	20.36008	200	120	127	140	131	140	0	258	208	0	0
	scat			79.31900	200	126	127	149	137	140	0	262	208	0	0
138	D525	M	TATR	20.35689	188	120	127	140	131	164	0	258	0	149	0
	scat			79.31194	191	129	127	149	139	164	0	270	0	153	0

139	D527	M	TATR	20.36672	188	126	125	140	137	155	0	262	214	144	225
	scat			79.30233	188	129	127	149	137	159	0	266	217	149	225
140	D1279	M	TATR	20.19128	188	129	125	149	141	159	169	262	214	149	209
	scat			79.40983	206	129	141	153	144	159	184	262	223	153	209
141	D2151	U	TATR	20.13200	0	114	119	0	139	0	198	287	0	149	213
	scat			79.51592	0	123	129	0	139	0	198	287	0	161	213
142	D2152	F	TATR	20.24264	188	0	127	153	139	159	171	262	214	145	209
	scat			79.39608	206	0	127	157	142	159	184	270	217	145	225
143	D2154	F	TATR	20.12525	0	0	123	149	139	176	171	270	208	153	213
	scat			79.42564	0	0	125	157	146	176	180	278	208	153	225
144	D2155	U	TATR	20.36044	191	123	119	153	142	0	171	250	208	149	213
	scat			79.32042	197	126	125	153	144	0	171	258	211	161	225
145	D2156	U	TATR	20.36042	0	0	125	149	142	149	169	258	208	145	0
	scat			79.32711	0	0	127	157	144	149	180	287	208	149	0
146	D2165	M	TATR	20.12783	200	114	125	149	142	149	171	262	217	141	209
	scat			79.37464	206	129	129	153	146	159	184	266	232	149	225
147	D2166	F	TATR	20.21858	203	114	125	0	142	149	0	258	208	145	225
	scat			79.36353	206	114	125	0	144	176	0	266	217	161	225
148	D1362	M	BTR	23.69406	206	123	127	149	137	164	169	270	211	153	217
	scat			80.99422	209	126	127	157	141	172	180	270	223	157	217
149	D1368	M	BTR	23.66342	206	123	127	140	137	164	169	266	211	128	217
	scat			81.01433	209	126	127	149	146	164	169	270	211	153	221
150	D1720	F	BTR	23.66386	206	114	129	140	137	164	169	266	211	0	217
	scat			81.02831	209	123	129	149	146	164	169	270	211	0	221
151	D1721	F	BTR	23.67683	206	114	129	149	137	164	169	266	211	0	221
	scat			81.02103	209	123	129	157	146	164	169	270	211	0	221
152	D1726	F	BTR	23.69614	203	114	129	140	137	164	169	270	208	0	217
	scat			81.02850	203	123	129	149	146	164	190	270	211	0	217
153	D1736	F	BTR	23.71336	203	126	129	157	141	159	169	270	208	0	205
	scat			81.03544	206	126	141	157	146	172	180	270	211	0	217
154	D1737	F	BTR	23.71536	203	114	129	140	137	155	171	0	229	0	217
	scat			81.03719	203	123	129	149	146	168	190	0	229	0	217
155	D1762	M	BTR	23.67142	188	114	129	140	137	159	180	270	0	0	217
	scat			80.90700	209	123	141	149	137	164	180	270	0	0	217

156	D1769	M	BTR	23.61833	203	123	129	140	141	159	169	270	220	0	217
	scat			80.95022	206	126	129	149	146	172	180	270	226	0	217
157	D1722	M	BTR	23.70239	203	123	129	149	139	168	169	270	211	0	217
	scat			80.97142	206	126	129	157	146	172	190	270	226	0	217
158	D1775	M	BTR	23.68131	203	123	129	149	137	164	169	266	211	0	217
	scat			81.00350	206	123	129	153	139	168	190	270	211	0	221
159	D1777	F	BTR	23.66828	206	114	129	149	137	164	169	266	211	0	221
	scat			81.01817	209	123	129	157	146	164	169	270	211	0	225
160	D1778	F	BTR	23.66261	206	114	125	149	137	151	169	266	211	128	217
	scat			81.01661	209	123	129	157	146	164	178	270	211	128	217
161	D1792	F	BTR	23.71531	203	123	129	149	137	164	171	270	208	153	217
	scat			81.01206	203	126	129	157	146	164	190	270	211	157	217
162	D1795	M	BTR	23.70583	203	114	129	140	137	164	171	270	208	153	217
	scat			81.00003	203	123	129	144	146	164	190	270	211	157	217
163	D1796	M	BTR	23.70175	203	114	129	149	137	164	171	266	211	128	217
	scat			80.99497	206	123	129	149	139	168	190	270	211	128	221
164	D1810	F	BTR	23.68431	203	123	129	140	141	159	171	258	223	157	217
	scat			80.99186	206	126	129	157	146	172	180	270	226	161	217
165	D1822	M	BTR	23.67417	203	123	129	149	137	159	171	266	208	0	205
	scat			81.00703	203	126	141	157	146	168	190	270	211	0	217
166	D1823	F	BTR	23.67417	203	123	129	140	137	159	171	270	211	0	217
	scat			81.00703	209	126	129	149	137	168	190	270	211	0	217
167	D1825	F	BTR	23.67417	203	123	129	149	135	159	171	270	211	0	217
	scat			81.00703	209	126	129	157	137	168	190	270	211	0	221
168	D1827	F	BTR	23.68122	203	123	129	157	141	159	169	270	223	0	0
	scat			81.00353	206	126	129	157	146	172	180	270	226	0	0
169	D587	F	BTR	23.71497	206	126	129	149	137	159	171	266	211	153	217
	blood			81.02656	209	126	141	157	137	172	180	270	226	153	217

Table A4. Mean *Q* values of population assignments to each of the four STRUCTURE clusters for all tiger individuals. Bold values are the higher proportion of assignments for each sampling area. Putative migrant individuals identified in this study are shaded in grey.

Sl No.	Sampled locality	ID	locprior=1				locprior=0			
			MST	P	K	B	MST	P	K	B
1	M	D1598	**0.444**	**0.512**	0.008	0.036	**0.398**	**0.524**	0.010	0.068
2	M	D1599	**0.880**	0.098	0.008	0.014	**0.876**	0.082	0.008	0.034
3	M	D1622	**0.774**	**0.203**	0.007	0.017	**0.770**	0.189	0.007	0.034
4	M	D1843	**0.549**	**0.390**	0.051	0.010	**0.275**	**0.527**	0.186	0.013
5	M	D1862	**0.381**	**0.570**	0.020	0.029	**0.852**	0.129	0.007	0.011
6	M	D1892	**0.544**	**0.418**	0.028	0.010	**0.658**	**0.289**	0.042	0.011
7	M	D1900	**0.830**	0.138	0.017	0.014	**0.897**	0.064	0.019	0.019
8	M	D1911	**0.630**	**0.335**	0.024	0.010	**0.506**	**0.433**	0.050	0.011
9	M	D2056	**0.924**	0.060	0.006	0.010	**0.895**	0.076	0.007	0.022
10	M	D2057	**0.912**	0.073	0.006	0.008	**0.898**	0.083	0.006	0.013
11	M	D2058	**0.456**	**0.516**	0.010	0.017	0.147	**0.758**	0.019	0.076
12	M	D2060	**0.912**	0.065	0.009	0.014	**0.879**	0.073	0.011	0.038
13	M	D1837	**0.912**	0.073	0.006	0.009	**0.895**	0.083	0.006	0.015
14	M	D2073	**0.909**	0.076	0.006	0.009	**0.898**	0.083	0.006	0.013
15	M	D2075	**0.898**	0.085	0.006	0.010	**0.892**	0.078	0.006	0.024
16	S	D1476	0.131	**0.694**	0.171	0.004	**0.791**	0.071	0.133	0.005
17	S	D1480	0.131	**0.658**	0.204	0.007	**0.800**	0.043	0.135	0.021
18	S	D1926	**0.652**	0.129	0.214	0.008	**0.193**	**0.648**	0.127	0.033
19	S	D1962	**0.979**	0.008	0.009	0.004	**0.510**	**0.470**	0.013	0.008
20	S	D1987	**0.669**	0.106	0.217	0.008	**0.481**	**0.325**	0.180	0.014
21	S	D2002	**0.837**	0.058	0.100	0.005	**0.485**	**0.497**	0.010	0.008
22	S	D2003	**0.855**	0.037	0.103	0.005	**0.530**	**0.456**	0.009	0.005
23	S	D2022	**0.970**	0.009	0.016	0.005	0.148	**0.695**	0.153	0.004
24	S	D1963	**0.860**	0.037	0.098	0.005	**0.516**	**0.464**	0.013	0.008
25	S	D2020	**0.835**	0.039	0.122	0.005	**0.510**	**0.464**	0.020	0.007
26	S	D2030	**0.824**	0.043	0.128	0.005	**0.500**	**0.475**	0.019	0.006
27	P	D532	0.028	**0.913**	0.058	0.002	0.025	**0.957**	0.008	0.010
28	P	D431	0.006	**0.933**	0.058	0.002	0.006	**0.975**	0.010	0.009
29	P	D1122	0.011	**0.813**	0.175	0.002	0.044	**0.819**	0.129	0.008
30	P	PSG1	0.007	**0.949**	0.042	0.002	0.007	**0.982**	0.006	0.005
31	P	PSG2	0.062	**0.793**	0.142	0.003	0.112	**0.817**	0.058	0.012
32	P	D954	0.006	0.241	**0.750**	0.002	0.009	0.019	**0.963**	0.009
33	P	D955	0.008	0.175	**0.803**	0.014	0.035	0.009	**0.889**	0.067
34	P	D958	0.006	0.190	**0.801**	0.004	0.033	0.016	**0.936**	0.015
35	P	D970	0.012	**0.872**	0.113	0.003	0.012	**0.954**	0.023	0.011
36	P	D1024	0.019	**0.671**	0.307	0.003	0.301	**0.369**	0.308	0.023
37	P	D1026	0.006	**0.902**	0.079	0.012	0.090	**0.764**	0.026	0.120
38	P	D1029	0.011	**0.908**	0.074	0.007	0.011	**0.951**	0.011	0.026
39	P	D1039	0.010	**0.681**	0.307	0.002	0.194	**0.372**	0.421	0.013
40	P	D1043	0.009	**0.886**	0.104	0.002	0.008	**0.969**	0.015	0.007
41	P	D1051	0.011	**0.636**	0.351	0.002	0.048	**0.759**	0.186	0.006
42	P	D1052	0.006	**0.935**	0.058	0.001	0.008	**0.977**	0.010	0.005
43	P	D1064	0.013	**0.882**	0.104	0.002	0.027	**0.939**	0.027	0.007

44	P	D1066	0.009	**0.928**	0.060	0.002	0.066	**0.914**	0.012	0.008
45	P	D1073	0.017	**0.753**	0.227	0.002	0.035	**0.879**	0.074	0.011
46	P	D1075	0.026	**0.530**	**0.439**	0.005	0.065	**0.237**	**0.663**	0.034
47	P	D1076	0.007	**0.903**	0.088	0.002	0.008	**0.966**	0.019	0.008
48	P	D1079	0.014	**0.840**	0.143	0.003	0.173	**0.736**	0.062	0.028
49	P	D1381	0.213	**0.466**	**0.316**	0.004	**0.698**	**0.150**	0.133	0.019
50	P	D1383	0.068	**0.510**	**0.420**	0.003	**0.495**	**0.137**	**0.360**	0.008
51	P	D1390	0.009	**0.629**	**0.361**	0.001	0.019	**0.522**	**0.453**	0.005
52	P	D1393	0.020	**0.435**	**0.540**	0.006	0.089	**0.083**	**0.807**	0.021
53	P	D1394	0.032	**0.539**	0.416	0.013	0.191	**0.315**	0.456	0.039
54	P	D1395	0.032	**0.652**	0.314	0.002	0.186	**0.432**	0.369	0.013
55	P	D1399	0.015	**0.260**	**0.723**	0.002	0.087	**0.022**	**0.885**	0.007
56	P	D1400	0.041	**0.399**	**0.558**	0.002	0.146	**0.110**	**0.738**	0.006
57	P	D571	0.017	**0.836**	0.143	0.004	0.559	**0.405**	0.021	0.016
58	P	D573	0.145	**0.680**	0.174	0.002	0.459	**0.520**	0.016	0.005
59	P	D574	0.010	**0.948**	0.038	0.003	0.075	**0.899**	0.007	0.018
60	P	D576	0.008	**0.924**	0.066	0.001	0.010	**0.971**	0.013	0.006
61	P	D577	0.012	**0.900**	0.077	0.010	0.454	**0.478**	0.021	0.047
62	P	D578	0.016	**0.830**	0.151	0.003	0.033	**0.867**	0.088	0.012
63	P	D580	0.008	**0.940**	0.048	0.004	0.008	**0.971**	0.007	0.014
64	P	D581	0.031	**0.804**	0.156	0.009	0.509	**0.406**	0.036	0.049
65	P	D1109	0.060	**0.829**	0.108	0.003	0.615	**0.360**	0.012	0.013
66	P	D1111	0.006	**0.931**	0.061	0.002	0.006	**0.975**	0.010	0.009
67	P	D1114	0.009	**0.901**	0.088	0.002	0.535	**0.433**	0.025	0.008
68	P	D1580	0.015	**0.897**	0.085	0.003	0.555	**0.418**	0.017	0.010
69	P	D1581	0.012	**0.846**	0.140	0.002	0.547	**0.415**	0.030	0.008
70	P	D1582	0.245	**0.457**	0.295	0.003	0.501	**0.468**	0.022	0.010
71	P	D1584	0.008	**0.941**	0.049	0.002	0.009	**0.977**	0.008	0.006
72	P	D1585	0.007	**0.940**	0.052	0.002	0.014	**0.970**	0.010	0.006
73	P	D1587	0.007	**0.946**	0.044	0.003	0.478	**0.497**	0.008	0.017
74	P	D1588	0.006	**0.942**	0.049	0.003	0.446	**0.515**	0.014	0.024
75	P	D1591	0.013	**0.905**	0.079	0.002	0.549	**0.428**	0.014	0.009
76	P	D1592	0.194	**0.560**	0.243	0.003	0.448	**0.511**	0.031	0.010
77	P	D1594	0.316	**0.420**	0.261	0.002	0.807	0.086	**0.097**	0.011
78	KPC	D1330	0.069	0.213	**0.667**	0.050	0.175	0.264	**0.313**	0.249
79	KPC	D1332	0.067	0.135	**0.783**	0.015	0.212	0.032	**0.746**	0.010
80	KPC	D1337	0.091	0.190	**0.695**	0.024	0.500	0.039	**0.436**	0.025
81	KPC	D1297	0.044	**0.406**	**0.530**	0.020	0.075	**0.683**	**0.215**	0.026
82	KPC	D1336	0.227	0.144	**0.609**	0.020	0.764	0.020	**0.205**	0.011
83	K	D1299	0.007	0.029	**0.926**	0.038	0.215	0.072	**0.593**	0.120
84	K	D254	0.005	0.010	**0.976**	0.009	0.012	0.007	**0.975**	0.005
85	K	D528	0.016	0.013	**0.963**	0.008	0.114	0.040	**0.841**	0.005
86	K	D398	0.005	0.015	**0.970**	0.009	0.014	0.019	**0.962**	0.005
87	K	D403	0.005	0.029	**0.957**	0.009	0.043	0.180	**0.771**	0.007
88	K	D426	0.005	0.019	**0.966**	0.010	0.021	0.059	**0.914**	0.007
89	K	D441	0.019	0.027	**0.917**	0.037	0.529	0.051	**0.369**	0.051
90	K	D430	0.007	0.019	**0.948**	0.027	0.050	0.041	**0.824**	0.085
91	K	D401	0.004	0.011	**0.975**	0.010	0.007	0.009	**0.978**	0.006
92	K	D407	0.005	0.013	**0.967**	0.015	0.011	0.014	**0.959**	0.016
93	K	D399	0.004	0.013	**0.974**	0.009	0.009	0.009	**0.977**	0.005
94	K	D402	0.004	0.013	**0.973**	0.010	0.009	0.012	**0.972**	0.007
95	K	D406	0.005	0.030	**0.934**	0.031	0.024	0.054	**0.850**	0.072

96	K	D272	0.005	0.052	**0.929**	0.015	0.024	0.080	**0.884**	0.012
97	K	D273	0.005	0.012	**0.973**	0.010	0.011	0.009	**0.973**	0.007
98	K	D288	0.019	0.013	**0.953**	0.016	0.124	0.021	**0.829**	0.026
99	K	D290	0.005	0.014	**0.969**	0.011	0.030	0.019	**0.940**	0.011
100	K	D296	0.008	0.022	**0.959**	0.010	0.084	0.073	**0.832**	0.010
101	K	D489	0.004	0.014	**0.973**	0.008	0.010	0.011	**0.974**	0.005
102	K	D503	0.005	0.019	**0.967**	0.009	0.017	0.014	**0.963**	0.006
103	K	D504	0.007	0.025	**0.934**	0.035	0.282	0.180	**0.464**	0.074
104	K	D507	0.013	0.014	**0.964**	0.010	0.084	0.020	**0.890**	0.006
105	K	D510	0.007	0.022	**0.958**	0.014	0.068	0.022	**0.898**	0.013
106	K	D586	0.003	0.022	**0.969**	0.006	0.014	0.008	**0.959**	0.019
107	K	D789	0.038	0.205	**0.735**	0.023	0.014	0.220	**0.760**	0.006
108	K	D795	0.006	0.019	**0.958**	0.017	0.056	0.039	**0.875**	0.030
109	K	D1131	0.004	0.014	**0.974**	0.008	0.009	0.011	**0.976**	0.004
110	K	D1137	0.006	0.016	**0.928**	0.050	0.048	0.025	**0.784**	0.143
111	K	D1140	0.008	0.034	**0.936**	0.023	0.070	**0.556**	**0.344**	0.030
112	K	D1144	0.004	0.013	**0.973**	0.010	0.010	0.012	**0.971**	0.007
113	K	D1162	0.027	0.012	**0.926**	0.035	0.497	0.017	**0.408**	0.077
114	K	D1164	0.004	0.014	**0.974**	0.007	0.009	0.012	**0.976**	0.004
115	K	D1165	0.004	0.011	**0.974**	0.011	0.008	0.007	**0.975**	0.010
116	K	D1168	0.004	0.013	**0.973**	0.009	0.009	0.017	**0.969**	0.006
117	K	D1170	0.004	0.015	**0.973**	0.008	0.010	0.011	**0.974**	0.005
118	K	D1172	0.005	0.019	**0.966**	0.010	0.023	0.025	**0.945**	0.007
119	K	D1174	0.005	0.014	**0.954**	0.027	0.018	0.020	**0.897**	0.065
120	K	D1176	0.004	0.012	**0.972**	0.011	0.008	0.012	**0.972**	0.009
121	K	D1179	0.009	0.011	**0.953**	0.027	0.079	0.011	**0.855**	0.055
122	K	D1180	0.005	0.025	**0.958**	0.012	0.028	0.071	**0.890**	0.010
123	K	D1182	0.005	0.018	**0.966**	0.011	0.052	0.025	**0.914**	0.010
124	K	D1183	0.004	0.019	**0.965**	0.011	0.021	0.032	**0.937**	0.009
125	K	D1185	0.010	0.036	**0.940**	0.014	0.105	0.167	**0.708**	0.020
126	K	D1193	0.008	0.018	**0.961**	0.014	0.063	0.060	**0.856**	0.021
127	K	D1194	0.004	0.011	**0.976**	0.009	0.010	0.007	**0.976**	0.006
128	K	D1196	0.005	0.014	**0.950**	0.031	0.033	0.019	**0.860**	0.089
129	K	D1198	0.004	0.014	**0.953**	0.029	0.013	0.013	**0.902**	0.072
130	K	D1200	0.004	0.028	**0.958**	0.010	0.045	0.077	**0.854**	0.024
131	K	D1605	0.006	0.035	**0.939**	0.020	0.033	0.075	**0.872**	0.021
132	K	D1298	0.016	0.154	**0.820**	0.009	0.313	0.182	**0.487**	0.018
133	A	D1217	0.006	0.139	**0.848**	0.006	0.059	0.030	**0.903**	0.008
134	A	D1284	0.005	0.071	**0.917**	0.007	0.017	0.012	**0.962**	0.009
135	A	D1285	0.007	0.106	**0.879**	0.009	0.087	0.073	**0.826**	0.014
136	A	D1637	0.009	0.083	**0.903**	0.006	0.013	0.038	**0.943**	0.005
137	T	D524	**0.619**	0.026	0.345	0.010	**0.767**	0.032	0.192	0.009
138	T	D525	**0.587**	0.022	**0.380**	0.010	**0.664**	0.023	**0.300**	0.013
139	T	D527	**0.776**	0.017	0.197	0.010	**0.893**	0.083	0.013	0.012
140	T	D1279	**0.651**	0.019	**0.303**	0.027	**0.640**	0.199	0.074	0.087
141	T	D2151	**0.821**	0.013	0.159	0.007	**0.976**	0.012	0.007	0.005
142	T	D2152	**0.735**	0.014	0.244	0.007	**0.913**	0.040	0.043	0.005
143	T	D2154	**0.610**	0.024	**0.355**	0.012	**0.460**	0.158	**0.358**	0.024
144	T	D2155	**0.760**	0.015	0.219	0.006	**0.964**	0.013	0.018	0.005
145	T	D2156	**0.667**	0.019	0.300	0.015	**0.894**	0.032	0.055	0.019
146	T	D2165	**0.777**	0.016	0.201	0.007	**0.836**	0.139	0.019	0.006
147	T	D2166	**0.729**	0.019	0.244	0.008	**0.840**	0.115	0.038	0.007

148	B	D1362	0.004	0.007	0.044	**0.945**	0.008	0.014	0.017	**0.961**
149	B	D1368	0.004	0.006	0.030	**0.960**	0.008	0.013	0.009	**0.970**
150	B	D1720	0.005	0.004	0.019	**0.972**	0.006	0.008	0.006	**0.980**
151	B	D1721	0.005	0.004	0.024	**0.966**	0.008	0.010	0.008	**0.974**
152	B	D1726	0.004	0.004	0.020	**0.972**	0.006	0.007	0.005	**0.982**
153	B	D1736	0.004	0.004	0.069	**0.923**	0.007	0.009	0.038	**0.946**
154	B	D1737	0.014	0.005	0.024	**0.957**	0.026	0.015	0.008	**0.951**
155	B	D1762	0.025	0.005	0.033	**0.938**	0.054	0.016	0.011	**0.918**
156	B	D1769	0.004	0.004	0.053	**0.939**	0.010	0.009	0.026	**0.955**
157	B	D1722	0.004	0.003	0.024	**0.970**	0.005	0.005	0.007	**0.983**
158	B	D1775	0.011	0.005	0.051	**0.933**	0.023	0.016	0.026	**0.935**
159	B	D1777	0.013	0.009	0.029	**0.949**	0.023	0.032	0.010	**0.936**
160	B	D1778	0.008	0.009	0.101	**0.883**	0.021	0.022	0.107	**0.851**
161	B	D1792	0.004	0.004	0.019	**0.973**	0.005	0.006	0.005	**0.984**
162	B	D1795	0.006	0.010	0.022	**0.962**	0.012	0.020	0.007	**0.962**
163	B	D1796	0.005	0.004	0.027	**0.964**	0.008	0.007	0.009	**0.976**
164	B	D1810	0.005	0.006	0.100	**0.890**	0.018	0.014	0.089	**0.879**
165	B	D1822	0.004	0.004	0.042	**0.950**	0.007	0.008	0.017	**0.968**
166	B	D1823	0.004	0.004	0.017	**0.976**	0.005	0.006	0.005	**0.984**
167	B	D1825	0.005	0.005	0.036	**0.954**	0.014	0.011	0.013	**0.962**
168	B	D1827	0.004	0.004	0.040	**0.952**	0.006	0.009	0.018	**0.967**
169	B	D587	0.004	0.003	0.021	**0.973**	0.005	0.004	0.006	**0.985**

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Abdo Z, Crandall KA and Joyce P (2004) Evaluating the performance of likelihood methods for detecting population structure and migration.*Molecular Ecology*, 13, 837-851.

Aguilar A, Jessup DA, Estes J and Garza JC (2008) The distribution of nuclear genetic variation and historical demography of sea otters. *Animal Conservation*, 11, 35–45.

Allendorf FW, Luikart G and Aitken SN (2013) *Conservation and the Genetics of Populations*, *Second Edition*, Wiley-Blackwell, UK.

Alroy J (2001) A multispecies overkill simulation of the end-Pleistocene megafaunal mass extinction. *Science*, 292, 1893-1896.

Ambrose SH (1998) Late Pleistocene human population bottlenecks, volcanic winter, and differentiation of modern humans. *Journal of Human Evolution*, 34(6), 623-651.

Anderson CR, Lindzey FG, McDonald DB (2004) Genetic structure of cougar populations across the Wyoming basin: metapopulation or megapopulation. *Journal of Mammalogy*, 85, 1207–1214.

Balloux F and Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology,* 11, 2953-2962.

Beaumont M, Barratt EM, Gotelli D *et al.* (2001) Genetic diversity and introgression in the Scottish wildcat. *Molecular Ecology,* 15, 319-336.

Beaumont MA and Rannala B (2004) The Bayesian revolution in genetics.*Nature Reviews Genetics*, 5, 251–261.

Beerli P (2008) MIGRATE version 3.0: a maximum likelihood and Bayesian estimator of gene flow using the coalescent. Available at http://popgen.sc.fsu.edu/Migrate/Migrate-n.html

Beerli P (2009) How to use MIGRATE or why are Markov chain Monte Carlo programs difficult to use? *Population Genetics for Animal Conservation* (eds: Bertorelle G, Bruford MW, Hauffe HC, Rissoli A and Vernesi C) vol 17 of Conservation Biology, Cambridge University Press, Cambridge, UK, pp. 42-79.

Beerli P (2010) MIGRATE version 3.2 documentation. Department of Scientific Computing, Florida State University, Tallahassee, Florida.

Beerli P and Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of National Academy of Sciences,* 98, 4563-4568.

Beier P and Noss RF (1998) Do habitat corridors provide connectivity? *Conservation Biology*, 12, 1241-1252.

Bergl RA and Vigilant L (2007) Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). *Molecular Ecology*, 16, 501-506.

Bethke R, Taylor M, Amstrup S and Messier F (1996) Population delineation of polar bears using satellite collar data. *Ecological Applications*, 6, 311-317.

Bhagavatula J and Singh L (2006) Genotyping faecal samples of Bengal tiger *Panthera tigris tigris* for population estimation: A pilot study. *BMC Genetics,*7, 48, doi:10.1186/1471-2156-7-48

Bidlack AL, Reed SE, Palsbøll PJ and Getz WM (2007) Characterization of a western North American carnivore community using PCR-RFLP of cytochrome b obtained from fecal samples. *Conservation Genetics* 8, 1511-1513.

Bonnet E and Van de Peer Y (2002) zt: a software tool for simple and partial Mantel tests. *Journal of Statistical software,* 7, 1-12.

Boom R, Sol CJA, Salimans MMM, Lansen CL, Wertheim-van Dillen PME and Nordaa JVD (1990) Rapid and simple method for purification of nucleic-acids. *Journal of Clinical Microbiology,* 28, 495-503.

Borthakur U, Barman RD, Das C, Basumatary A, Talukdar A, Ahmed MF and Bharali R (2011) Noninvasive genetic monitoring of tiger (*Panthera tigris tigris*) population of Orang National Park in the Brahmaputra floodplain, Assam, India. *European Journal of Wildlife Research*, *57*, 603-613.

Boyce, MS, McDonald LL (1999) Relating populations to habitats using resource selection functions. *Trends in Ecology and Evolution*, 14, 268-272.

Brongersma LD (1935) Notes on some recent and fossil cats, chiefly from the Malay Archipelago.*Zoologische Mededeelingen*, 18, 1-89.

Bruford MW, Hanotte O, Brookfield JFY and Burke T (1998) Multi-locus and single-locus DNA fingerprinting. Pp. 287–336 in Hoelzel AR, ed. *Molecular Genetic Analysis of Populations*. Oxford University Press, New York.

Butler JM (2002) *Information on the Cat STR Multiplex Developed at NIST and a Protocol for its Use on the ABI 310 and ABI 3100 Instruments*. A Technical Report submitted to the National Institute of Justice, USA.

Butler JM, David VA, O'Brien S J and Menotti-Raymond M (2002) The MeowPlex: a new DNA test using tetranucleotide STR markers for the domestic cat. *Profiles in DNA*, 5, 7-10.

Campbell V and Strobeck C (2006) Fine-scale genetic structure and dispersal in Canada lynx (*Lynx canadensis*) within Alberta, Canada. *Canadian Journal of Zoology*, 84, 1112-1119.

Chakraborty R, Kimmel M, Strivers DN, Davison LJ and Deka R (1997) Relative mutation rates at di-, tri- and tetranucleotide microsatellite loci. *Proceedings of National Academy of Sciences,* 94, 1041-1046.

Champion HG and Seth SK (1968)*The Forest Types of India*. Government of India Press, New Delhi.

Chandran MDS (1997) On the ecological history of the Western Ghats. *Current Science,* 73, 146–155.

Chapron G, Miquelle DG, Lambert A, Goodrich JM, Legender S and Clobert J (2008) The impact on tigers of poaching versus prey depletion. *Journal of Applied Ecology* 45, 1667–1674.

Charruau P, Ferndandes C, Orozco-Ter Wengel P, Peters J, Hunter L, Ziaie H, Jourabchian A, Jowkar H, Schaller G, Ostrowski S, Vercammen P, Grange T, Schlötterer C, Kotze A, Geigl E-M, Walzer C and Burger PA (2011) Phylogeography, genetic structure and population divergence time of cheetahs in Africa and Asia: evidence for long-term geographic isolates. *Molecular Ecology*, 20, 706-724.

Check E (2006) Thetiger'sretreat. *Nature*, 441, 927–930

Chen C,Durand E, Forbes F and François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, 7(5), 747-756.

Chessier RK, Rhodes OEJ, Sugg DW and Schnabel A (1993) Effective sizes for subdivided populations.*Genetics*, 135, 1221-1232.

Chiucchi JE and Gibbs HL (2010) Similarity of contemporary and historical gene flow among highly fragmented populations of an endangered rattlesnake. *Molecular Ecology*, 19(24), 5345-5358.

Cho YS, Hu L, Hou H, Lee H, *et al.* (2013) The tiger genome and comparative analysis with lion and snow leopard genomes. *Nature Communications*, doi: 10.1038/ncomms3433.

Ciucci P, Reggioni W, Maiorano L and Boitani L (2009) Long-Distance Dispersal of a Rescued Wolf from the Northern Apennines to the Western Alps. *The Journal of Wildlife Management*, 73(8), 1300-1306.

Corander J, Sirén J and Arjas E (2008) Bayesian spatial modeling of genetic population structure.*Computational Statistics*, 23, 111–129.

Cornuet JM and Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, 144, 2001-2004.

Cossíos D and Angers B (2006) Identification of Andean felid species using PCR-RFLP.*Mastozooogía Neotropical*, 13, 239-244.

Cracraft J, Feinstein J, Vaughn J and Helm-Bychowski K (1998) Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. *Animal Conservation*, 1(2), 139-150.

Crandall KA, Binindaemonds ORP, Mace GM and Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, 15, 290 –295.

Creel S, Spong G, Sands JL, Rotella J, Zeigle J, Joe L and Smith D (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes.*Molecular Ecology*, 12(7), 2003-2009.

Crooks K (2002) Relative Sensitivities of Mammalian Carnivores to Habitat Fragmentation.*Conservation Biology*, 2, 488-502.

Crooks KR and Sanjayan MA (Eds.) (2006) *Connectivity conservation* (No. 14).Cambridge University Press.

Croteau EK, Heist EJ and Nielsen CK (2010) Fine-scale population structure and sex-biased dispersal in bobcats (*Lynx rufus*) from southern Illinois. *Canadian Journal of Zoology*, 88, 536-545.

DeFries RS, Rudel T, Uriarte M and Hansen M (2010) Deforestation driven by urban population growth and agricultural trade in the twenty-first century.*Nature Geoscience*, 3, 178–181 doi:10.1038/ngeo756.

Dewoody J, Nason JD and Hipkins VD (2006) Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes*, 6, 951–957.

Dinerstein E, Loucks C, Wikramanayake E, Ginsberg J, Sanderson E, Seidensticker J, *et al.* (2007) The fate of wild tigers. *BioScience*, 57(6), 508-514.

Divyabhanusinh (1999) *The end of a trail: the cheetah in India.* Oxford University Press, New Delhi.

Dobhal R (1994) Forestry for food: Challenges for 2000 AD and beyond. *Current Science*, 67(2), 71-73.

Donnelly P (1995) Non-independence of matches at different loci in DNA profiles: quantifying the effect of close relatives on the match probability. *Heredity*, 75, 26-34.

Driscoll CA, Menotti-Raymond MA, Nelson G, Goldstein D, O'Brien SJ (2002) Genomic microsatellites as evolutionary chronometers: a test in wild cats. *Genome Research*, 12, 414–423.

Driscoll CA, Yamaguchi N, Bar-Gal GK *et al.* (2009) Mitochondrial phylogeography illuminates the origin of the extinct Caspian tiger and its relationship to the Amur Tiger. *PLoS One*, 4, e4125.

Dutta T, Sharma S, Maldonado JE, Wood TC, Panwar HS and Seidensticker J (2012) Fine-scale population genetic structure in a wide-ranging carnivore, the leopard (*Panthera pardus fusca*) in Central India. *Diversity and Distributions*, 19, 760-771.

Dutta T, Sharma S, Maldonado JE, Wood TC, Panwar HS and Seidensticker J (2013) Gene flow and demographic history of leopards (*Panthera pardus*) in the Central Indian Highlands. *Evolutionary Applications*, 6, 949-959 (doi:10.1111/eva.12078).

Earl DA and vonHoldt BM (2009) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* DOI: 10.1007/s12686-011-9548-7. http://taylor0.biology.ucla.edu/struct_harvest/index.php

Ellegren H (2000) Microsatellite mutations in the germline: implications for evolutionary inference.*Trends in Genetics*, 16, 551-558.

Ernest HB, Penedo MC, May BP, Syvanen M and Boyce WM (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology,* 9, 433-442.

Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, 2611-2620.

Evett IW and Weir BS (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sinauer, Sunderland, UK.

Excoffier L, Laval G and Schneider S (2005) Arlequin (version 3.0): an integrated package for population genetics data analysis. *EvolutionaryBioinformatics Online*, 1, 47-50.

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among haplotypes: applications to human mitochondrial DNA restriction data. *Genetics,* 131, 479-491.

Farrell LE, Roman J and Sunquist ME (2000) Dietary separation of sympatric carnivores identified by molecular analysis of scats.*Molecular Ecology,* 9, 1583-1590.

Fernandes A (2012) *How coal mining is thrashing tigerland*. Greenpeace, Bengaluru, India.115pp. www.greenpeace.org/india.

Fleischer RC, Perry EA, Muralidharan K, Stevens EE and Wemmer CM (2001) Phylogeography of the Asian elephant (*Elephas maximus*) based on mitochondrial DNA. *Evolution*, 55(9), 1882-1892.

Flerov KK and Biron A (1960) *Fauna of USSR mammals*.Israel Program for Scientific Translations.

Forest (Conservation) Act (1980) Handbook of Forest (Conservation) Act, 1980 (With Amendments made in 1988), Forest (Conservation) Rules, 2003 (With Amendments made in 2004), Guidelines & Clarifications (Up to June, 2004), Government of India, Ministry of Environment & Forest, New Delhi. 126pp. http://www.arunachalpwd.org/pdf/Hanbook%20on%20Forest%20(Conservation)%20Act,%201980%20(updated%202004).pdf (Date accessed March 29, 2013).

Forsyth J (1919) *The highlands of central India, notes of their forests, wild tribes, natural history and sports*. Chapman and Hall, London.

Frankham R, Ballou JD and Briscoe D (2002) *Introduction to Conservation Genetics*.Cambridge University Press, Cambridge, UK.

Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK and Kohn MH (1998) Empirical evaluation of preservation methods for faecal DNA. *Molecular Ecology*,7, 1423-1428.

Garza JC and Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, 10, 305-318.

Gee EP (1964) *The Wildlife of India*. Collins, London.

Geist V (1971) The relation of social evolution and dispersal in ungulates during the Pleistocene, with emphasis on the old world deer and the genus *Bison*. *Quaternary Research*, 1(3), 285-315.

Girod C, Vitalis R, Leblois R and Freville H (2011) Inferring population decline and expansion from microsatellite data: a simulation-based evaluation of the Msvar method. *Genetics*, 188, 165-179.

Gittleman JL, Funk SM, Macdonald DW and Wayne RK (eds.) (2001) *Carnivore conservation*. Cambridge: Cambridge University Press. 690 p.

Goldstein DB and Schlötterer C (1999) *Microsatellites: Evolution and applications*. Oxford University Press, Oxford.

Goossens B, Funk SM, Vidal C, Latour S, Jamart A, Ancrenaz M, *et al.* (2002) Measuring genetic diversity in translocation programmes: principles and application to a chimpanzee release project. *Animal Conservation*, 5(3), 225-236.

Goossens B and Salgado-Lynn M (2013) Advances and difficulties of molecular tools for carnivore conservation in the tropics. *Raffles Bulletin of Zoology*,Supplement No. 28, 43–53.

Gopal R and Shukla R (2001) *Management Plan of Kanha Tiger Reserve for the Period of 2001-02 to 2009-10*. Kanha Tiger Reserve, Mandla.

Gopal R, Qureshi Q, Bharadwaj M, Singh RKJ and Jhala YV (2010) Evaluating the status of the endangered tiger *Panthera tigris* and its prey in Panna Tiger Reserve, Madhya Pradesh, India. *Oryx*, 44, 383–398.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www2.unil.ch/popgen/softwares/fstat.htm

Greenwood PJ (1980) Mating systems, philopatry, and dispersal in birds and mammals.*Animal Behaviour*, 28, 1140-1162.

Haila Y (2002) A conceptual genealogy of fragmentation research: from island biogeography to landscape ecology. *Ecological Applications,* 12, 321–334.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hanski IA (1994) A practical model of metapopulation dynamics.*Journal of Animal Ecology*, 63, 151-162.

Hanski I and Gilpin M (1991) Metapopulation dynamics – brief history and conceptual domain.*Biological Journal of the Linnean Society,* 42, 3–16.

Hanski IA and Gilpin ME (1997) *Metapopulation biology: Ecology, Genetics and Evolution*. Academic Press, Orlando, Florida.

Hardy OJ, Charbonnel N, Freville H and Heuertz M (2003) Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics*, 163, 1467-1482.

Hemmer H (1971) Fossil mammals of Java. II Zur Fossilgeschicte des Tigers (*Panthera tigris* (L.)) in Java. *Koninklijke Nederlandse Akademie van Wetenschappen, Series B*, 74, 35-52.

Hemmer H (1987) The phylogeny of the tiger (*Panthera tigris*). In Tigers of the World: Pp. 28-35 in The Biology, Biopolitics, and Management of an Endangered Species, ed. RL Tilson and US Seal, Park Ridge, New Jersey, Noyes Publications.

Hendrickson SL, Mayer GC, Wallen EP and Quigley K (2000) Genetic variability and geographic structure of three subspecies of tigers (*Panthera tigris*) based on MHC class I variation. *Animal Conservation*, 3(2), 135-143.

Henry P, Miquelle D, Sugimoto T, McCullough DR, Caccone A and Russello MA (2009) *In situ* population structure and *ex situ* representation of the endangered Amur tiger. *Molecular Ecology*, 18, 3173–3184.

Heptner VG and Sludski AA (1992) *Mammals of the Soviet Union, volume II, part 2, Carnivora (Hyaenas and Cats*). (English translation, ed. RS Hoffman).Washington DC, Smithsonian Institution Libraries and the National Science Foundation.

Hunter L (2011) *Carnivores of the World*.Princeton University Press, Princeton, New Jersey, 240 pp.

Iyengar A, Babu VN, Hedges S, Venkataraman AB, Maclean N and Morin PA (2005) Phylogeography, genetic structure, and diversity in the dhole (*Cuon alpinus*). *Molecular Ecology*, 14, 2281-2297.

Jakobsson M and Rosenberg N (2007) *CLUMPP*: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23, 1801-1806.

Janecˇka JE, Jackson R, Yuquang Z, Diqiang L, Munkhtsog B, Buckley-Beason V and Murphy WJ (2008) Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. *Animal Conservation*, doi:10.1111/j.1469-1795.2008.00195.x

Jhala YV, Gopal R and Qureshi Q (eds.) (2008) *Status of the tigers, co-predators, and prey in India.* National Tiger Conservation Authority, Ministry of Environment and Forests, Government of India, New Delhi and Wildlife Institute of India, Dehradun. TR08/001 pp164.http://oldwww.wii.gov.in/publications/statusof_tigers2008.pdf

Jhala YV, Qureshi Q and Gopal R (2011a) Can the abundance of tigers be assessed from their signs? *Journal of Applied Ecology*, 48, 14-24.

Jhala YV, Qureshi Q, Gopal R and Sinha PR (eds.) (2011b) *Status of the tigers, co-predators, and prey in India, 2010.* National Tiger Conservation Authority, Ministry of Environment and Forests, Government of India, New Delhi and Wildlife Institute of India, Dehradun. TR2011/003 pp-302. http://www2.wii.gov.in/publications/researchreports/2011/tiger/staus_tiger_2010.pdf

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E and O'Brien SJ (2006) The late Miocene radiation of modern Felidae: a genetic assessment. *Science*, 311(5757), 73-77.

Joshi A, Vaidyanathan S, Mondol S, Edgaonkar A and Ramakrishnan U (2013) Connectivity of Tiger (*Panthera tigris*) Populations in the Human-Influenced Forest Mosaic of Central India. *PLoS ONE*, 8 (doi: 10.1371/journal.pone.0077980)

Kalinowski ST, Taper ML and Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology,* 16, 1099-1006.

Karanth KU and Nichols JD (1998) Estimation of tiger densities in India using photographic captures and recaptures. *Ecology,* 79, 2852-2862.

Karanth KU and Sunquist ME (2000) Behavioural correlates of predation by tiger (*Panthera tigris*) leopard (*Panthera pardus*) and dhole (*Cuon alpinus*) in Nagarahole, India. *Journal of Zoology,* 250, 255-265.

Karanth KU and Gopal R (2005) An ecology-based policy framework for human-tiger coexistence in India. Pp. 373-387 in *People and Wildlife: Conflict or Coexistence?* (Editors: R. Woodroffe, S. Thirgood and A. Rabinowitz). Cambridge University Press.

Kenny JS, Smith JLD, Starfield AM and McDougal CW (1995) The long-term effect of tiger poaching on population viability. *Conservation Biology*, 9, 1127-1133.

Kim JH, Antunes A, Luo SJ *et al.* (2006) Evolutionary analysis of a large mtDNA translocation (*numt*) into the nuclear genome of the *Panthera* genus species. *Gene* 366, 292-302.

Kitchener AC (1999) Tiger distribution, phenotypic variation and conservation issues.Pp. 19-39 in J. Seidensticker, S. Christie, and P. Jackson, editors.*Riding the tiger: Tiger conservation in human dominated landscapes*.Cambridge University Press, Cambridge, UK.

Kitchener AC and Dugmore AJ (2000) Biogeographical change in the tiger, *Panthera tigris.* Animal Conservation, 3, 113-124.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX and Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of National Academy of Sciences*, 86, 6196-6200

Koenig WD, VanVuren D and Hogge PN (1996) Detectability, philopatry and the distribution of dispersal distances in vertebrates. *Trends in Ecology and Evolution*, 11, 514-517.

Koliyal A (1997) Extraction of Non-Timber Forest Produce from Selected Tree Species in Betul Forest Division and its Impact on the Population Structure of these Species, unpublished M.Sc.thesis. Dehradun: Wildlife Institute of India.

Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics*, 22(6), 768-770

Kumar A and Wright B (1999) Combating tiger poaching and illegal wildlife trade in India. Pp. 243-251 in J. Seidensticker, S. Christie, and P. Jackson, editors.*Riding the tiger: Tiger conservation in human dominated landscapes*.Cambridge University Press, Cambridge, UK.

Kyle CJ and Strobeck C (2002) Connectivity of peripheral and core populations of North American wolverines. *Journal of Mammalogy*, 83, 1141-1150.

Lande R (1988) Genetics and demography in biological conservation.*Science*, 241, 1455–1460.

Levins R (1970) Pp. 77-107 in Gerstenhaber M, ed. *Some Mathematical Problems in Biology*.American Mathematical Society, Providence, Rhode Island.

Linkie M, Chapron G, Martyr DJ, Holden J, Leader-Williams N (2006) Assessing the viability of tiger subpopulations in a fragmented landscape. *Journal of Applied Ecology*, 43,576-586.

Lopez JV, Cevario S and O'Brien SJ (1996) Complete Nucleotide Sequences of the Domestic Cat (*Felis catus*) Mitochondrial Genome and a Transposed mtDNA Tandem Repeat (*Numt*) in the Nuclear Genome. *Genomics*, 33(2), 229-246.

Lowe WH and Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology*, 19, 3038-3051.

Luikart G, Sherwin WB, Steele BM and Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology*, 7, 963–974.

Luo S-J, Kim J-H, Johnson WE, VanderValt J, Martenson J, Yukhi N, Miquelle DG, Uprykhina O, Goodrich JM, Quigley HB, Tilson R, Brady G, Martelli P, Subramaniam V, McDougal C, Hean S, Huang S-H, Pan W, Karanth UK, Sunquist M, Smith JLD and O'Brien SJ (2004) Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLoS Biology* 2(12), e442 doi:10.1371/journal.pbio.0020442

Luo SJ, Johnson WE, Martenson J *et al.* (2008) Subspecies genetic assignments of worldwide captive tigers increase conservation value of captive populations.*Current Biology*, 18, 592–596.

MacArthur RH and Wilson EO (1967) *The theory of island biogeography*. Princeton University Press, Princeton, New Jersey.

Manamendra-Arachchi K, Pethiyagoda R, Dissanayake R and Meegaskumbura M (2005) A second extinct big cat from the Late Quaternary of Sri Lanka. *Raffles Bulletin of Zoology*, Supplement No. 12, 423-434.

Manel S, Schwartz MK, Luikart G and Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, 18, 189-197.

Mani MS (ed.) (1974) *Ecology and biogeography in India*.Junk, The Hague.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27, 209-220.

Marshall TC, Slate J, Kruuk LEB and Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology,* 7,639- 655.

Mazak VJ (1981) *Panthera tigris. Mammalian Species*, 152,1-8.

Meirmans PG (2012) The trouble with isolation by distance. *Molecular Ecology*, 21, 2839-2846.

Menotti-Raymond M, David VA, Lyons LA, Schäffer AA, Tomlin JF, O'Brien SJ (1999) A Genetic Linkage Map of Microsatellites in the Domestic Cat (*Felis catus*). *Genomics,* 57, 9-23.

Miquelle DG, Smirnov EN, Merrill Tw, Myslenkov AE, Quigley HB, Hornocker MG and Schleyer B (1999) Pp. 71-99 in J. Seidensticker, S. Christie, and P. Jackson, editors. *Riding the tiger: Tiger conservation in human dominated landscapes*.Cambridge University Press, Cambridge, UK.

Misra VN (2001) Prehistoric human colonization of India.*Journal of Biosciences*, 26, 491-531

Mondol S, Bruford MW and Ramakrishnan U (2013) Demographic loss, genetic structure and the conservation implications for Indian tigers. *Proceedings of the Royal Society B: Biological Sciences,* 280, doi:10.1098/rspb.2013.0496

Mondol S, Karanth KU and Ramakrishnan U (2009a) Why the Indian Subcontinent Holds the Key to Global Tiger Recovery.*PLoS Genetics*, 5(8): e1000585. doi:10.1371/journal.pgen.1000585

Mondol S, Karanth KU, Kumar NS, Gopalaswamy AM, Andheria A and Ramakrishnan U (2009b) Evaluation of non-invasive genetic sampling methods for estimating tiger population size. *Biological Conservation*, 142, 2350-2360.

Moritz C (1994) Defining evolutionarily significant units for conservation.*Trends in Ecology and Evolution*, 9, 373-375.

Moritz C, Lavery S and Slade B (1995) Using allele frequency and phylogeny to define units for conservation and management. *American Fisheries Society Symposium*, 17, 249-262.

Mukherjee S, Ashalakshmi CN, Home C and Ramakrishnan U (2010) An evaluation of the PCR-RFLP technique to aid molecular-based monitoring of felids and canids in India. *BMC Research Notes*, 3, 159

Mukherjee N, Mondol S, Andheria A and Ramakrishnan U (2007) Rapid multiplex PCR based species identification of wild tigers using non-invasive samples. *Conservation Genetics*, 8, 1465-1470.

Murphy MA, Waits LP and Kendall KC (2000) Quantitative evaluation of fecal drying methods for brown bear DNA analysis. *Wildlife Society Bulletin* 28, 951-957.

Nagata J, Aramilev VV, Belozor A, Sugimoto T and McCullough DR (2005) Fecal genetic analysis using PCR-RFLP of cytochrome b to identify sympatric carnivores, the tiger *Panthera tigris* and the leopard *Panthera pardus*, in far eastern Russia. *Conservation Genetics* 6, 863–865, doi 10.1007/s10592-005-9038-0.

Narain S, Panwar HS, Gadgil M and Thapar V (2005) *Joining the dots: The report of the Tiger Task Force.*Project Tiger, Union Ministry of Environment and Forests, New Delhi.

Navidi W, Arnheim N and Waterman MS (1992) A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. *American Journal of Human Genetics,* 50, 347-359.

Neigel JE (2002) Is F_{ST} obsolete? *Conservation Genetics*, 3, 167-171.

Noss RF (1987) Corridors in real landscapes: a reply to Simberloff and Cox. *Conservation Biology*, 1, 159-164.

Paabo S, Poinar H, Serre D *et al.* (2004) Genetic analyses from ancient DNA. *Annual Review of Genetics*, 38, 645–679.

Pabla HS, Carlisle L, Cooper D, Cooke J, Nigam P, Sankar K, Srivastav A, Negi HS, Patil CK, Aggarwal S, Mishra A, Gupta S, Srivastav AB, Chauhan KS and Sarath C (2011) *Reintroduction of Gaur (Bos gaurus gaurus) in Bandhavgarh Tiger Reserve, India*. Technical Report 73p.Madhya Pradesh Forest Department, & Beyond and Wildlife Institute of India, Dehradun.

Paetkau DH (1999) Using genetics to identify intraspecific conservation units: a critique of current methods.*Conservation Biology*, 13, 1507-1509.

Paetkau D, Calvert W, Stirling I and Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology,* 4, 347-354.

Paetkau D and Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, 3, 489-495.

Paetkau D, Slade R, Burden and Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation –based exploration of accuracy and power. *Molecular Ecology,* 13, 55-65.

Panwar HS (1987) Project Tiger: the reserves, the tigers and their future. Pp. 110-117 in Tilson RL and Seal US eds. *Tigers of the World: the biology, biopolitics, management and conservation of an endangered species.*Noyes Publications, Park Ridge, New Jersey.

Peakall R and Smouse PE (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research.*Molecular Ecology Notes,* 6, 288-295.

Pearse DE and Crandall KA (2004) Beyond F_{ST}: Analysis of population genetic data for conservation. *Conservation Genetics*, 5, 585-602.

Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet-Beer E, Robinson S, Vasaquez-Carillo C, Pauli JN and Palsboll PJ (2012) Reliability of genetic bottleneck tests for detecting recent population declines. *Molecular Ecology*, 21, 3403-3418.

Pinjarkar V (2011) SC refuses to grant relief in NH7 four- laning along Pench. The Times of India, Nagpur.http://articles.timesofindia.indiatimes.com/2011-04- 06/nagpur /29387911_1_laning-mansar-cuts-tiger-corridor. (Date accessed March 28, 2013).

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L and Estoup A (2004) GENECLASS2: a software for genetic assignment and first generation migrants detection. *Journal of Heredity,* 95, 536-539.

Piry S, Luikart G and Cornuet J-M (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity,* 90, 502-503.

Pompanon F, Bonin A, Bellemain E and Taberlet P (2005) Genotyping Errors: causes, consequences and solutions.*Nature Reviews Genetics,* 6, 847-859.

Prasad S and Enzel Y (2006) Holocene paleoclimates of India.*Quaternary Research*, 66, 441-453

Pritchard JK, Stephens M and Donelly P (2000) Inferences of population structure using multilocus genotyping data. *Genetics,* 155, 945-959.

Pritchard J, Wen W and Falush D (2009) Documentation for STRUCTURE software: Version 2.3. Department of Human Genetics, University of Chicago, Chicago and Department of Statistics, University of Oxford, Oxford.

Proctor MF (2003) Genetic analysis of movement, dispersal and population of grizzly bears in southwestern Canada.Ph.D. thesis, University of Calgary, Alberta.

Proctor MF, McLellan BN, Strobeck C and Barclay RMR (2005) Genetic analysis reveals demographic fragmentation of grizzly bears yielding vulnerably small populations. *Proceedings of the Royal Society B: Biological Sciences,* 272, 2409-2416.

Pulliam HR (1988) Sources, sinks, and population regulation.*The American Naturalist*, 132, 652-661.

Purvis A, Agapow PM, Gittleman JL and Mace GM (2000) Nonrandom Extinction and the Loss of Evolutionary History.*Science*, 288, 328–330.

Pusey AE (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals.*Trends in Ecology and Evolution*, 2, 295-299.

Pusey AE and Wolf M (1996) Inbreeding avoidance in mammals.*Trends in Ecology and Evolution*, 11, 201-206.

Queller DC and Goodnight KF (1989) Estimating relatedness using genetic markers.*Evolution,* 43, 258-275.

Quintero J, Roca R, Morgan AJ and Mathur A (2010) *Smart Green Infrastructure in Tiger Range Countries: A Multi-Level Approach*. Global Tiger Initiative, GTI-SGI working group, technical paper. http://www.globaltigerinitiative.org/download/GTI-Smart-Green-Infrastructure-Technical-Paper.pdf (accessed January 28, 2013).

Ramachandran R (2011) Ministry’s ‘no’ to Neutrino Observatory project in Nilgiris. *The Hindu*http://www.thehindu.com/2009/11/21/stories /2009112154952000.htm (date accessed March 23, 2013).

Rampino MR and Self S (1992) Volcanic winter and accelerated glaciation following the Toba super-eruption. *Nature*, 359(6390), 50-52.

Rangarajan M (1996) *Fencing the forest: conservation and ecological change in India’s Central Provinces, 1860-1914.*Oxford University Press, Delhi, India.

Rangarajan M (2001) *India’s wildlife history: an introduction.* Permanent Black, New Delhi.

Rannala B and Mountain JL (1997) Detecting immigration by using multilocus genotypes.*Proceedings of the National Academy of Sciences, USA*, 94, 9197-9201.

Raymond M and Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86,248-249.

Reddy PA, Gour DS, Bhavanishankar M, Jaggi K, Hussain SM, Harika K and Shivaji S (2012) Genetic Evidence of Tiger Population Structure and Migration within an Isolated and Fragmented Landscape in Northwest India.*PLoS ONE*, 7(1): e29827. doi:10.1371/journal.pone.0029827

Rodgers WA and Panwar HS (1988) Planning a wildlife protected area network in India. II Volume. Project FO: IND/82/003. FAO, Dehradun.

Rosenberg NA (2004) *Distruct*: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4, 137-138.

Rosenberg NA, Burke T, Elo K *et al.* (2001) Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. *Genetics*, 159, 699-713.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance.*Genetics*, 145, 1219-28.

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, 8, 103-106.

Row JR, Gomez C, Koen EL, Bowman J, Murray DL and Wilson PJ (2012) Dispersal promotes high gene flow among Canada lynx populations across mainland North America. *Conservation Genetics*, 13, 1259-1268.

Rozen S and Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz, S. and Misener, S.), pp. 365–386, Humana Press, Totowa, New Jersey. http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

Sanderson E, Forrest J, Loucks C, Ginsberg J, Dinerstein E, Seidensticker J, Leimgruber P, Songer M, Heydlauff A, O’Brien T, Bryja G, Klenzendorf S and Wikramanayake E (2006) *Setting Priorities for the Conservation and Recovery of Wild Tigers: 2005–2015.The Technical Assessment*.WCS, WWF, Smithsonian, and NFWF-STF, New York – Washington, D.C.

Saunders DA, Hobbs RJ and Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology*, 5, 18–32.

Schaller G (1996) Carnivores and conservation biology.Pages 1-10 in J.L. Gittleman (ed.) *Carnivore behavior, ecology and evolution, Volume 2*, Cornell University Press, Ithaca, New York, USA.

Schwartz MK, Copeland JP, Anderson NJ, Squires JR, Inman RM, McKelvey KS and Cushman SA (2009) Wolverine gene flow across a narrow climatic niche. *Ecology*, 90, 3222-3232.

Seidensticker J, Christie S and Jackson P (eds.) (1999) *Riding the Tiger: Tiger conservation in human-dominated landscapes,* Cambridge University Press, Cambridge.

Seidensticker J, Dinerstein E, Goyal SP*et al.* (2010) Tiger range collapse and recovery at the base of the Himalayas. Pp. 305-324 in DW Macdonald and AJ Loveridge (eds.) *Biology and Conservation of Wild Felids*, Oxford University Press, Oxford.UK.

Sharma S, Dutta T, Maldonado JE, Wood TC, Panwar HS and Seidensticker J (2012) Spatial genetic analysis reveals high connectivity of tiger (*Panthera tigris*) populations in the Satpuda-Maikal landscape of Central India. *Ecology and Evolution,* 3, 48-60.

Sharma S, Dutta T, Maldonado JE, Wood TC, Panwar HS and Seidensticker J (2013) Forest corridors maintain historical gene flow in a tiger metapopulation in the highlands of central India. *Proceedings of the Royal Society B:Biological Sciences,* 280, 20131506 (1767) doi:10.1098/rspb.2013.1506

Sharma R, Stuckas H, Bhaskar R, Rajput S, Khan I, Goyal SP and Tiedemann R (2009) mtDNA indicates profound population structure in Indian tiger (*Panthera tigris tigris*). *Conservation Genetics*, 10, 909-914.

Sharma R, Stuckas H, Moll K, Khan I, Bhaskar R, Goyal SP and Tiedemann R (2008) Fourteen new di-and tetranucleotide microsatellite loci for the critically endangered Indian tiger (*Panthera tigris tigris*). *Molecular Ecology Resources*, 8, 1480-1482.

Simberloff D (1988) The contribution of population and community biology to conservation science. *Annual Review of Ecology and Systematics*, 19, 437–511.

Slate J, Marshall TC and Pemberton JM (2000) A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Molecular Ecology,* 9, 801-808

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139, 457-462.

Small MP, Stone KD and Cook JA (2003) American marten (*Martes americana*) in the Pacific Northwest: population differentiation across a landscape fragmented in time and space. *Molecular Ecology*, 12, 89-103.

Smith JLD (1993) The role of dispersal in structuring the Chitwan tiger population. *Behaviour*, 124, 165-195.

Smith JLD and McDougal C (1991) The contribution of variance in lifetime reproduction to effective population size in tigers. *Conservation Biology*, 5, 484-490.

Smith JLD, McDougal C, Ahearn SC, Joshi A and Conforti C (1999) Metapopulation structure of tigers in Nepal. Pages 176-191 in J. Seidensticker, S. Christie, and P. Jackson, editors.*Riding the tiger: Tiger conservation in human dominated landscapes*.Cambridge University Press, Cambridge, UK.

Smouse PE, Long JC and Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence.*Systematic Zoology*, 35, 627-632.

Soulé ME (1985) What is conservation biology? *BioScience*, 35(11), 727-734.

Spong G and Hellborg L (2002) A near-extinction event in lynx: do microsatellite data tell the tale? *Conservation Ecology*, 6(1), 15 [online] URL: http://www.consecol.org/vol6/iss1/art15

Stoner DC, Rieth WR, Wolfe ML, Mecham MB and Neville A (2008) Long-Distance Dispersal of a Female Cougar in a Basin and Range Landscape.*The Journal of Wildlife Management*, 72(4), 933-939.

Sugimoto T, Nagata J, Aramilev VV, Belozor A, Higashi S and McCullogh D (2006) Species and sex identification from faecal samples of sympatric carnivores, Amur leopard and Siberian tiger, in the Russian Far East. *Conservation Genetics*, 7, 799-802.

Sunquist ME (1981) *The social organization of tigers (Panthera tigris) in Royal Chitawan National Park, Nepal*. Washington, DC: Smithsonian Institution Press.

Sunquist ME, Karanth KU and Sunquist F (1999) Ecology, behaviour and resilience of the tiger and its conservation needs. Pages 5-18 in J. Seidensticker, S. Christie, and P. Jackson, editors.*Riding the tiger: Tiger conservation in human dominated landscapes*.Cambridge University Press, Cambridge, UK.

Swisher CC, Curtis GH, Jacob T, Getty AG and Suprijo A (1994) Age of the earliest known hominids in Java, Indonesia. *Science*, 263(5150), 1118-1121.

Taberlet P and Luikart G (1999) Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society,* 68, 41-55.

Taylor MK, Akeeagok S, Andriashek D, Barbour W, Born EW, Calvert W, Cluff D, Laake J, Rosing-Asvid A, Stirling I and Messier F (2001) Delineating Canadian and Greenland polar bear (*Ursus martimus*) populations by cluster analysis of movement. *Canadian Journal of Zoology*, 79, 690-709.

Thompson DJ and Jenks JA (2005) Research Notes: Long-distance Dispersal by a Subadult Male Cougar from the Black Hills, South Dakota. *Journal of Wildlife Management*, 69(2), 818-820.

Thompson DJ, Fecske DM, Jenks JA and Jarding AR (2009) Food habits of recolonizing cougars in the Dakotas: prey obtained from prairie and agricultural habitats. *The American Midland Naturalist*, 161(1), 69-75.

Tilson RL and Nyhus PJ (2010) *Tigers of the world: the science, politics, and conservation of Panthera tigris, Second edition*. Academic Press, London.

Tilson RL and Seal US (1987) *Tigers of the world.*Noyes Publications, Park Ridge, New Jersey.

Valière N (2002) GIMLET: a computer program for analyzing genetic individual identification data. *Molecular Ecology Notes,* 2, 377–379.

vanOosterhout C, Hutchinson WF, Wills DP and Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4, 535-538.

Vattakavan J (2010) *Fragmentation threat in Kanha-Pench corridor: Implications of the Gondia-Jabalpur railway line on corridor connectivity and tiger dispersal*. WWF-India, New Delhi,19pp.

Venkataraman AB and Johnsingh AJT (2004) The behavioural ecology of dholes in India. *The biology and conservation of wild canids* (Editors: DW Macdonald and C Sillero-Zubiri). Pp 323-336, Oxford University Press

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology,* 10, 249-256.

Walston J, Robinson JG, Bennett EL, Breitenmoser U, da Fonseca gab, Goodrich J *et al.* (2010) Bringing the tiger back from the brink – the six percent solution. *PLoS Biology*, 8, e1000485 doi:10.1371/journal.pbio.1000485.

Wang IJ, Savage WK and Shaffer HB (2009) Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (*Ambystoma californiense*). *Molecular Ecology*, 18, 1365-1374.

Wasser SK, Houston CS, Koehler GM, Cadd GG and Fain SR (1997) Techniques for application of fecal DNA methods to field studies of Ursids*.* *Molecular Ecology*, 6, 1091-1098.

WCMC (1992) *Global Biodiversity: Status of the Earth's Living Resources*. Chapman & Hall, London.

Weir BS and Cockerham CC (1984) Estimating F-statistics for the analysis of population structure.*Evolution*, 38, 1358-1370.

Wentzel J, Stephens JC, Johnson W*et al.* (1999) Subspecies of tigers: molecular assessment using 'voucher' specimens of geographically traceable individuals. Pp. 40-49 in J. Seidensticker, S. Christie, and P. Jackson, editors.*Riding the tiger: Tiger conservation in human dominated landscapes*. Cambridge University Press, Cambridge, UK

Whitlock MC and McCauley (1999) Indirect measures of gene flow and migration. *Heredity*, 82, 117-125.

Wilcox BA and Murphy DB (1985) Conservation strategy: The effects of fragmentation on extinction. *The American Naturalist*, 125, 879-887.

Williamson JE, Heubinger RM, Sommer JA, Louis EE and Barber RC (2002) Development and cross-species amplification of 18 microsatellite markers in the Sumatran tiger (*Panthera tigris sumatrae*). *Molecular Ecology Notes,* 2, 110-112.

Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from microsatellite loci. *Conservation Genetics*, 6, 551–562.

Wilson GA and Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics,* 163, 1177-1191.

Woodroffe R (2000) Predators and people: using human densities to interpret declines of large carnivores. *Animal Conservation*, 3, 165-173.

Woodroffe R and Ginsberg JR (1998) Edge effects and the extinction of populations inside protected areas. *Science*, 280, 2126-2128.

Woodroffe R, Thirgood S and Rabinowitz A (eds.) (2005) *People and Wildlife: Conflict or Coexistence?*Cambridge University Press.497 p.

Wright S (1951) The genetic structure of populations. *Annals of Eugenics*, 15, 323-354.

Wright S (1965) The interpretation of population structure by F-statistics with special reference to systems of mating. *Evolution*, 19, 395-420.

Wu J-H, Lei Y-L, Fang S-G and Wan Q-H (2008) Twenty one novel tri- and tetranucleotide microsatellite loci for the Amur tiger (*Panthera tigris altaica*). *Conservation Genetics*, 10, 567-570.

WWF–India (2012) *The return of tigers to Kopijhola, Madhya Pradesh, India: a tigress camera trapped in the Kopijhola forest block signifies its importance as a corridor.* WWF–India Satpura Maikal Landscape Program.http://www.wwfindia.org/news_facts/?uNewsID=7600

Yoganand K, Rice CG, Johnsingh AJT and Seidensticker J (2006) Is the sloth bear in India secure? A preliminary report on distribution, threats and conservation requirements. *Journal of the Bombay Natural History Society*, 103 (2-3), 172-181.

Young AG and Clarke GM (2000) *Genetics, Demography and Viability of Fragmented Populations*. Cambridge University Press, Cambridge.

Zhang ZH, Zhang WP, Yue BS *et al.* (2006) Twelve polymorphic microsatellite loci for the South China tiger *Panthera tigris amoyensis. Molecular Ecology Notes*, 6, 24-26.