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Original Research Article

Genetic structure of tigers (*Panthera tigris tigris*) in India and its implications for conservation

Vishnupriya Kolipakam [a, b], Shweta Singh [b], Bhawana Pant [b], Qamar Qureshi [a, b], Yadvendradev V. Jhala [a, b, *]

[a] National Tiger Conservation Authority — Wildlife Institute of India Tiger Cell, Wildlife Institute of India, Chandrabani, Dehradun, 248001, Uttarakhand, India
[b] Wildlife Institute of India, Chandrabani, Dehradun, 248001, Uttarakhand, India

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ABSTRACT

Identifying and prioritising naturally occurring within-species diversity, which may correlate with local adaptations or vicariance, is an integral part of conservation planning. Using non-invasive sampling and a panel of 11 microsatellites on 158 individual tigers from a pan India sample, our evaluation revealed three population clusters in India: unique North-Eastern tigers, a combined cluster of Western Ghats, Western India and Terai tigers, and a mixed cluster from Central India. At further population division, tigers from Odisha, Valmiki and southern Western Ghats were distinct. Central Indian tigers were most diverse, but showed the highest level of local structuring, suggestive of human induced fragmentation. We show that tigers in India are genetically structured and some clusters are unique. Considering a combined analysis of population size, genetic diversity and uniqueness, tigers from the North-East hills, and southern Western Ghats emerge as conservation priorities. We propose reintroductions and supplementation of tigers be done among the same broad genetic clusters. Restoration and management of habitat corridors is vital for anthropogenically fragmented Central Indian populations. This study suggests a paradigm shift from indiscriminately doubling tiger numbers to prioritising conservation of naturally occurring diversity amongst tigers, to retain their full evolutionary potential, while managing to mitigate anthropogenic induced genetic structuring.

1. Introduction

There is a general consensus that the planet is losing its biodiversity at a rapid rate due to human impacts (Dirzo et al., 2014; Hilton-Taylor, 2000; McGill et al., 2015). Conservation efforts need to be targeted not only at preserving species but also for conserving genetic diversity within a species. Genetic diversity can arise due to adaptive evolution (Endler, 1986; Ritland, 2000), or vicariance due to long-term historical isolation (Avise, 2000). Genetic diversity that results in behavioural, morphological and physiological plasticity is the ingredient for evolutionary processes to operate (Moczek et al., 2011;

* Corresponding author. Department of Animal Ecology & Conservation Biology, Wildlife Institute of India, Chandrabani, Dehradun, 248001, Uttarakhand, India.
E-mail addresses: vishnupriya@wii.gov.in (V. Kolipakam), shweta@wii.gov.in (S. Singh), bhawana@wii.gov.in (B. Pant), qnq@wii.gov.in (Q. Qureshi), jhalay@wii.gov.in (Y.V. Jhala).

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Schlichting and Smith, 2002). It is therefore important to recognise such diverse units within species and provide them with focussed conservation attention (Carvalho et al., 2017; Moritz, 2002).

Tigers are charismatic top predators that serve as umbrella species for the conservation of Asia's forest biodiversity. Tigers exhibit high level of plasticity in terms of habitats they occupy, prey they predate on, and climates they inhabit. For preserving tigers, their functional role in the ecosystem, and their evolutionary potential, it would be important to first understand the underlying genetic diversity of their populations caused due to these adaptations as well as natural historical events, and plan conservation strategies to preserve this diversity. Poaching for body parts, prey depletion and habitat loss are widely cited as causes of global tiger declines (Dinerstein et al., 2007). About 40,000 tigers were estimated to inhabit the Indian subcontinent before the colonial period (Pocock, 1929). Tiger populations within each of the forested landscapes of the North East, Central India, Western Ghats and the Terai probably shared a common genepool until recent times since these landscapes had contiguous forests. Currently tiger populations cohabit forests with anthropogenic use (Carter et al., 2013), a are small and isolated (Wikramanayake et al., 2011). Gene flow between tiger populations even within the same landscape is being restricted by modern agriculture, industry and associated infrastructure development (Yumnam et al., 2014). Despite the odds of housing 17.5% of the world's human population within 2.4% of the world's surface area, India boasts of harbouring over 70% of the world's extant wild tiger population, currently numbering about 2200 (Jhala et al., 2015). With a growing human population and rapid economic and infrastructure development, tiger populations are under increased threat. India's tiger conservation policy needs to incorporate strategies that accommodate its rapid development agenda. The conservation policy should be founded on a) preservation of viable source populations of tigers within each landscape unit (Bisht et al., 2019), b) ensuring a metapopulation structure by delineating and restoring corridors between populations within landscapes, and c) identification and subsequent preservation of extant genetic diversity at appropriate spatial scales.

Extant genetic structure in a population is brought about either through a) accumulation of variation as an adaptive response to the environment that a species inhabits (Slatkin, 1987), b) historical lineage of the species and biogeographic barriers to gene flow (Irwin, 2002) or c) in response to isolation caused by human induced habitat fragmentation followed by lack of gene-flow for prolonged periods of time (Fahrig, 2003). Conservation management requires tools that assist in differentiating natural vs. human induced genetic structuring. In case of the latter, management interventions, preferably by restoring habitat (corridor) connectivity or assisted geneflow between populations, needs to be implemented (Aitken and Whitlock, 2013). In extreme cases where inbreeding depression is documented, conservation management may even need to consider genetic rescue for population persistence (Hedrick and Fredrickson, 2010). Analysis using spatially explicit modern genetic tools, combined with information on biogeography and ecology, would enable identification of genetic clustering caused by natural versus anthropogenic causes (Slatkin, 1987). For effective conservation of tigers, it would be essential that each of the naturally occurring genetic cluster has sufficient number of tigers for long-term persistence; either as a single population or as a metapopulation. Effects of human induced isolation would need to be mitigated within genetic clusters, especially if population sizes are small (Proctor et al., 2005). Recent studies using whole genome data have demonstrated the need to identify inherent genetic clusters, even at a sub-species level, in order to develop a holistic conservation strategy (Liu et al., 2018; Luo et al., 2019).

In this study, we non-invasively sample tigers from across their range in India. We use a panel of 11 microsatellite loci on 158 individuals, to delineate population clusters. We evaluate and identify conservation priorities based on genetic variation, uniqueness and status of tiger populations. Previous studies on tigers in India have either been limited by low sample size, restricted to a particular landscape (Reddy et al., 2012; Sharma et al. 2011, 2013b; Yumnam et al., 2014), or have used inappropriate species specific markers (Mondol et al., 2009; Mukherjee et al., 2007; Maroju et al., 2016). By addressing these earlier limitations, the information generated herein provides a critical baseline on how tiger populations are genetically structured in India. We look at signatures of genetic structure within landscape units, which enable us to understand parameters of gene-flow, admixture and also help in identifying populations within clusters that require special conservation attention. The status of tiger in India has been evaluated every four years, since 2006 (Jhala et al. 2008, 2011, 2015). We use this information on the status of tigers that includes population spatial extent and size, in combination with their genetic data (divergence and diversity), to prioritise populations for conservation investment. Additionally, our results guide managers in making decisions on interventions of supplementation, genetic rescue and in selecting source populations for translocations.

2. Methods

Faecal samples of carnivores (scats) were collected from across India, covering 34 tiger populations from all major tiger landscapes (Jhala et al., 2011), between December 2013–December 2014. Scats were collected from field in plastic zip pouches containing silica, later aliquoted and kept in −20 °C freezer in 2 ml screw cap vials/double bagged zip pouches with silica gel. Samples from different tiger populations were divided into ecologically and biogeographically meaningful landscape clusters as follows: a) Central India and Eastern Ghats (CEG) – Odisha (Simlipal, Satkosia), Bandhavgarh, Gurughasidas, Palamau, Achanakmar, Kanha, Pench, Indravati, Tipeshwar, Udanti, Umred, b) Sunderbans (SBN), c) North-East (NE) – Namdapha, Dibang, Buxa, Manas, Kaziranga, d) Terai – Rajaji, Corbett, Dudhwa, Valmiki, e)Western India (WI) – Ranthambore, Sariska, f) Northern Western Ghats (N-WG) – Goa, Sahyadri, Anshi Dandeli, Bhadra, Biligiri Ranganatha Temple, Bandipur, Mudumalai, and, g) Southern Western Ghats (S-WG)- Anamalai, Periyar and Kalakkad Mundanthurai.

Details of genomic DNA extraction, and subsequent PCR for species identification is explained in Supplement S1. Samples positively identified as those belonging to tigers were then genotyped using the following 11 microsatellite loci: FCA304, F85,

F53, FCA441, FCA424, F124, FCA954, F96 (Menotti-Raymond et al. 1999, 2005), E6 and E7 (Bhagavatula and Singh, 2006), and 6HDZ700 (Williamson et al., 2002). These microsatellite loci were selected due to their high amplification rate, high polymorphic information content (PIC), and successful demonstration of data generation from faecal DNA samples of tigers (Joshi et al., 2013; Mondol et al. 2009, 2013; Reddy et al., 2012; Sharma et al., 2013a; Yumnam et al., 2014). PCR reactions for each of these microsatellite loci were performed in a single-plex reaction and multiplexed post-PCR for genotyping. The details of the PCR reactions for microsatellite genotyping are mentioned in Supplement S1.

The power of the eleven microsatellites to distinguish amongst closely related individuals was determined by calculating the probability of identity statistic (P_{ID}), computed in GIMLET (Valière, 2002). Each microsatellite loci was replicated at least thrice following the multiple−tube approach (Taberlet et al., 1996), in order to finalise the consensus genotype and correct for false alleles. In rare cases where no consensus was obtained with three replicates, samples were further amplified till consensus was reached. Genotyping was scored and cross-checked by two researchers independently to come to a conclusion on allele genotypes. Unique multilocus genotypes were identified using CERVUS 3.0 (Kalinowski et al., 2007). Samples that showed mismatches up to two loci were re-examined and if required re-analysed, for possible genotyping errors before assigning them as unique individuals. We computed the number of alleles per locus *(n),* observed (H_{obs}) and expected heterozygosity (H_{exp}) using Arlequin (Excoffier et al., 2005). PIC and tests for deviation from Hardy-Weinberg Equilibrium (HWE) were calculated using Genepop for web (Raymond and Rousset, 2002). Genetic distance measures between population clusters − G_{ST} (modified for multiple alleles by Nei (1973) and G'_{ST} (Hedrick and Goodnight, 2005) were estimated using R packages (Team, 2014) of adegenet (Jombart, 2008) and mmod (Winter, 2012). G'_{ST} was computed to address the limitations of G_{ST} (Jost, 2008; Meirmans and Hedrick, 2011).

2.1. Population genetic structure

We applied Bayesian clustering of multilocus genotypes to assign individuals to populations, and inferred the number of parental populations (K), for a sample using program STRUCTURE (Pritchard et al., 2000). The parameters used were, $K = 2$ to $K = 10$, 50,000 burn-in, and 10,000,000 Markov chain Monte Carlo (MCMC) iterations, with 10 replicates for each K value. The number of biological populations were determined by using ΔK statistic (Evanno et al., 2005), with the online version of Structure Harvester 5 v0.56.1 (Earl, 2012). One has to also note that STRUCTURE picks the K value at the hierarchically topmost level of genetic structure in a sample, as one increases the value of K, important information on admixture and gene-flow is acquired (Evanno et al., 2005). Therefore, we explored higher values of K to look at population differentiation across apriori defined landscapes. STRUCTURE runs were performed using the admixture model and correlated allele frequencies, with location priors. The assignment values of the samples were used with the program, DISTRUCT (Rosenberg, 2004), to produce graphs of STRUCTURE output.

2.2. Extent of allele sharing through discriminant analysis of principal component scores (DAPC)

Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010), implemented through the package *adegenet* in R was also used to understand the extent of allelic sharing in the sampled populations. DAPC is a model-free approach and extracts information by transforming the alleles into uncorrelated components using principal components analysis (PCA). A discriminant analysis is then applied to the principal components retained, to maximize the variation between population clusters and minimize the variation within apriori assigned groups. The *a.score* and *a.optim.score* functions were applied to identify the optimal number of principal components to be retained and the landscape scale clusters were used as a*priori* populations for the DAPC, based on which the number of discriminant functions was chosen (maximum number of population clusters minus one). The number of PCs retained was further cross-checked by an optimization procedure implemented in the *adegenet* package of R, which was performed with 100 replicates.

2.3. Influence of space on population genetic affinities through spatial principal component analysis (sPCA)

To investigate the potential relationship between geographic distances and genetic variation, a spatial principal component analysis (sPCA) (Jombart et al., 2008) was carried out using the algorithm implemented in the R software package *ade4* (Dray and Dufour, 2007) and *adegenet*. This approach does not require Hardy-Weinberg or linkage equilibria which were prerequisites for STRUCTURE analysis (Caye et al., 2016). sPCA incorporates the dimension of space in its analysis and therefore enables the detection of spatial genetic patterns which remain cryptic to non-spatial methods (Jombart et al., 2008). The method summarizes spatial autocorrelation amongst populations by using Moran's index on a set of allelic frequencies. We used data on tiger distribution and abundance across India (Jhala et al., 2015), overlaid on forest cover (Qureshi et al., 2014), to define population clusters that were either continuous tiger distribution or were within contiguous forest patches. We specified a connection network based on ground realities of habitat permeability to tiger movement determined by circuit theory (Yumnam et al., 2014), for the sampled populations. The resultant independent components quantified by sPCA are both positive and negative as they optimize the product between the spatial autocorrelation of populations and their genetic variation. A high positive sPCA loading indicates that separation by space explains the observed genetic differences and indicates global structure. While a high negative loading on sPCA indicates that neighbours are genetically more different that can be explained by spatial distance and are exhibiting local structure. A Monte-carlo based test with 10,000 iterations

was used in *global.rtest* implemented in the *adegenet* package, to test for statistical significance of observed allelic pattern against a random spatial distribution.

2.4. Prioritisation of populations for conservation investment

Priorities for conservation investment, based on genetic diversity and divergence were calculated using the Petit's Index (Petit et al., 1998). This index calculates a sub-population's contribution to the total diversity of the population, and the uniqueness of alleles within the sub-population, to rank the conservation value of each sub-population from a genetic perspective. Along with this index, we took into account information on the current population size for prioritising populations. Smaller populations are ranked above larger populations, due to the more imminent threat of extinction to the former and is therefore a meaningful ecological indicator to prioritise populations for immediate conservation attention. We combine this information on population size, along with the Petit's Index, to further prioritise populations.

3. Results

A total of 1147 samples were collected during the sampling season from 36 tiger reserves. Out of these, 718 samples successfully amplified with either tiger or leopard specific primers, resulting in a total of 341 tiger positive scats (Fig. 1). The cumulative P_{ID} value of the microsatellite marker panel was 1.29×10^{-17} (Table 1), and we were able to identify 187 unique individual tigers. After discarding individuals which had missing data at more than 3 loci, data from 158 individual tigers were used for further population genetic analysis. Presence of fewer than 11% null alleles were estimated on average and these were not consistent across loci or populations. Allelic dropout rates were negligible (average of 0.007%), with a maximum of 20% and this was not consistent across loci (Table S1). No evidence of stuttering error and short allele dominance was found for any of the loci. For the Hardy-Weinberg equilibrium test, only locus F124 deviated from equilibrium, mainly due to the allele frequencies in the population of Dudhwa tiger reserve. All the loci showed polymorphism in all landscapes, with high levels of genetic diversity across the landscapes. The PIC value for the alleles ranged from 0.7 to 0.9. Central India, in proportion to its sample size, had the highest mean number of alleles of 12.27, followed by North-East and N-WG with a mean number of 9.8 and 9.6 alleles respectively (Table 2). Western India showed an excess of observed heterozygosity when compared to the expected.

3.1. Population genetic structure across landscapes

Populations showed significant genetic structuring between and also within landscapes. The mean G_{ST} value among all the population pairs was 0.053 ± 0.028 and the mean G'_{ST} value was 0.433 ± 0.146 (Table 3). STRUCTURE result showed maximum differentiation at $K = 3$ populations, inferred by lnP(K) and ΔK (Fig. 2a). At this K, differentiation of the North-East populations as a separate cluster explained majority of the observed genetic structure in Indian tiger populations. Majority of the North-Eastern tiger individuals were assigned to a single cluster ($Q > 0.9$). The Terai, Western Indian and Western Ghat tiger populations emerge as a second cluster while the Central Indian tigers formed one large panmictic third cluster sharing alleles with North-East, Western Ghat and Terai populations. Distance measures indicate that the Terai populations were closest to the Western Ghat populations. The least genetic distance of the North-Eastern population was with that of Central India ($G_{ST} = 0.027$, $G'_{ST} = 0.332$; Table 3).

With increase in K (6–10), North-Eastern tigers split into two genetic clusters; a) the highland populations and b) the tigers of the North-Eastern plains. With increasing K values (Fig. 2b-d), Central Indian tigers further split into several smaller clusters. Western Indian tigers formed a unique cluster with affinity to both central Indian and the Terai populations (Fig. 2). Indices of G_{ST} and G'_{ST} also suggest that the Western Indian tigers are genetically closest to the Terai ($G_{ST} = 0.038$, $G'_{ST} = 0.291$, Table 3), and CEG tigers ($G_{ST} = 0.048$, $G'_{ST} = 0.426$; Table 3). From amongst the Terai populations at $K \geq 6$, Valmiki formed a unique cluster at $Q \geq 0.79$, while the remaining Terai tigers were assigned to a separate single cluster with $Q \geq 0.97$. Tigers south of the Palghat Gap in the Western Ghats (S-WG) formed a unique cluster at a $Q \geq 0.60$. While Western Ghat tigers north of the Palghat Gap (N-WG) could be differentiated into two clusters which had decreasing affinity from north to south with tigers from Central India. Interestingly, genetic distance (Table 3) between tigers from S-WG and N-WG was more than the genetic distance between tigers from the S-WG and Terai (Table 3). Sundarban tigers could not be distinguished as separate from the of Central Indian tiger cluster, even at $K = 10$ (Fig. 2d). Simlipal-Palamau tigers formed a unique cluster within central India at $K \geq 6$ (Fig. 2c and d).

3.1.1. Extent of allele sharing through discriminant analysis of principal component scores (DAPC)

The number of PCs to be retained from *optim.a.score* function varied between 24 and 30, while the cross-validation technique with 100 replicates estimated optimal PCs to be 30. Therefore, 30 PCs were retained for the final analysis. The North-Eastern tigers formed a separate ellipse from the rest of the tigers. The remaining tiger populations showed varied levels of overlap (Fig. 3). Western Ghat tigers south of the Palghat Gap (S-WG) formed a distinct ellipse from Western Ghat tigers north of the Palghat gap (N-WG), but overlapped with the population ellipse of Terai Tigers. Western Indian tigers were seen distributed closer to the Central, N-WG and Terai populations. While Sundarban tigers formed a unique ellipse closer to CEG and North-East tigers. Ellipses of CEG, N-WG and Western Indian tigers overlapped substantially in discriminant space.

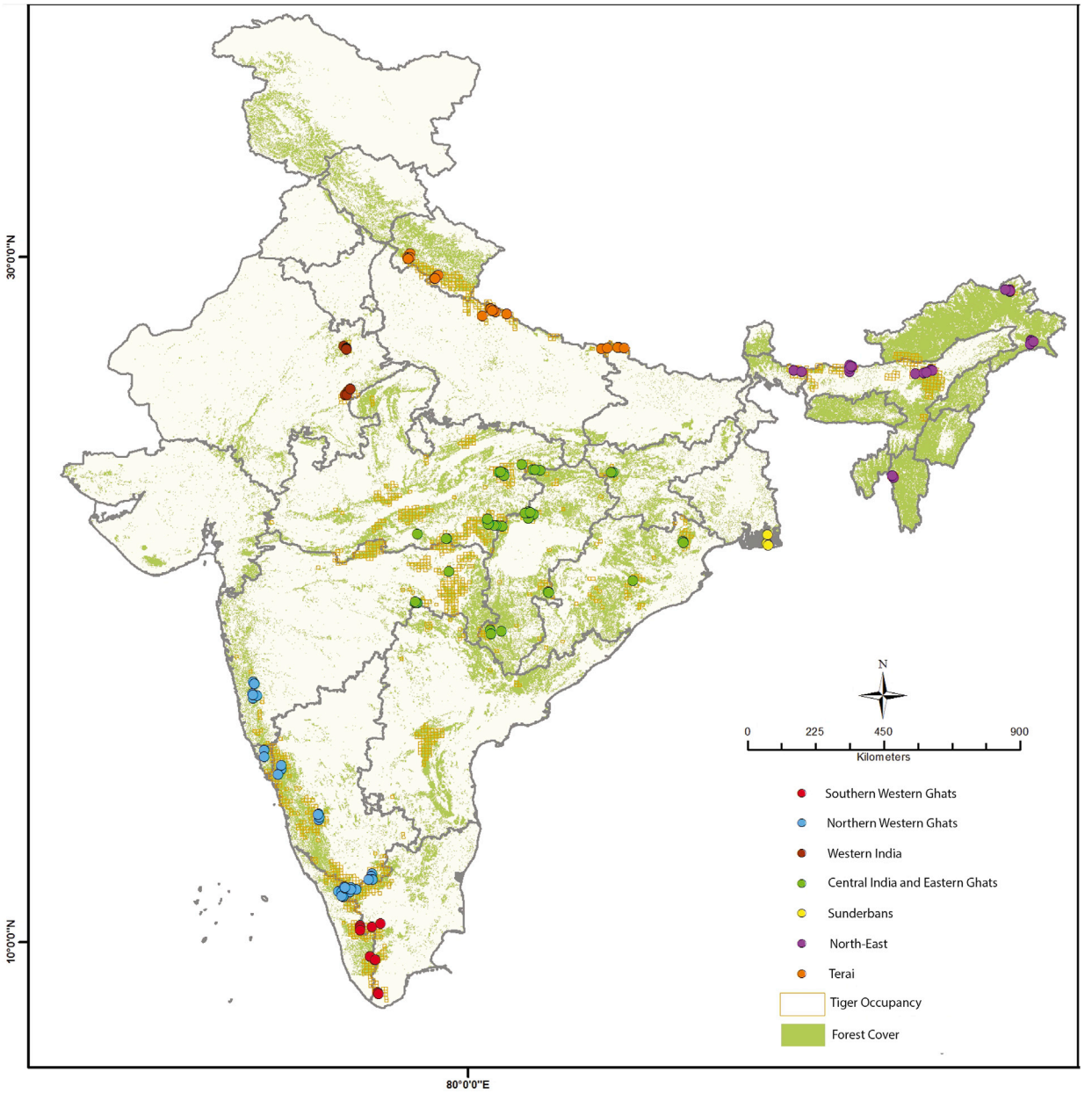


Fig. 1. Tiger distribution, sample locations, on forest cover map of India. Source for tiger distribution: Jhala et al. (2015).

3.2. *Influence of space on population genetic affinities through spatial principal component analysis (sPCA)*

Tests of significance for the sPCA detected a significant global pattern ($p < 0.001$) as well as a local pattern ($p < 0.05$). We retained the first two global axes and first local axis based on their eigenvalues. Tigers from the NE and Western Ghats adhered to the global pattern as their genetic variation was explained by geographic distance. A steep global cline between NE, Central, Terai and WG tiger populations is observed. Tigers from Central India showed local patterns of differentiation which were diffused (Fig. 4, Fig. S1). Tigers from NE and Odisha emerged to be genetically distinct (Fig. 4).

Table 1
Non-exclusion probability of Identity (Cumulative), Polymorphic information content (PIC), Expected Heterozygosity (H_{exp}) and Observed Heterozygosity (H_{obs}) of each loci, across 158 tiger individuals from 39 tiger reserves.

S.No	Loci	NE-SI	PIC	H_{exp}	H_{obs}
1	FCA304	2.50E-002	0.870	0.88	0.90
2	6HDZ700	1.50E-003	0.785	0.81	0.85
3	F85	1.07E-004	0.763	0.79	0.45
4	F53	5.11E-006	0.813	0.83	0.70
5	FCA441	3.48E-007	0.770	0.79	0.62
6	F124	4.52E-009	0.911	0.92	0.53
7	FCA424	2.98E-010	0.775	0.80	0.50
8	E7	9.54E-012	0.854	0.87	0.76
9	FCA954	1.24E-013	0.908	0.92	0.73
10	E6	1.61E-015	0.909	0.92	0.68
11	F96	1.29E-017	0.821	0.93	0.70

Table 2
Landscape sample size (N), Mean number of alleles per locus (MNA), Allelic richness (AR), Observed Heterozygosity (H_{obs}), Expected Heterozygosity (H_{exp}), Inbreeding Coefficient (F_{IS}). CEG – Central India & Eastern Ghats, NE – Northeast, WI- Western India, N-WG – Northern Western Ghats, S-WG – Southern Western Ghats.

Landscape	Populations	N (158)	MNA	AR	H_{obs}	H_{exp}	F_{IS}
CEG	Odisha (Simlipal, Satkosia), Bandhavgarh, Guru Ghasidas, Palamau, Achanakmar, Kanha, Pench, Indravati, Tipeshwar, Umred, Udanti	44	12.27	4.34	0.683	0.860	0.208
Sunderbans	Sunderbans	3	2.81	2.82	0.757	0.648	−0.220
NE	Namdapha, Dibang, Buxa, Manas, Kaziranga	27	9.81	4.05	0.705	0.821	0.144
Terai	Rajaji, Dudhwa, Corbett, Valmiki	26	7.81	3.80	0.655	0.784	0.168
WI	Ranthambore, Sariska	11	5.27	3.28	0.766	0.711	−0.080
N-WG	Goa, Sahyadri, Anshi Dandeli, Bhadra, Biligiri Ranganatha Temple, Bandipur, Mudumalai	33	9.63	4.09	0.698	0.832	0.164
S-WG	Anamalai, Periyar and Kalakkad Mundanthurai (KMTR)	10	5.91	3.66	0.549	0.768	0.300

Table 3
Genetic distances between Indian tiger landscape population pairs. Values below the diagonal represent Nei's G_{ST} and above the diagonal represent G'_{ST}. Also see Table S3.

Populations	CEG	Sunderbans	NE	Terai	WI	N-WG	S-WG
CEG	0	0.461	0.332	0.305	0.426	0.235	0.408
Sunderbans	0.058	0	0.599	0.571	0.658	0.636	0.532
NE	0.027	0.084	0	0.447	0.582	0.445	0.523
Terai	0.028	0.086	0.046	0	0.291	0.255	0.233
WI	0.048	0.117	0.073	0.038	0	0.429	0.504
N-WG	0.019	0.087	0.040	0.025	0.051	0	0.366
S-WG	0.039	0.083	0.057	0.027	0.070	0.038	0

CEG – Central India & Eastern Ghats, NE – Northeast, WI- Western India, N-WG – Northern Western Ghats, S-WG – Southern Western Ghats.

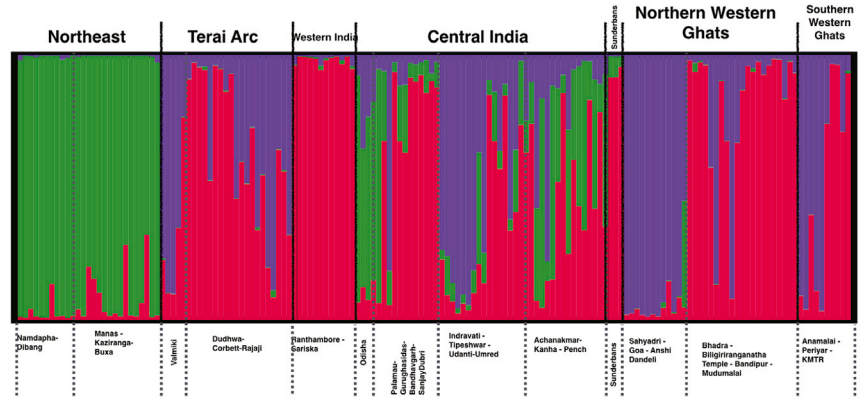
3.3. Population genetic structure within each tiger inhabited landscape

3.3.1. CEG landscape

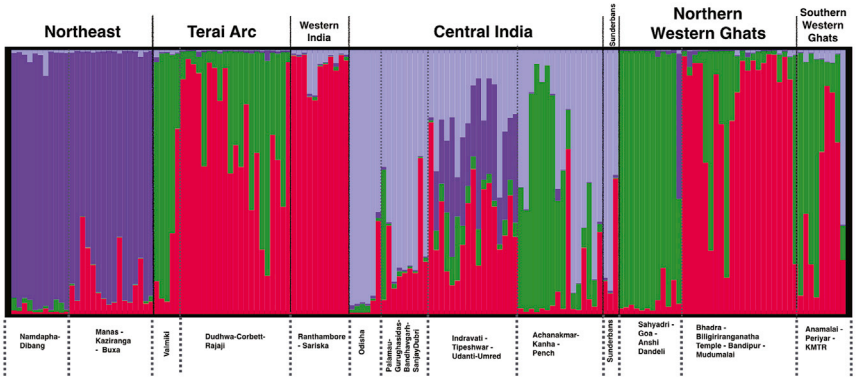
Western Indian and Sunderban tigers were also included within CEG landscape due to their biogeographic proximity and genetic affinity. For CEG landscape, K = 6 was inferred as the optimum K at which these populations showed maximum differentiation (Fig. 5a). At this K value, the tigers from Odisha formed a separate cluster with genetic affinity to Palamau tigers. While Sunderban tigers separate out as a cluster, there exists substantial allele sharing with tigers from Achanakmar-Kanha and Pench, and to a lesser extent with Odisha tigers (Fig. 5a). Tigers from Achanakmar, Kanha, and Pench formed a single cluster.

3.3.2. Terai landscape

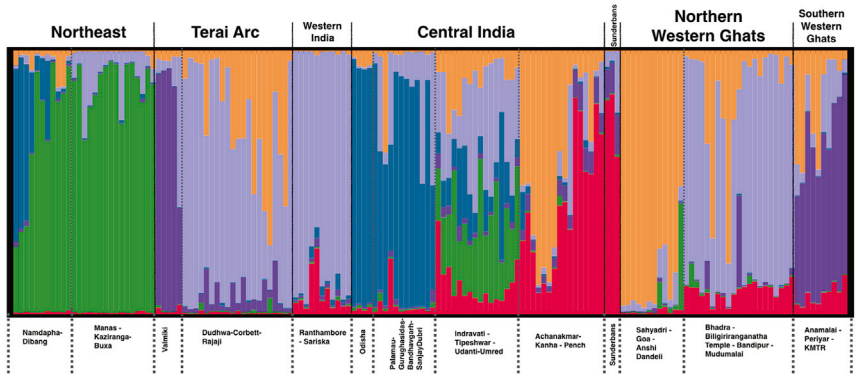
Since Western Indian populations are geographically proximal to the Terai populations, and as there was a clear signal of genetic admixture in the global analysis, we included Western Indian tigers for this analysis as well. The value of K that maximized the genetic differentiation was determined to be three. At this K = 3 value, there were three clear clusters representing a) Valmiki, b) Dudhwa-Corbett-Rajaji complex, and c) Western Indian complex of Ranthambore and Sariska, with negligible signatures of admixture amongst the clusters (Q values ≥97%, Fig. 5b).



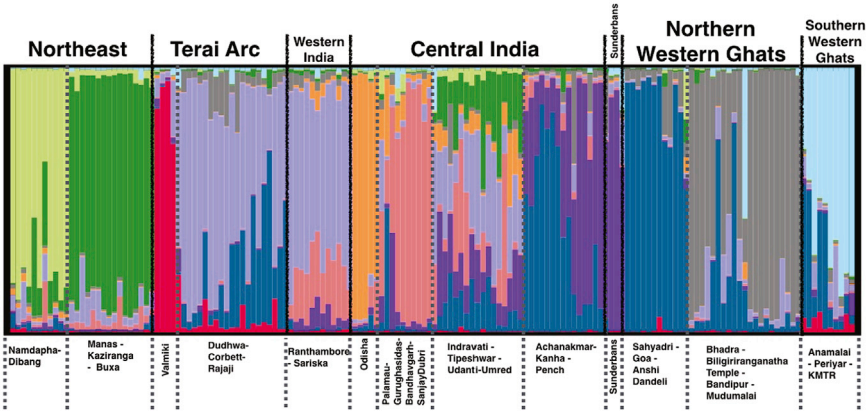
(a) K=3



(b) K=4



(c) K=6



(d) K=10

Fig. 2. Barplot indicating genetic structure of 158 individual tigers across landscapes using STRUCTURE runs at K = 3, K = 4, K = 6 and K = 10. Each individual is represented by a vertical bar, and the coloured length of each bar indicates the probability of membership in each cluster.

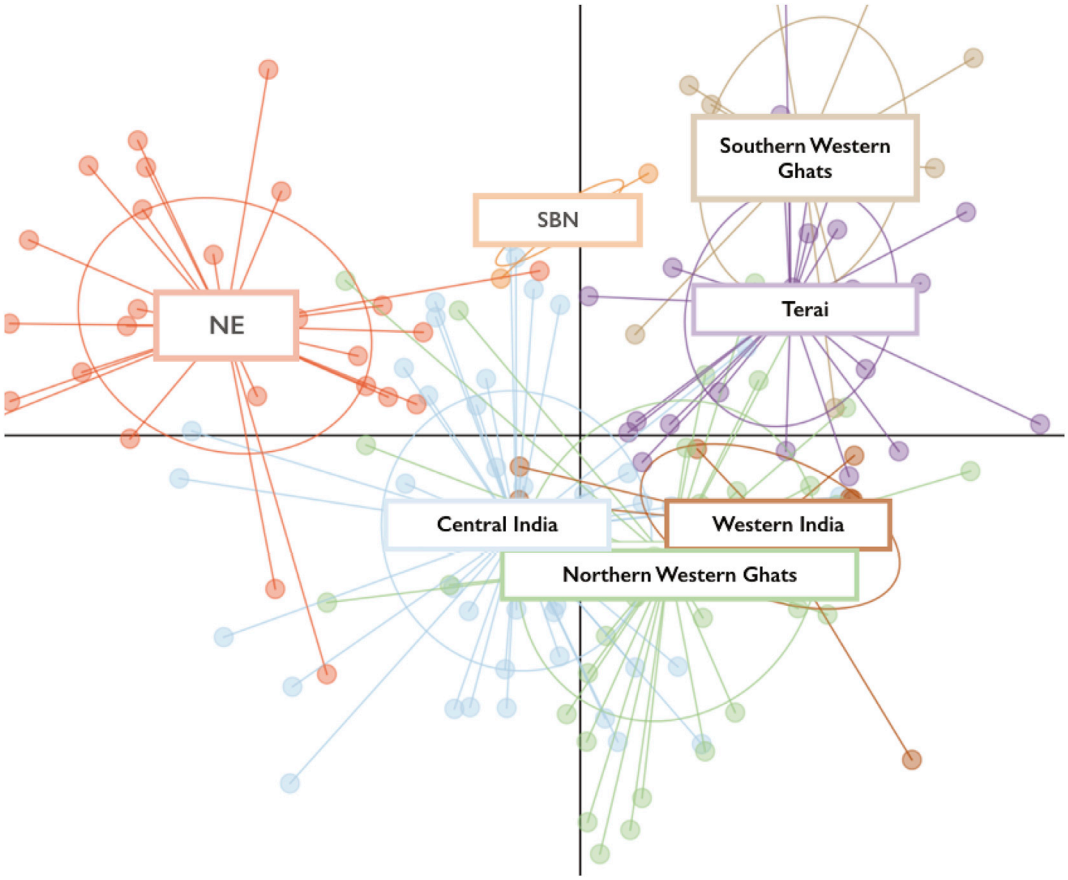


Fig. 3. Discriminant analysis of 30 principal components (DAPC), of 158 individual tigers from seven landscapes in India.

3.3.3. Western Ghats landscape

Best representation of genetic differentiation was inferred at K = 3 for tigers of the Western Ghats (Fig. 5c). The three clusters delineated were the north Western Ghat tigers (Sahyadri-Goa-Anshi Dandeli), the central Western Ghat tigers (Bhadra-Biligiri Ranganathaswami temple–Bandipur-Mudumalai) and tigers south of the Palghat gap (Anamalai-Periyar-KMTR).

3.4. Conservation prioritisation

Based on genetic diversity and divergence, the landscapes of conservation priority were ranked as follows: 1) CEG, 2) NE, 3) NWG (Table 4). After incorporating the aspect of population size, which is indicative of population vulnerability to extinction, the populations of 1) NE, 2) SWG, and 3) Sunderbans need to be prioritized for immediate conservation efforts (Fig. 6).

4. Discussion

Most large carnivore populations have shown global declines (Weber & Rabinowitz, 1996) in the recent past. Tigers in particular have lost 93% of their global range and declined by 45% in the past two decades alone (Sanderson et al., 2010). The Government of India committed significantly for tiger conservation by launching Project Tiger in the early 1973 (Panwar, 1987). Subsequently, increased demand of tiger body parts in China and SE Asia depleted wild tiger populations across India (Check, 2006). The first country-wide scientific assessment in 2006 (Jhala et al., 2008) showed a significant decline in tigers compared to the presumed official estimates. The Indian Government created the National Tiger Conservation Authority and substantially increased funding support, and formulated policy for prioritising tiger conservation (Narain et al., 2005). It is important that conservation investment should ensure survival not only of tigers as a species, but also conserve and maintain its extant genetic diversity. Foremost target for ensuring survival of tigers would be to secure and protect existing viable source populations within each landscape (Bisht et al., 2019; Walston et al., 2010). This has been achieved within most tiger landscapes of India (example Corbett within the Terai (Bisht et al., 2019), Kanha/Pench/

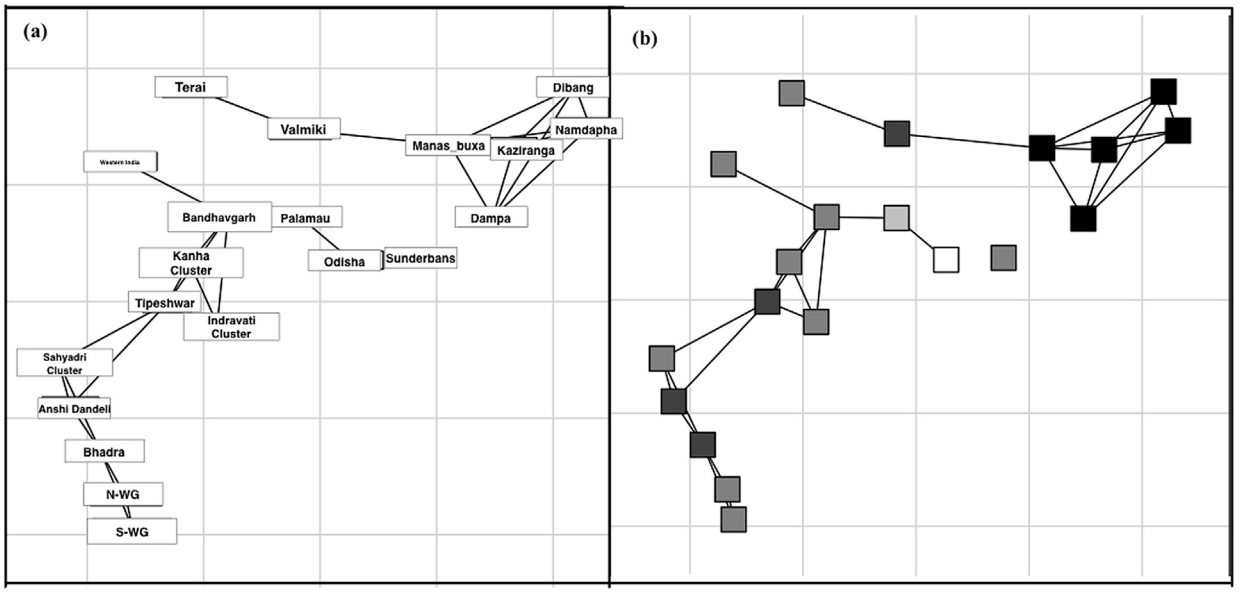


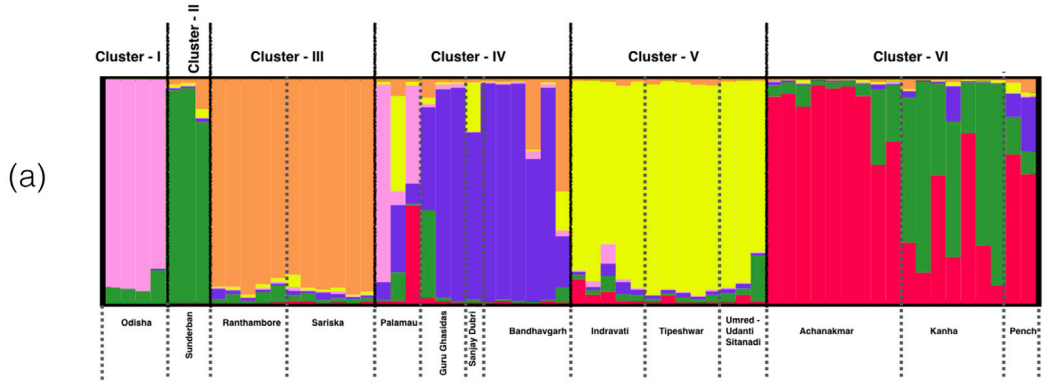
Fig. 4. a) Network connections of tiger populations in India for spatial principal component analysis (sPCA) b) genetic clines as determined by sPCA analysis, wherein Odisha and north-east populations stand out as unique.

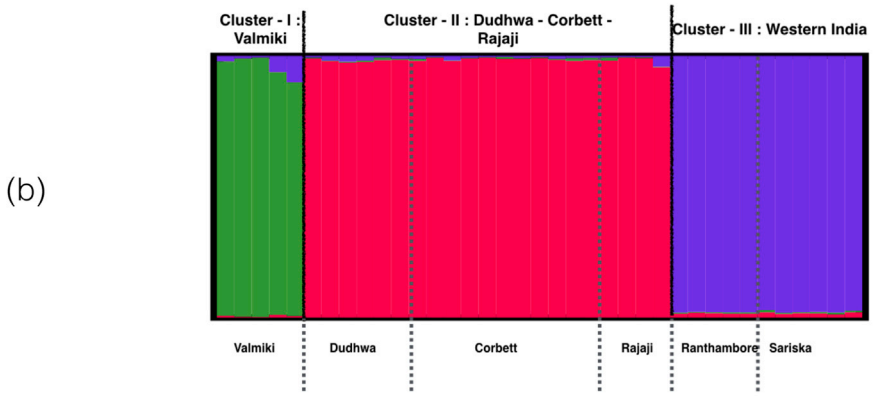
Bandhavgarh in Central India, Bandipur-Mudumalai-Nagarhole-Sathyamangalam-Wyanad in Western Ghats, Kaziranga in North-East). The focus now, after ensuring sufficient resources to protecting existing sources, would be to secure the extant genetic variation in the Indian tiger genepool (Liu et al., 2018; Luo et al., 2019). Towards this end, our results provide valuable information to target and prioritise conservation of tiger populations across India.

Our results suggest that Indian tigers possess comparatively high levels of genetic diversity (Table 2, Fig. S2) than reported earlier (Luo et al., 2004). Indian tiger diversity (MNA 7.6 ± 3.2) (Fig. S2) was higher than that recorded for other large felids like Amur tiger (*Panthera tigris altaica*; MNA 3.47 ± 1.22), African lions (*Panthera leo leo*; MNA 5 ± 1.75), cheetah (*Acinonyx jubatus*; MNA 4.92 ± 2.87), and North American puma (*Puma concolor*; MNA 3.08 ± 1) but comparable to South American puma (*Puma concolor*; MNA 7 ± 1.76). The central Indian tigers probably house the maximum diversity amongst all extant tiger populations at MNA of 12.27. Recent whole genome analysis at 10X depth also reflects the divergent nature of the Indian tiger subspecies. This is likely due to divergence from other tiger sub-species following a vicariant event post the last glacial maxima (Luo et al., 2019).

We used three different approaches to analyse population structure within Indian tigers. Our results from STRUCTURE, DAPC and sPCA, all converge to show that tigers from North-East were unique and distinct. Further, tigers from southern Western Ghats, Odisha and Valmiki showed unique genetic signatures. The North-Eastern landscape is one of the most globally biodiverse regions (Myers et al., 2000). Our study also forms the first genetic study with samples from Namdapha Tiger reserve and Dibang valley, where low densities of tigers were expected (Datta et al., 2008). The results from STRUCTURE and DAPC, in consonance with earlier studies (Sharma et al. 2009, 2011), clearly show the genetic separation of the North-Eastern tigers rom the rest of the Indian tiger populations. The results of Petit's index also highlight the importance of North-East tigers due to their divergent genetic constitution (Table 4). At further subdivisions in STRUCTURE, the highland populations of Dibang and Namdapha differentiate from that of the plains and foot hill populations of Kaziranga-Manas-Buxa complex. A plausible reason for the distinction of North-Eastern tigers from the rest of the Indian tigers could due to sharing of genetic material with the Indochinese tiger population (*Panthera tigris corbetti*) which borders the north-eastern states. Given the current results, combined with the low density and small tiger numbers in the north-eastern hill region (Jhala et al., 2015), the North-Eastern tiger populations merit the status of a special population unit of high conservation value. For managerial purposes, the two sub-clusters of the North-Eastern landscape needs to be maintained, i.e., a) the Hills and b) flood plains and foothills.

The CEG landscape harbours the second largest metapopulation of tigers in the country and many populations in the landscape have been categorised as a Class-I site for conservation priority (Dinerstein et al., 2007; Sanderson et al., 2010). The CEG tigers encompass the entire spectrum of diversity recorded from almost all Indian tigers with the exception of tigers from North-east Hills, Valmiki and southern Western Ghats (Fig. 1d), and is prioritized as the most diverse population for conservation, based on results from Petit's index (Table 4). It is believed that tigers colonized the Indian sub-continent from the North-East through connected habitats (Driscoll et al., 2009). A reason for this high diversity could be that CEG lies at the cross-roads of tiger colonisation and central to admixture between genetic clusters. We found evidence of strong sub-structure (Figs. 2 and 5), with major tiger populations like Kanha-Achanakmar having poor exchange of genes with





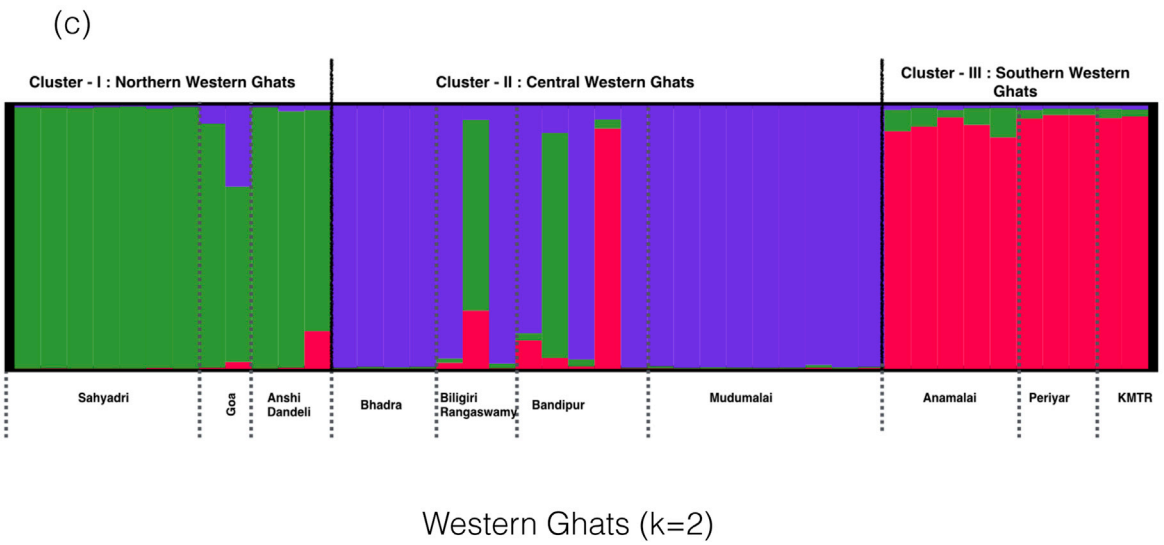


Fig. 5. Barplot indicating genetic structure at "best" K of individual tigers within each landscapes using STRUCTURE. Each individual is represented by a vertical bar, and the coloured length of each bar indicates the probability of membership in each cluster.

Table 4
Diversity, divergence, Petit's Index, and population size of tiger occupied landscapes in India. NE-Northeast, S-WG – Southern Western Ghats, West-Western India, SBN- Sunderbans, CEG – Central India & Eastern Ghats, N-WG – Northern Western Ghats. * Population size from Jhala et al., (2015).

Landscape	Diversity	Divergence	Petit's Index	Population size and (SE)*
CEG	3.08	−0.82	2.26	643 (557–729)
NE	1.61	0.32	1.93	201 (174–212)
N-WG	1.82	−1.02	0.80	687 (599–752)
S-WG	−0.30	0.38	0.08	98 (86–109)
SBN	−4.42	0.47	−3.95	76 (62–96)
West	−2.17	1.62	−0.55	45 (39–51)
Terai	0.38	−1.72	−1.33	485 (427–543)

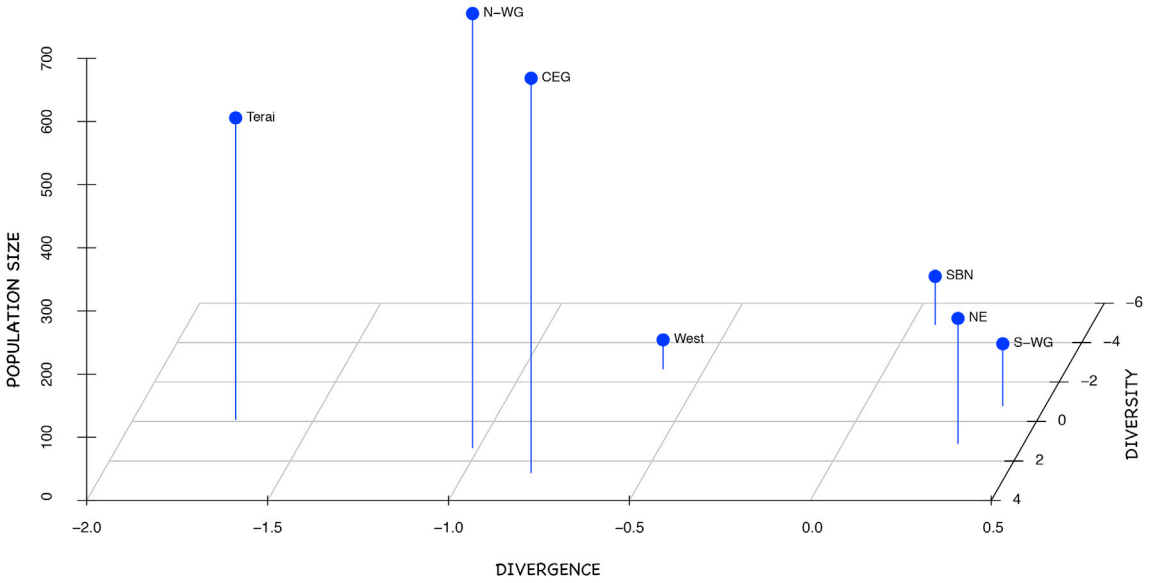


Fig. 6. Diversity, divergence, and population size of tigers in different landscapes of India. NE – Northeast, S-WG – Southern Western Ghats, West – Western India, SBN - Sunderbans, CEG – Central India & Eastern Ghats, N-WG – Northern Western Ghats.

neighbouring populations like Bandhavgarh. These results are in consonance with Yumnam et al. (2014). The immediate point of concern in the CEG cluster is the existence of distinct population clusters. This local structure is likely an artefact of human induced habitat fragmentation in this historically important agricultural belt along the Narmada river basin. For the long-term survival of tigers in this landscape, with burgeoning human pressure, it is important to manage them as a meta-population, which would reduce the risk of extinction.

Also in CEG, at higher sub-divisions, the Odisha tigers separate out as a unique cluster, apart from some similarity with Palamau tigers (Figs. 2d, 4 and 5) and distantly with Bandhavgarh. Interestingly, this is the only locality from where melanistic tigers are currently recorded (Jhala et al., 2015). Recent status assessment puts the population of these tigers to below 25 individuals, primarily occurring in a single population within Simlipal Tiger Reserve (Jhala et al., 2015). This genetic uniqueness and critical status puts the Odisha tigers on a priority for conservation investment. These depleted populations could potentially be supplemented from Bandhavgarh, which is genetically the closest source population.

Sunderban tigers shared allelic diversity with the CEG cluster of Achanakmar-Pench-Kanha cluster, which is likely due to a historical signal of founding population or recent isolation, and is in agreement with a recent study by Singh et al. (Singh et al., 2015). This result is surprising since the Sundarban tigers are morphologically distinct and smaller from mainland tigers (Barlow et al., 2010). Sundarban tigers did show a high level of divergence, but not diversity (Table 4). Further research based on a larger sample and screening of functional genes may be required to address this enigma.

The results of DAPC and STRUCTURE analysis indicated that the Western Indian tigers share allelic space with tigers from CEG and with Terai as well (Figs. 3 and 5). Recent studies have suggested that the Western Indian landscape is rapidly losing its link with other tiger populations in CEG (Reddy et al., 2012). The Aravalli Hill range of western India connecting the Gangetic Plains of Terai landscape, is now under intense agriculture but was forested as recently as the 15th century AD, and could explain the affinity of WI tigers with Terai. A negative F_{IS} value indicates that the population has recovered from a bottleneck recently, and from historical records we know this to be true (Sadhu et al., 2017). While inbreeding due to this bottleneck is expected (Frankham et al., 2002), ecological studies have revealed no signs of inbreeding depression (Sadhu

et al., 2017). This population offers an opportunity to study the genetic consequences of isolation, small population size and inbreeding in a wild tiger population.

At the highest level of structuring, the Terai Arc landscape tigers are seen as one large contiguous gene-pool (Fig. 2). But at further divisions and after taking into account spatial configuration, there is evidence of sub-structure, with tigers from Valmiki separating out from the rest of the tiger populations in the region (Figs. 2d and 5b). The Valmiki tiger population and its tiger habitat is continuous with Chitwan National Park and Parsa Wildlife Sanctuary. A recent study of Nepal tiger populations (Thapa et al., 2018) shows tigers from Chitwan to be distinct from tigers of western Terai. Thus, it seems likely that this tiger population in eastern Terai is genetically different from tigers in western Terai and from tigers further east (North-Eastern foot hills and flood plains). Unlike central India where local structure has likely arisen due to human induced habitat and population fragmentation, Valmiki is part of a large contiguous tiger habitat extending into Nepal as Chitwan NP and Parsa WLS within which tiger occupancy is also continuous, often exchanging individuals as evidenced by camera trapping (Chanchani et al., 2014). Thus, the uniqueness of Valmiki tigers is not likely due to isolation and drift, but more likely to be a vicariant event and therefore of conservation importance (Rutledge et al., 2010). Given our results, managers should treat Valmiki-Chitwan as a genetically distinct sub-unit from the rest of the Terai arc populations, while considering translocations and supplementations.

The Western Ghats landscape complex, a biodiversity hotspot (Myers et al., 2000), harbours the largest number of tigers in the country, and the largest contiguous tiger population in the world, estimated at 450 individuals (Jhala et al., 2015). The northern Western Ghat tigers share some genetic similarity with CEG (Fig. 2), and this is in consonance with the current and historical tiger distribution pattern (where the forested Vindhya hill range from the Deccan plateau meets the Western Ghats). sPCA and STRUCTURE analysis reveal three large sub-clusters at the population level (Figs. 3 and 5c) in which, the populations south of Palghat gap seem disconnected from the other two clusters, with limited gene-flow (Figs. 2d and 5c). The reason for S-WG population's isolation could be that either the dense human population of Palghat gap acting as a contemporary barrier between the two populations or the pattern is a historical relic from tiger colonisation of the Western Ghats. Results of the DAPC analysis showed the S-WG populations to be more closely clustered to the Terai populations, than the N-WG population. Following the colonisation of the Western Ghats, where historical connectivity with Terai existed, a population loss or decline in areas between and further re-colonisation could lead to the current observed pattern. A coalescent analysis testing different plausible hypotheses, along with additional data from mtDNA, is needed to understand the underlying mechanism causing this pattern. For managerial purposes, for now, S-WG should be treated as a separate population cluster. Within S-WG populations, tiger density is not as high as the Central WG populations, with no single population exceeding 22 tigers (Jhala et al., 2015). The total number of tigers in the cluster were estimated to be 98 (SE: 86–109). Given their genetic uniqueness and fragmented nature of the populations, these populations should be a conservation priority (Table 4). Restoring and managing habitat corridors that connect the various tiger reserves, Parambikulam-Anamalai with Periyar and Kalakkad Mundanthurai Tiger Reserves in S-WG cluster should be given priority, as these populations can only persist for the long-term if managed as a metapopulation.

Understanding the underlying genetic diversity is an important baseline for informed conservation practice. Through this information, we are able to identify genetic clusters, populations harbouring unique diversity and subsequently those in need of significant conservation attention. This information guides managers in planning tiger reintroductions and sourcing tigers for supplementing depleting populations. Tiger populations of Central India and Southern Western Ghats were observed to have high local structure, and existing tiger populations here are small and fragmented. Habitat fragmentation caused by humans is a major cause for concern across tiger landscapes, especially so within these two landscapes. Habitat restoration and management within identified corridors (Qureshi et al., 2014) are essential elements for managing these populations in a metapopulation framework for long-term persistence of tigers here. Our results emphasise the uniqueness of North-Eastern tigers which segregate at highest level of population distinction. Subsequently, at landscape scales Odisha, Valmiki and Southern Western Ghat tigers have unique traits. Given the small number of tigers in all of these unique populations, conservation efforts should target these on a priority, so as to preserve tiger genetic diversity in its entirety. Central Indian tigers were the most diverse and encompassed diversity from across most Indian tiger populations, therefore by investing conservation effort for these tigers would give maximum returns in terms of preserving tiger genetic diversity. Enabling habitat connectivity between populations to maintain large meta-populations is essential e.g. in Central India, even though it harbours maximum diversity, has many sub-clusters which are geographically proximal but possibly lack gene-flow between them (Yumnam et al., 2014). Focussing on protecting corridors between such populations would maximize the chances of population survival in this landscape. More sampling and coalescent based analyses are required to investigate the processes that drive the patterns we have discovered here.

5. Conclusion

Within India, priority conservation efforts need to focus on NE populations, (especially the NE-hill populations), Simlipal-Palamau (Odisha) tigers and tigers from southern Western Ghats. All these three areas currently have low tiger numbers, yet harbour unique diversity that is currently under-represented and not prioritized for conservation investments. Current conservation investment is maximum in high profile tiger reserves with large tiger populations like Kanha, Corbett, Bandipur, amongst others. Our research highlights the need to extend the focus of the current conservation paradigm to ensure conservation of all representative extant genetic diversity amongst tigers so that evolutionary lineages are not lost forever.

Author Contributions

YVJ & QQ conceived the study, raised the resources and collected the samples, VK, SS, & BP did the laboratory analysis, VK analysed the data with inputs from YVJ & QQ, VK & YVJ and wrote the paper, all authors reviewed the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00710.

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