Global Ecology and Conservation 32 (2021) e01888

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco

Pan-India population genetics signifies the importance of habitat connectivity for wild Asian elephant conservation

Rahul De [a], Reeta Sharma [a], Priya Davidar [b,c], N. Arumugam [c], Arulmozhi Sedhupathy [c], Jean-Philippe Puyravaud [b], K. Muthamizh Selvan [d], P. P. Abdul Rahim [c], Swati Udayraj [c], Jyotirmayee Parida [c], Dinesh Kumar Digal [c], Rajapandian Kanagaraj [e], Kashmira Kakati [f], Parag Nigam [a], A. Christy Williams [g], Bilal Habib [a], Surendra Prakash Goyal [a,*]

[a] *Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand, 248001, India*
[b] *Sigur Nature Trust, Masinagudi, Chadapatti, Tamil Nadu, 643223, India*
[c] *Pondicherry University, Kalapet, Pondicherry, 605014, India*
[d] *Project Elephant Division, Ministry of Environment, Forest & Climate Change, New Delhi, 110003, India*
[e] *French Institute of Pondicherry, Saint Louis Street, Pondicherry, 605001, India*
[f] *Independent researcher, Guwahati, 781021, India*
[g] *WWF-Myanmar, Y15/C, Than Taman Street, Dagon Township, Yangon, 11191, Myanmar*

ARTICLE INFO

Keywords:
Elephas maximus
Microsatellites
Genetic diversity
Population structure
Conservation priority
Climate change

ABSTRACT

Asian elephants are endangered while they have faced ~70% population decline in India in the last 60 years. Climate change projections indicate exacerbation of ongoing habitat loss (>40%) by 2070, potentially impacting genetic structure of wild elephants across India. Therefore, we provide consolidated baseline data on genetic diversity and structure of elephants across four eco-regions of India, i.e., north-western (NW), north-eastern (NE), east-central (ECI), and southern India (SI), to identify populations at greater risk of further divergence. We genotyped 169 faecal samples across 14 microsatellites with 90.0% overall success rate. The genetic diversity levels were moderate and varied between the eco-regions (H_E =0.57–0.74). Allelic richness was higher in NE (3.73–3.78) and SI (3.62–3.71). We observed a high inbreeding coefficient in NE (F_{IS}=0.55–0.58) compared to the other elephant populations, probably due to the presence of related individuals in our samples. Genetic differentiation between populations using F_{ST} statistics (F_{ST}=0.06–0.18) was significant. Bayesian and multivariate analyses identified three major genetic clusters in India – NW, NE, and combined ECI-SI, mostly consistent with their geographic distribution. We also observed an unexpected pattern of high genetic distance between adjacent populations. This fine-scale genetic structure suggests the presence of barriers (natural and anthropogenic) and complex social organisation. Additionally, incipient sub-structuring within NE and SI indicates potential genetic discontinuity. These results highlight the importance of maintaining genetic diversity, particularly of NE and ECI populations, by retaining habitat connectivity and ensuring gene flow for effective elephant conservation in India.

* Corresponding author.
E-mail address: goyalsp@wii.gov.in (S.P. Goyal).

https://doi.org/10.1016/j.gecco.2021.e01888
Received 6 April 2021; Received in revised form 16 September 2021; Accepted 20 October 2021
Available online 26 October 2021

1. Introduction

Management of threatened species in the Anthropocene has become a prime necessity to maintain their ecological functions through trophic linkages and inter-species interactions. Anthropogenic land use change is a prominent causative factor for several species' local and global extinction during the last century (Dirzo et al., 2014). Large bodied mammalian species are at high extinction risk due to anthropogenic impacts (Ripple et al., 2017). Moreover, dwindling population sizes below demographic tipping points can drive local extinction (de Silva and Leimgruber, 2019).

Among the large body-sized mammals of south and south-east Asia, elephants (*Elephas maximus*) can be considered a keystone species for their role in ecosystem functioning in Asian tropical forests (Baskaran, 2013; Campos-Arceiz and Blake, 2011; Sukumar, 2003). Asian elephants, the second-largest land-dwelling animal, require ample space, forage and water to maintain sustainable populations (Sukumar, 2003; Williams et al., 2010). Over historical time, they have been obliterated from most of its historical range while facing continuous population decline, thereby being enlisted as 'Endangered' by IUCN (Williams et al., 2020), placed in 'Appendix I' of CITES, and categorized as a 'Schedule I' species in the Wildlife (Protection) Act, 1972 of India. The current Asian elephant range is highly fragmented (Leimgruber et al., 2003) due to land use change.

India harbours ~60% of the current global elephant population (Jathanna et al., 2015), although extensive habitat loss and fragmentation threaten its long-term population viability. Padalia et al. (2019) estimate that ~25% of elephant habitat has been destroyed since the 1930 s and deforestation has been associated with increasing incidents of human-elephant interactions (Puyravaud et al., 2019). Hence, the protection of the remaining elephant habitat is vital for the long-term viability of this species. Wild Asian elephants occur in four disjunct eco-regions in India – north-western, north-eastern, east-central and southern region. Amongst these, southern and north-eastern regions together hold the vast majority (~81%) of the Indian elephant population (PED-MoEFCC, 2017). Although a total area of ~70,000 km^2 has been demarcated as elephant reserves, an administrative category, only a fraction of these reserves (27% by area) enjoy the formal legal status of protected areas. The elephant habitats face fragmentation of varying magnitude, while > 100 corridors have been identified using expert opinion to maintain connectivity between these landscapes (Menon et al., 2017). Besides, elephants may lose > 40% of their current suitable habitat by 2070 in the Indian sub-continent due to climate change along with further loss of connectivity between populations (Kanagaraj et al., 2019).

Population fluctuations, habitat destruction, fragmentation and global warming alter the spatial genetic linkages of species, potentially leading to demographic collapse caused by genetic drift, loss of genetic diversity and inbreeding (Frankham et al., 2010). Studies predict that recent habitat loss and fragmentation may cause genetic differentiation in elephants within a short time period, though historical patterns of gene flow may confound the effect of genetic drift (Goossens et al., 2016). Asian elephants have been extensively studied with respect to habitat use, population demography, ranging patterns and human-elephant interaction (Choudhury, 1999; Goswami et al., 2014; Johnsingh et al., 1990; Koirala et al., 2016; Lakshminarayanan et al., 2016; Madhusudan et al., 2015; Menon and Tiwari, 2019; Naha et al., 2019; Pollard et al., 2008; Saaban et al., 2020; Sarker and Røskaft, 2014; Thapa et al., 2019). Conversely, population genetic structure and its connection to social dynamics and demographic changes have received less attention (Ahlering et al., 2011; Chakraborty et al., 2014; Flagstad et al., 2012; Goossens et al., 2016; Moßbrucker et al., 2015; Thitaram et al., 2015; Vidya et al., 2007, 2005a; Vidya and Sukumar, 2005; Zhang et al., 2015). So far, a single study (Vidya et al., 2005a) has been conducted to provide information on the pan-India baseline of genetic diversity and population genetic structure of the Asian elephant. On the other hand, the population genetic structure, demography, social dynamics, level of hybridization and forensic tracking, as well as the impact of poaching on the African forest and savannah elephants have been extensively studied, forming the basis of effective conservation actions (Ahlering et al., 2012; Archie et al., 2006; Archie and Chiyo, 2012; de Flamingh et al., 2015; Ishida et al., 2016; Johnson et al., 2019; Mondol et al., 2015; Munshi-South, 2011; Santos et al., 2019; Wasser et al., 2015; Whitehouse and Harley, 2001).

Moreover, several recent advancements have taken place in the field of conservation genetics (Allendorf et al., 2013; Ortega and Maldonado, 2020), reiterating that previously generated data using dissimilar molecular markers across studies are incompatible for planning conservation actions for wide-ranging species. Harmonized genetic information across a species' distribution range is critical for defining conservation units (CU) and informed management of populations, thereby retaining evolutionary processes and genetic diversity (Crandall et al., 2000). The use of modern population genetic approaches, e.g. Bayesian and multivariate individual-based clustering and consideration of landscape variables while analyzing genetic structures, can facilitate understanding factors and processes shaping the fine-scale population genetic structure of wide-ranging species across different bioclimatic zones.

Therefore, the objective of this study was to genetically characterize the elephant populations from four discrete eco-regions across India and to delineate conservation units using contemporary analytical approaches. Specifically, we (1) evaluate the genetic diversity; (2) analyse differentiation among populations; (3) test whether population genetic structure is explained by isolation by distance, or barriers to gene flow.

2. Material and methods

2.1. Study area

The current study encompasses the elephant populations inhabiting four eco-regions of India viz. (i) the north-western (NW) and (ii) north-eastern (NE) Himalayan foothills, (iii) the central Indian-Eastern Ghats region (ECI), and (iv) the Western Ghats of southern India (SI). The north-western region supports ~2000 elephants, and the north-eastern population holds ~10,000 individuals. In comparison, the central Indian-Eastern Ghats and the Western Ghats of southern India population estimates are about 3000 and 12,000

elephants, respectively (PED-MoEFCC, 2017). These populations occur across a wide range of habitats, including evergreen forests, moist and dry deciduous forests, scrub savannah and alluvial grasslands.

2.2. Collection of non-invasive faecal samples and DNA extraction

We employed a non-invasive faecal sampling approach to isolate DNA from the outer layers of a single fresh elephant dung bolus per pile (n = 169), collected opportunistically from the four eco-regions (Table 1) with spatial representation across administrative units (e.g. forest beats and ranges). We stored the samples in sterile vials submerged in 95% ethanol or over silica gel in the field. We subdivided the four eco-regions into the following sampling units (n = 7), i.e. NW1, NW2, NE1, NE2, ECI, SI1 and SI2, based on geographical distance and discontinuities (Fig. 1, Table 1). The sampling units covered a total of 16 localities, as listed in Table 1. Sampling efforts were designed to avoid multiple samplings of individuals by not including similar bolus size and consistency in proximity. We dried the faecal samples in a hot air oven at 56 °C before storage over silica gel until further processing.

We scraped the top layer of the partial boluses, containing sloughed off intestinal epithelial cells, with sterile surgical blades into polypropylene tubes containing stool lysis buffer supplied with QIAamp DNA Stool Mini Kit (Qiagen GmbH). After overnight incubation at 56 °C water bath, we isolated and purified DNA from the faecal samples using a silica membrane column-based method following the manufacturer recommended protocol, with the modification of overnight incubation. We eluted genomic DNA in sterile polypropylene tubes using 180 μl TE buffer. DNA extraction procedures were carried out in dedicated low-DNA isolation facilities with negative controls to track and prevent contamination.

2.3. Microsatellite amplification and fragment analysis

We selected 14 microsatellite markers (Table 2) designed either for Asian or African elephants (Kongrit et al., 2008; Nyakaana and Arctander, 1998), which had previously been used successfully in Asian elephants (Ahlering et al., 2011; Chakraborty et al., 2014; Moßbrucker et al., 2015). To reduce processing time, cost and chances of manual errors, we multiplexed these markers for co-amplification based on their reported fragment length and dye labels into four panels (Supplementary Table S1). Each reaction consisted of 5 μl Qiagen Multiplex PCR Master-mix, 10 μg bovine serum albumin (BSA), 1.0 μl of multiplexed primer panel (equal proportions of 10 μM primers; forward primers labelled with Applied Biosystems fluorescent dye set), 2 μl genomic DNA of variable concentration, and nuclease-free water to bring the reaction volume to 10 μl. The polymerase chain reactions (PCR) were carried out in an Applied Biosystem Veriti thermocycler. The thermal cycling profile for Panel 1 (Supplementary Table S1) included initial denaturation at 95 °C for 15 min followed by 45 cycles of denaturation at 95 °C for 30 s, touchdown annealing at 62–52 °C for one minute – a drop of 2 °C every four cycles up to 20th cycle and 52 °C for rest of the 25 cycles, and extension at 72 °C for 40 s followed by a final extension at 60 °C for 30 min before hold at 4 °C. Reaction conditions for Panels 2,3 and 4 were similar except for a fixed annealing temperature (58 °C) instead of the touchdown annealing. We replicated the reactions a total of four times with each DNA isolate following a multi-tube approach (Taberlet et al., 1996) to reach a consensus in genotyping. All reactions included positive and negative controls to account for contamination and PCR failure. A fraction of the PCR products (~10%) from all panels were run in 2% w/v agarose gel stained with SYBR green along with 100 bp size markers before visualization in a gel-documentation station to screen for PCR success. Each PCR product (1.0 μl) were then mixed with Hi-Di™ Formamide (8.93 μl) and GeneScan™ 500 LIZ™ size marker (0.07 μl) (Invitrogen) and denatured at 95 °C for 5 min before capillary injection in an ABI 3530XL Genetic Analyser using POP-7 polymer (Invitrogen) for fragment analysis.

Table 1
Details of elephant faecal samples used in this study.

S. No.	Eco-region	State	Protected Area/Forest Division	Sampling Unit	Number of samples used in this study
1.	North-west India	Uttarakhand	Shivalik Elephant Reserve	NW1	64
2.	(NW)	Uttar Pradesh	Katarniaghat Wildlife Sanctuary	NW2	6
3.	North-east India (NE)	West Bengal	Baikunthapur Forest Division	NE1	14
4.		Assam	Jeypore Reserve Forest	NE2	14
5.	East-central India	Odisha	Similipal Tiger Reserve	ECI	6
	(ECI)		Kuldiha Wildlife Sanctuary		10
6.	South India (SI)	Karnataka	Nagarhole National Park	SI1	4
			Biligiriranganatha Swamy Temple Wildlife Sanctuary		4
		Kerala	Wayanad Wildlife Sanctuary		9
		Tamil Nadu	Sathyamangalam Tiger Reserve		6
			Mudumalai Tiger Reserve		2
			Sigur Reserve Forest		5
			Coimbatore Forest Division		4
7.		Kerala	Periyar Tiger Reserve	SI2	6
		Tamil Nadu	Kalakkad Mundanthurai Tiger Reserve		5
			Anamalai Tiger Reserve		10

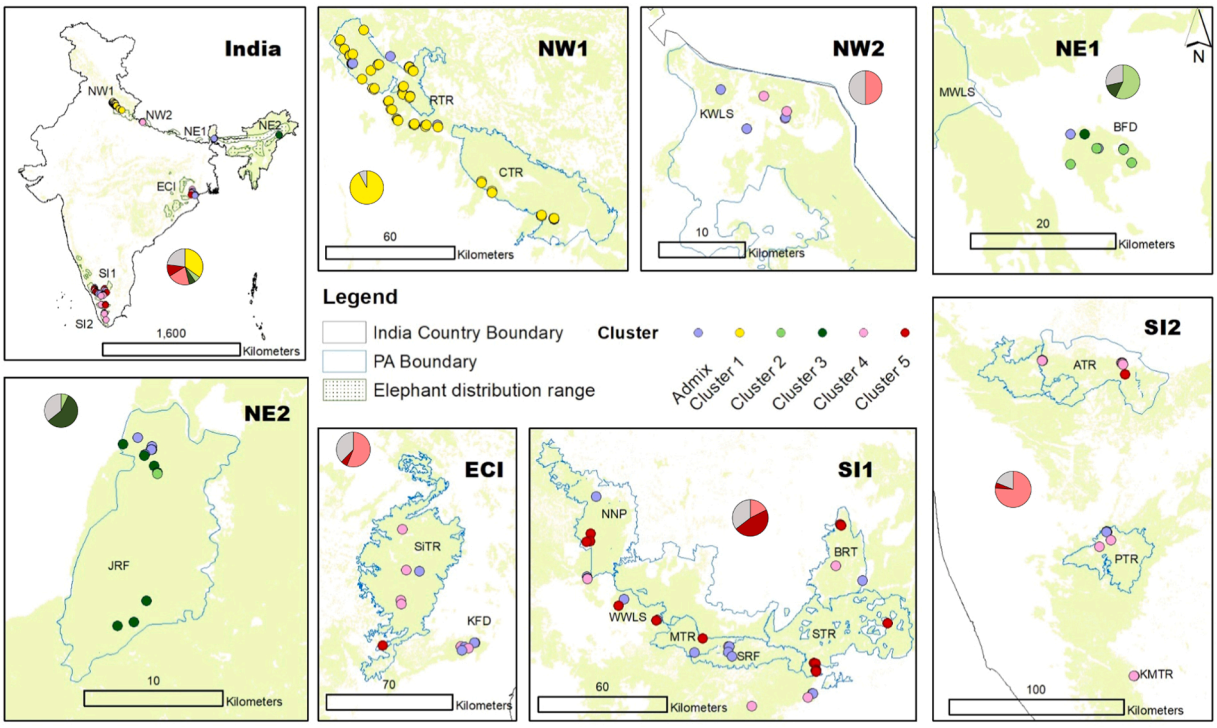

Fig. 1. Population genetic structure of the Asian elephant in India – assignment probabilities of the individuals to clusters as computed using STRUCTURE at K= 5. The pie charts depict the proportion of the individuals assigned to different clusters in the respective populations. Key: RTR – Rajaji Tiger Reserve (TR), CTR – Corbett TR, KWLS – Katarniaghat Wildlife Sanctuary (WLS), MWLS – Mahananda WLS, BFD – Baikunthapur Forest Division (FD), JRF – Jeypore Reserve Forest (RF), SiTR – Similipal TR, KFD – Kuldiha FD, NNP – Nagarhole National Park, WWLS – Wayanad WLS, SRF – Sigur RF, MTR – Mudumalai TR, STR – Sathyamangalam TR, BRT - Biligiriranganatha Swamy Temple WLS, ATR – Anamalai TR, PTR – Periyar TR, KMTR - Kalakkad Mundanthurai TR.

2.4. Microsatellite data analysis

We examined the resulting electropherograms using GENEMAPPER v5.0 (Applied Biosystems), performed automated allele scoring, followed by verifying each call manually. We binned the raw sizing data using AUTOBIN v0.9 Excel Macro (https://www6.bordeaux-aquitaine.inra.fr/biogeco_eng/Scientific-Production/Computer-software/Autobin). A consensus homozygous genotype was only recorded if the same allele amplified in at least three replicates out of the four, whereas we considered a consensus heterozygote if at least two replicates produced the same two sets of alleles (Morin et al., 2018, 2016; Ruiz-González et al., 2013; Sawaya et al., 2011). We discarded any genotype with two different sets of heterozygous consensus calls.

2.5. Microsatellite marker characteristics

We computed success rates for the individual microsatellite markers as the percentage of successful genotypes produced out of the total number of PCR. We estimated the polymorphism information content (PIC) of the markers using MolKin v3.0 (Gutiérrez et al., 2005). Frequencies of null alleles were calculated using the software FreeNA (Chapuis and Estoup, 2007) employing the EM algorithm (Dempster et al., 1977), while R package diveRsity (Keenan et al., 2013; RCore Team, 2019) was used to compute observed (H_O), expected (H_E) and unbiased expected heterozygosities (uH_E), allelic richness (AR) as well as to perform exact tests to detect departure from Hardy-Weinberg equilibrium (HWE). We calculated genotyping error rates in a maximum likelihood approach with 100,000 search steps using PEDANT v1.0 (Johnson and Haydon, 2009) from the first two replicates for each microsatellite loci. Cumulative probabilities of identifying unrelated individuals as a single individual (P_{ID}) and the probability of identifying siblings as unique individuals (P_{IDsib}) were calculated using GIMLET v1.3.3 (Valière, 2002).

2.6. Genetic diversity and population genetic structure

We computed the genetic diversity and differentiation parameters using the R package diveRsity (Keenan et al., 2013; RCore Team, 2019) for each sampled population. We used a Microsoft Excel macro, GenAlEx 6.5 (Peakall and Smouse, 2012, 2006), to identify private alleles (A_P) and to calculate their frequencies. We performed Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992; Michdaki and Excoffier, 1995) to infer the extent of population structuring using ARLEQUIN 3.5.2.2 (Excoffier et al., 2005).

Table 2
Observed characteristics of the microsatellite markers and genetic diversity indices in wild Indian elephants.

Marker	Success rate	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Unbiased expected heterozygosity (uH_E)	Frequency of null alleles	Allelic dropout (ADO) per genotype	False alleles (FA) per genotype	Polymorphism information content (PIC)	No. of private alleles
EMU03	92.31	0.46	0.73	0.74	0.14	0.09	0.07	0.69	4
EMU04	88.76	0.34	0.71	0.71	0.15	0.24	0.05	0.67	4
EMU07	92.90	0.35	0.73	0.73	0.17	0.19	0.00	0.69	3
EMU09	73.96	0.50	0.83	0.84	0.15	0.25	0.00	0.82	2
EMU10	98.82	0.43	0.76	0.76	0.17	0.20	0.00	0.73	3
EMU11	96.45	0.36	0.59	0.60	0.12	0.15	0.02	0.54	2
EMU12	94.67	0.56	0.84	0.84	0.10	0.06	0.00	0.82	6
EMU13	88.17	0.50	0.70	0.70	0.08	0.16	0.00	0.66	2
EMU14	84.02	0.48	0.82	0.83	0.15	0.04	0.05	0.80	5
EMU15	93.49	0.53	0.80	0.80	0.16	0.04	0.00	0.78	3
EMU17	81.66	0.53	0.84	0.84	0.14	0.17	0.03	0.82	4
LafMS02	87.57	0.41	0.72	0.72	0.17	0.09	0.04	0.68	1
LafMS03	95.86	0.42	0.66	0.66	0.14	0.06	0.00	0.64	3
LafMS05	91.72	0.46	0.68	0.68	0.11	0.17	0.01	0.65	6
Mean±SE	90.03 ± 1.78	0.45 ± 0.02	0.74 ± 0.02	0.75 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.02 ± 0.01	0.71 ± 0.02	3.43 ± 0.40

All markers deviated from the assumptions of Hardy-Weinberg Equilibrium.

Table 3
Genetic diversity parameters of the seven populations based on 14 microsatellite marker data across the elephant distribution range in India.

Eco-region (s)	Populations	Total no. of samples	Mean no. of alleles (MNA)	Allelic richness (AR)	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Unbiased expected heterozygosity (uH_E)	Inbreeding coefficient (F_{IS})	No. of Private allele observed (A_P)	Mean frequency of private alleles
North-west India (NW)	NW1	64	5.57	3.16	0.46	0.62	0.62	**0.25**	6	0.04
	NW2	6	3.71	3.15	0.51	0.57	0.63	0.10	1	0.33
North-east India (NE)	NE1	14	6.57	3.73	0.30	0.72	0.75	**0.58**	12	0.12
	NE2	14	6.07	3.78	0.33	0.74	0.77	**0.55**	8	0.11
East-central India (ECI)	ECI	16	5.07	3.35	0.43	0.64	0.66	**0.31**	4	0.03
South India (SI)	SI1	34	6.86	3.62	0.52	0.66	0.68	**0.20**	6	0.06
	SI2	21	5.79	3.71	0.51	0.65	0.66	**0.19**	11	0.03

Values in bold are statistically significant at $\alpha = 0.05$

Key: NW1: Shivalik ER (Rajaji TR, Lansdowne FD, Corbett TR), NW2: Katarniaghat WLS, NE1: Baikunthapur FD, NE2: Jeypore RF, ECI: Similipal TR and Kuldiha FD, SI1: Nilgiri Biosphere Reserve, SI2: Anamalai, Periyar and Kalakkad-Mundanthurai TR.

ER: Elephant Reserve, TR: Tiger Reserve, WLS: Wildlife Sanctuary, RF: Reserve Forest, FD: Forest Division

To examine the patterns of genetic structuring of elephants, we also employed multiple Bayesian approaches, viz. STRUCTURE v2.3 (Pritchard et al., 2000), TESS v2.3.1 (Chen et al., 2007) and GENELAND v4.0.8 (Guillot et al., 2005; RCore Team, 2019). We implemented discriminant analysis of principal components (DAPC) using the R package ADEGENET (Jombart, 2008; RCore Team, 2019) without any underlying assumptions of population genetic models such as HWE (Vergara et al., 2015). Additionally, we used the genetic landscape shape interpolation (GLSI) technique to visualize diversity patterns using the software ALLELES IN SPACE (AIS) (Miller, 2005). To understand the interrelationship of the seven sampled populations, we computed pairwise Nei's standard genetic distance (Ds) (Nei, 1978) at the population level using the software POPULATIONS v1.2.32 (Langella, 2002). We used FIGTREE v1.4.2 (Rambaut, 2014) to visualize and annotate the resulting dendrogram. We provide a detailed account of computational parameters, model selection and justification for all analytical methods mentioned in this section with the Supplementary Materials available online.

2.7. Assessment of conservation priority

Assessing relative contribution to global genetic diversity has recently been used to assign management priority of free-ranging and captive populations of threatened species (Kolipakam et al., 2019; Mannise et al., 2017; Sato et al., 2017). Similarly, we computed relative contributions of each sampled population to the aggregate genetic diversity of the elephants in India using two different approaches – mean genetic distance (GD) (Caballero and Toro, 2002) and total allelic richness (C) (Petit et al., 1998). Higher positive values for a population obtained using the GD based method indicates lower contribution, whereas a greater value of C specifies a higher contribution to aggregate diversity. We used the software MolKin v3.0 (Gutiérrez et al., 2005) to identify the elephant populations which are critical for retaining the current genetic diversity using both of the discussed methods.

Additionally, we examined the genetic diversity of each eco-region with respect to the projected habitat loss due to global climate change for Asian elephants under the least and the most aggressive scenarios (RCP 2.6 and RCP 8.5, respectively) (Mokhov and Eliseev, 2012) up to the next 50 years (Kanagaraj et al., 2019). To take the current situation of habitat connectivity into account, we also compared the total number of corridors and their priorities as reported (Menon et al., 2017) within each eco-region while considering the current official elephant population estimate (PED-MoEFCC, 2017) to assign conservation priorities.

3. Results

Fresh elephant faecal samples (n = 169) collected from the four eco-regions could be grouped into seven major populations (Table 1) based on a priori information available. Areawise, we effectively sampled ~15% (16,250 km^2) of the elephant habitat in India , estimated to be between 101,350 km^2 and 119,550 km^2 (Source: Ministry of Environment, Forests and Climate Change, Government of India; http://moef.gov.in/wp-content/uploads/2017/08/ehabitat.pdf).

3.1. Microsatellite marker characteristics

We genotyped the DNA extracts with 14 microsatellite markers and obtained consensus data of at least ten loci for all samples. The success rates across loci varied from 73.96% in EMU09 to 98.82% in EMU10, with a mean success of 90.03 ± 1.78% (Table 2). All markers were highly polymorphic (PIC>0.5) with a mean PIC of 0.71 ± 0.02. Null allele frequencies were moderate, varying between 0.08 and 0.17. H_E and uH_E for all the markers were higher than H_O while no loci conformed with the assumptions of HWE. The mean maximum-likelihood allelic dropout per genotype was 0.14 ± 0.01, while the false allele per genotype was 0.02 ± 0.01 (Table 2). All genotypes belonged to unique individuals (n = 169), with cumulative P_{ID} value for the 14 markers being 2.26×10^{-15} whereas P_{IDsib} was 2.74×10^{-06}.

3.2. Genetic diversity

Table 3 indicates that genetic diversity estimates varied among the four eco-regions, with substantial differences in the mean number of alleles and observed heterozygosities. Amongst the seven sampled populations, mean number of alleles (MNA) across loci was the highest in SI1 (6.86), while the lowest value was observed in NW2 (3.71) (Table 3). The NE2 population had the highest allelic richness (AR=3.78). We recorded moderate values of observed heterozygosity in southern, north-western and east-central populations (Ho=0.52–0.43) and comparatively lower H_O in the north-east (between 0.33 and 0.30). Consequently, NE1 (F_{IS}=0.58) and NE2 (F_{IS}=0.55) showed high fixation indices. Private alleles (A_P) constituted 27.9% of the total allelic richness (n = 172), while the number of A_P varied between one (NW2) and 12 (NE1) across the populations (Table 3). The mean frequencies of private alleles ranged from 0.03 (ECI) to 0.33 (NW2).

3.3. Population genetic structure

We tested for genetic structuring using multiple metrics, i.e. pairwise F_{ST} with ENA correction, D_{EST}, and AMOVA. A comparison of $F_{ST\ and}$ D_{EST} statistics allowed assessment of the genetic differentiation among the four elephant populations at varying spatial scales. The divergence was high, as expected, between the geographically distant (>2000 km) population pairs NW1-SI1 (D_{EST}=0.31; F_{ST}=0.18), and NW1-SI2 (D_{EST}=0.29; F_{ST}=0.17). However, we observed a moderate amount of genetic differentiation between populations located comparatively close to each other, i.e., NW1 and NW2 (distance~120 km, D_{EST}=0.18; F_{ST}=0.15), NE1 and the

NE2 (distance~650 km, D_{EST}=0.16; F_{ST}=0.12), SI1 and SI2 (distance~40 km, D_{EST}=0.07; F_{ST}=0.07), thereby suggesting limited gene flow between adjoining populations.

All the other pairwise $F_{ST\ and}$ D_{EST} values were moderate and statistically significant ($\alpha = 0.05$) based on 9999 bootstrap re-samplings (Fig. 2). AMOVA indicated that 15.9% of the total molecular variance was contributed by 'between-populations' differentiation, whereas 84.1% was caused by 'within-population' variation indicating population sub-structuring corroborating the deviation from HWE.

3.3.1. *Bayesian clustering approaches*

The spatially implicit individual-based clustering (IBC) approach implemented by the program STRUCTURE revealed the presence of three genetic clusters implied by a unimodal peak in the ΔK plot at K= 3 (Supplementary Fig. S1). This inference was supported by the L(K) method (Pritchard et al., 2000) as the rate of increase in L(K) reduced after K= 3 though the standard deviation substantially increased only after K= 5 (Supplementary Fig. S1). Therefore, we examined the individual assignment probabilities (Q) for K= 3, 4 and 5 for further information. At K= 3, 90.5% individuals (n = 169) were assigned with high probability (Q>0.8) viz. NW1 formed a separate genetic cluster, the north-eastern populations (NE1 and NE2) were assigned to the second cluster while ECI, SI1 and SI2 constituted the third cluster (Fig. 3a). The NW2 population, however, maintained mixed signatures. In addition to the clustering patterns of K= 3, 52.9% of the SI1 population (n = 34) were assigned to an emergent cluster at K= 4 with 81.1% assignment of the total individuals (n = 169). While examining the assignment probabilities at K= 5, we found 77.5% of individuals (n = 169) to be assigned (Q>0.8) while 57.1% of the NE1 samples (n = 14) showed the signature of an additional cluster.

According to the spatially explicit IBC algorithm in TESS, the optimal K was recorded as K_{MAX}= 4 based on the criteria of DIC values first reaching a plateau (Durand et al., 2009) for all three separate analyses with varying spatial interaction parameters (SIP=0.0, 0.6 and 0.9) (Supplementary Fig. S1). With four assumed populations, 88.2% of individuals (n = 149) were assigned to clusters with high probabilities (Q>0.8). NW1 formed a distinct genetic population while individuals from ECI, SI1 and SI2 were grouped into a separate cluster (Fig. 3b). NW2 population retained a mixed signature. The third and the fourth cluster were shared between NE1 and NE2 in different proportions.

Running correlated allele frequency models, GENELAND identified a total of eight genetic clusters without assuming admixture based on the clear mode (Guillot et al., 2005) at K= 8, plotting the posterior densities of the runs (Supplementary Fig. S1, Fig. 3c). NW1

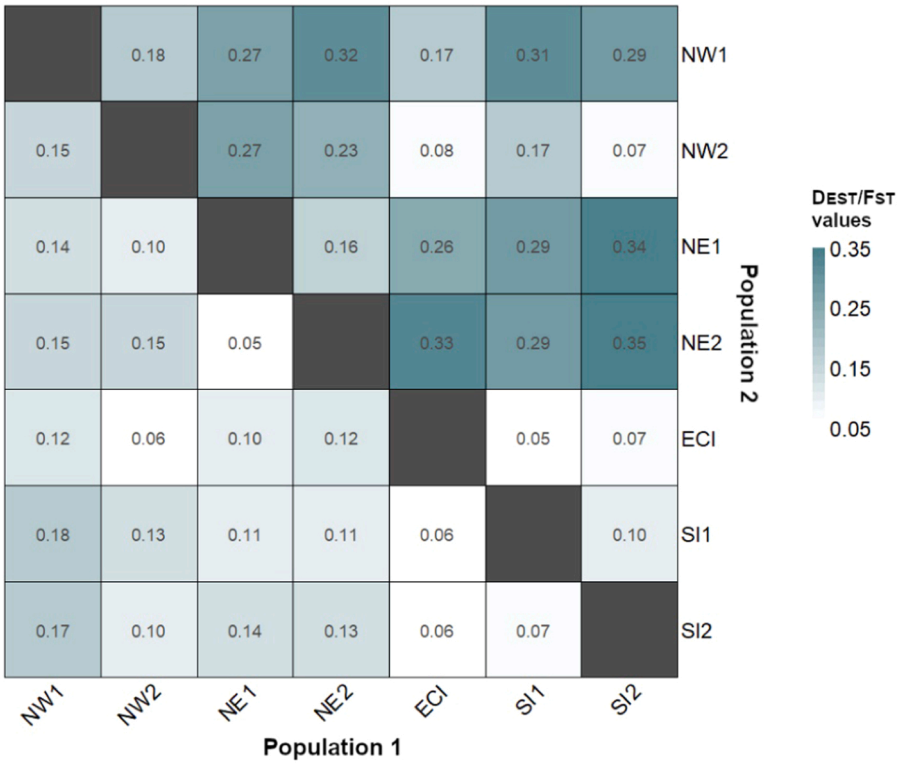

Fig. 2. Pairwise genetic differentiation across the seven elephant populations sampled in India. Above diagonal values are measures of D_{EST} (Jost, 2008), while below diagonal values indicate ENA corrected F_{ST} (Chapuis and Estoup, 2007; Weir, 1996). The lighter to darker colour gradient corresponds to increasing values of D_{EST}/ F_{ST}. All D_{EST}/ F_{ST} values are statistically significant at $\alpha = 0.05$. Key: NW1: Shivalik ER (Rajaji TR, Lansdowne FD, Corbett TR), NW2: Katarniaghat WLS, NE1: Baikunthapur FD, NE2: Jeypore RF, ECI: Similipal TR and Kuldiha FD, SI1: Nilgiri Biosphere Reserve, SI2: Anamalai, Periyar and Kalakkad-Mundanthurai TR. ER: Elephant Reserve, TR: Tiger Reserve, WLS: Wildlife Sanctuary, RF: Reserve Forest, FD: Forest Division.

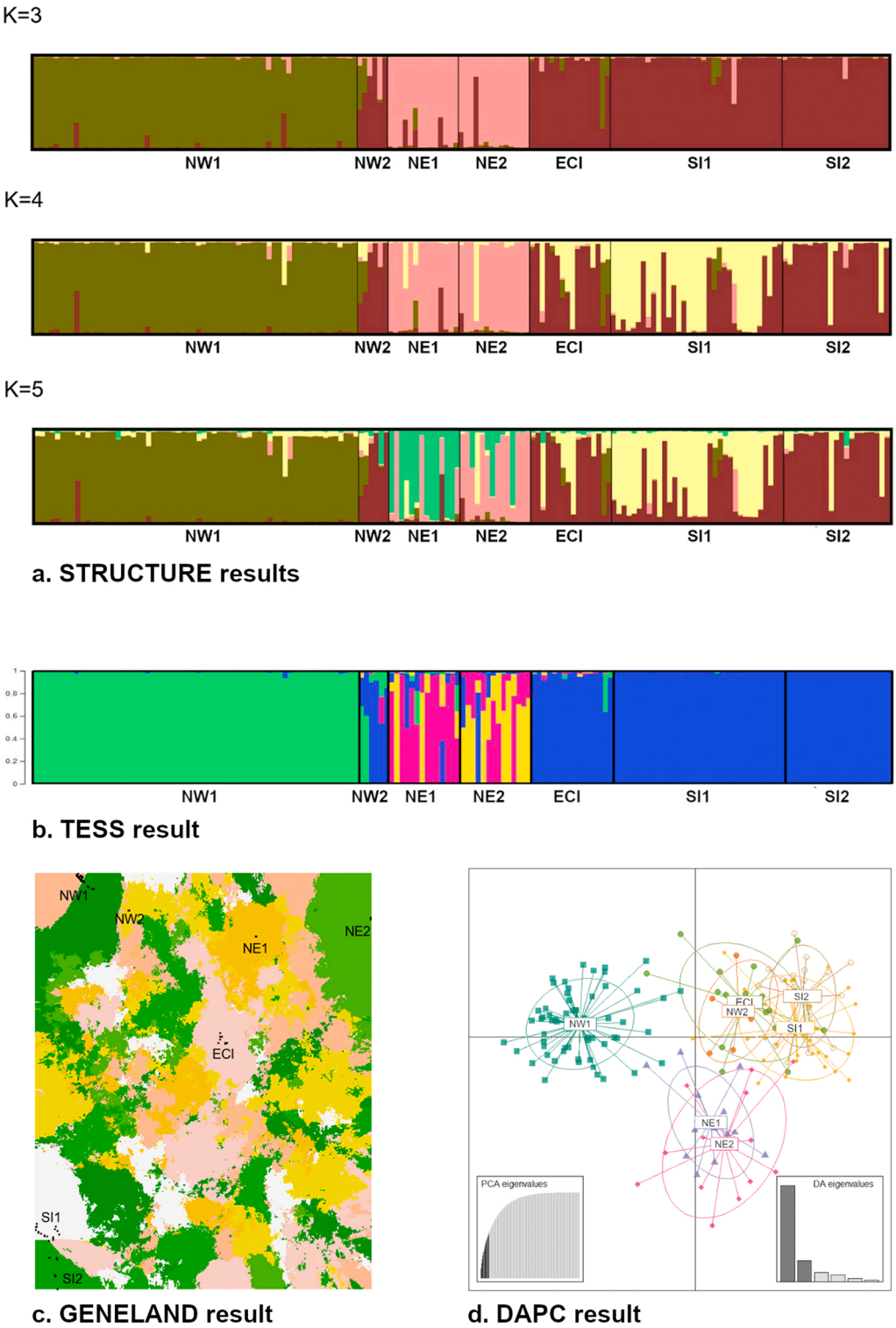

Fig. 3. Results of the different Bayesian individual-based clustering algorithms and multivariate analysis to understand population genetic structure of elephants across India – (a) STRUCTURE results for K= 3–5, (b) TESS results for Kmax= 4 with SIP 0.6, (c) GENELAND results at K= 8 and (d) DAPC results with a-priori information on the seven sampled populations. Key: NW1: Shivalik ER (Rajaji TR, Lansdowne FD, Corbett TR), NW2: Katarniaghat WLS, NE1: Baikunthapur FD, NE2: Jeypore RF, ECI: Similipal TR and Kuldiha FD, SI1: Nilgiri Biosphere Reserve, SI2: Anamalai, Periyar and Kalakkad-Mundanthurai TR. ER: Elephant Reserve, TR: Tiger Reserve, WLS: Wildlife Sanctuary, RF: Reserve Forest, FD: Forest Division.

was subdivided into two genetic clusters, the first corresponding to Rajaji Tiger Reserve (TR) and Haridwar Forest Division (FD) and the second cluster continued from Lansdowne FD to Corbett TR. Individuals from NW2, NE1, NE2 and ECI were assigned to exclusive clusters corresponding to each population. Out of the 34 individuals in SI1, 88.2% were assigned to a unique cluster. However, four individuals from Coimbatore FD, a part of the SI1 population, showed affinity to other clusters – one had similarity to the ECI while the other three were having the signature of SI2. All individuals of SI2 were assigned exclusively to the eighth genetic cluster.

3.3.2. *Non-Bayesian examination of population structure*

We tested the extent of population structuring based on the a-priori sampling populations using DAPC, which also provided an opportunity to verify the inference on differentiation obtained from Bayesian IBC approaches. Optimization of α-score through spline interpolation indicated that including information from 13 principal components (PC) would best explain clustering without over-fitting. We observed that NW1 formed an exclusive ellipse separate from the other populations (Fig. 3d). NE1 and NE2 formed mutually overlapping ellipses distant from the other populations. The NW2, ECI, SI1 and SI2 population ellipses were grouped together and could not be differentiated in discriminant space.

3.3.3. *Genetic landscape shape interpolation and genetic distances*

GLSI analysis revealed differential patterns of allele frequencies across the elephant habitats in India (Fig. 4). Regional clustering was prominent in NE1 and NE2 populations. Differentiation was also observed between sampling units NW1 and NW2. Moreover, a weak genetic cline divided the NW1 population into two segments longitudinally. ECI was differentiated from the rest of the populations, whereas moderate separation was observed between SI1 and SI2. Additionally, three weak latitudinally differentiated sub-clusters were found in SI2. A few non-sampled regions displayed spurious peaks, which were ignored subsequently (Fig. 4).

Exploring the dendrogram (Fig. 5) for the pairwise genetic distances (Ds) between the seven sampled populations, we observed three major genetic clusters – i) the NW and NE populations, ii) the ECI population, and iii) the SI population. The differentiation between these three clusters had 77% bootstrap support. We observed the formation of two sub-clusters (74% support), viz. NW1-NE1-NE2 and NW2, whereas further differentiation with 92% support separated NE1 and NE2. Within the SI population, the SI1-SI2 differentiation had 88% bootstrap support.

3.4. *The relative contribution of populations to aggregate diversity*

Based on the total contribution to Nei's gene diversity (GD) as well as allelic richness (C) methods, NE2 ranked highest, followed by the NE1 population. SI2 and SI1 switched ranks between third and fourth (Table 4). Fifth, sixth and seventh ranks were assigned to NW2, ECI and NW1 populations, respectively, based on both parameters computing contribution to global diversity. To understand the relative contributions at the landscape level, we compared GD and C values for the four eco-regions amongst the Indian elephant

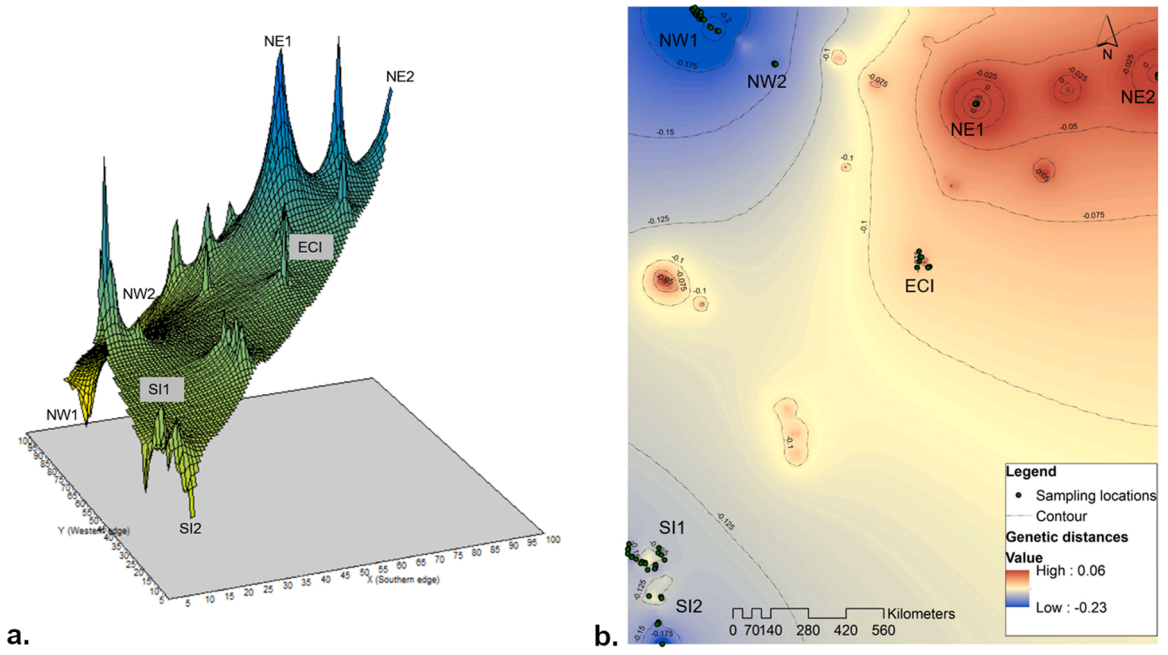

Fig. 4. Spatial genetic characterization across different populations of elephants in India (a) Genetic Landscape Shape Interpolation (GLSI) and (b) contours indicate genetic distance patterns over the geographical landscape. Key: NW1: Shivalik ER (Rajaji TR, Lansdowne FD, Corbett TR), NW2: Katarniaghat WLS, NE1: Baikunthapur FD, NE2: Jeypore RF, ECI: Similipal TR and Kuldiha FD, SI1: Nilgiri Biosphere Reserve, SI2: Anamalai, Periyar and Kalakkad-Mundanthurai TR. ER: Elephant Reserve, TR: Tiger Reserve, WLS: Wildlife Sanctuary, RF: Reserve Forest, FD: Forest Division.

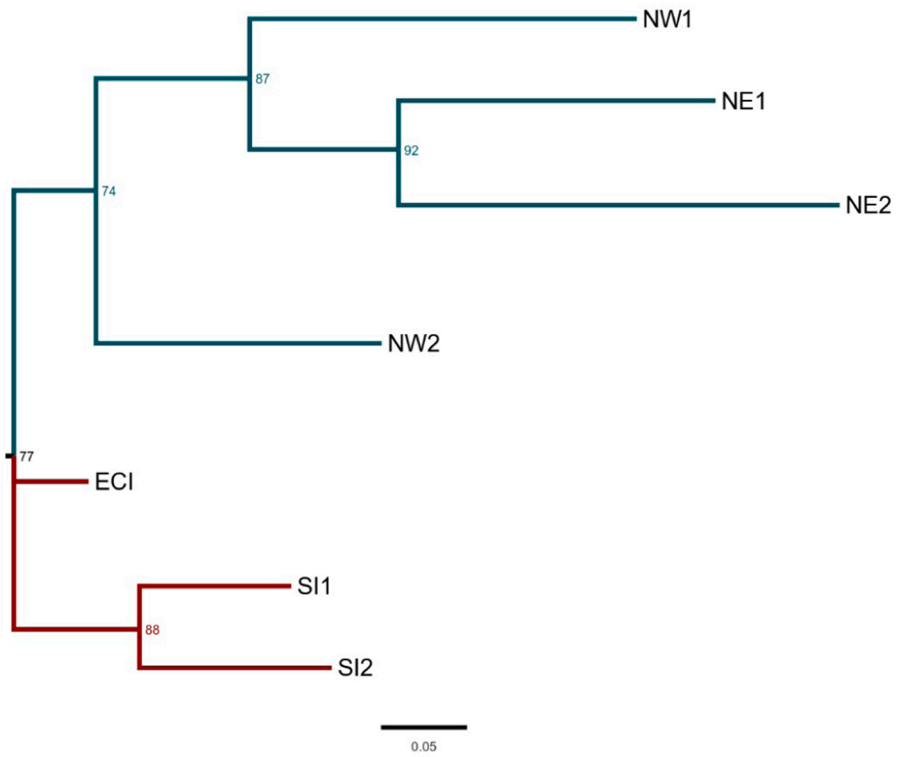

Fig. 5. Neighbour-joining dendrogram depicting Nei's standard genetic distances (Ds) (Nei, 1978) between the sampled populations. The values on the nodes denote percentage bootstrap support for the branching. The teal and dark red coloured branches indicate populations with α and β clade haplotypes (Vidya et al., 2005a), respectively (unpublished data). Key: NW1: Shivalik ER (Rajaji TR, Lansdowne FD, Corbett TR), NW2: Katarniaghat WLS, NE1: Baikunthapur FD, NE2: Jeypore RF, ECI: Similipal TR and Kuldiha FD, SI1: Nilgiri Biosphere Reserve, SI2: Anamalai, Periyar and Kalakkad-Mundanthurai TR. ER: Elephant Reserve, TR: Tiger Reserve, WLS: Wildlife Sanctuary, RF: Reserve Forest, FD: Forest Division.

habitat. In accordance with the fine-scale analysis, the north-eastern population (NE1-NE2) ranked highest in terms of contribution to genetic diversity, followed by southern (SI1-SI2), east-central (ECI) and north-western (NW1-NW2) populations.

4. Discussion

India harbours the largest wild population of Asian elephants in the world. We describe the genetic characteristics of the elephant populations in each of the four eco-regions along with the patterns and extent of population divergence in India. The DNA amplification success rate in our study was higher than most of the non-invasive studies using a comparable number of microsatellite markers in Asian elephants (Table 5). The mean per genotype error rate in this study was moderate and comparable to error rates reported in Asian elephants (Table 5). Our study suggests that the elephant populations retained significant levels of genetic diversity, and genetic structure differed both locally and across regions.

4.1. Genetic characteristics of elephants across India and delineation of conservation units

We found differential genetic diversity among the four eco-regions that might be related to their extent of suitable habitats, connectivity and demography while three distinct genetic clusters were identified across India. In comparison with other populations, we observed that Asian elephants in India had higher MNA (3.71–6.86) than free-ranging populations in Borneo (2.80), China (3.22–3.67) and Nepal (4.20) (Flagstad et al., 2012; Goossens et al., 2016; Zhang et al., 2015) but lower than Lao PDR (Ahlering et al., 2011) (Table 5). Crop-raiding elephants of Alur, Karnataka, India had a value of MNA= 4.00 (Chakraborty et al., 2014), which was lower than what we observed for the corresponding SI1 population (MNA=6.86). This difference in MNA compared to the current study could have been driven by our sampling scheme, having extensive spatial coverage across the Nilgiri Biosphere Reserve. The allelic richness in Borneo (1.83–3.21) was comparable to our findings (3.15–3.78) (Table 5). The H_O values from the present study were similar to the values obtained for elephant populations in China, Cambodia and Myanmar (H_O=0.36–0.58) and previously recorded values from India (H_O=0.29–0.57), though we report higher H_O than Borneo (H_O=0.25) and lower than Lao PDR (H_O=0.67) and Nepal (H_O=0.66) (Ahlering et al., 2011; Flagstad et al., 2012; Goossens et al., 2016; Gray et al., 2014; Kusza et al., 2018; Vidya et al., 2005a; Zhang et al., 2015). In spite of the chance of incurring ascertainment bias due to different sets of microsatellite markers used across the studies (Table 5), this comparison suggests that Asian elephants in India retain a level of genetic diversity similar to

Table 4
Relative contribution and conservation priority across different elephant populations and landscapes in India.

Scale	Assessed unit		Sampling area	Nei's gene diversity	Internal diversity (Caballero and Toro, 2002)	Mean distance (Caballero and Toro, 2002)	Caballero statistic	Internal diversity (Petit et al., 1998)	Divergence (Petit et al., 1998)	Petit statistic
Population	North-West India (NW)	NW1	Shivalik ER	0.75	3.34	-2.75	0.58	-2.83	-1.74	-4.56
		NW2	Katarniaghat WLS	0.74	0.34	-0.35	-0.02	-2.23	2.11	-0.12
	North-east India (NE)	NE1	Baikunthapur FD	0.73	-1.08	-0.57	-1.64	3.60	0.47	4.07
		NE2	Jeypore RF	0.73	-1.15	-0.73	-1.87	3.31	1.10	4.42
	East-central India (ECI)	ECI	Similipal TR and Kuldiha FD	0.74	-0.02	0.28	0.26	-0.92	0.72	-0.20
	South India (SI)	SI1	Nilgiri BR	0.74	-0.11	-0.48	-0.59	-0.31	2.14	1.82
		SI2	Anamalai, Periyar and Kalakkad-Mundanthurai TR	0.73	-0.29	-0.66	-0.95	-0.63	1.47	0.84
Landscape	North-west India (NW)	NW1 and NW2		0.75	4.45	-3.86	0.59	-6.21	-0.67	-6.88
	North-east India (NE)	NE1 and NE2		0.71	-3.03	-0.98	-4.01	10.10	2.01	12.11
	East-central India (ECI)	ECI		0.74	0.37	-0.11	0.26	-4.01	3.81	-0.20
	South India (SI)	SI1 and SI2		0.72	-0.62	-2.25	-2.87	0.11	5.17	5.28

Key: NW1: Shivalik ER (Rajaji TR, Lansdowne FD, Corbett TR), NW2: Katarniaghat WLS, NE1: Baikunthapur FD, NE2: Jeypore RF, ECI: Similipal TR and Kuldiha FD, SI1: Nilgiri Biosphere Reserve, SI2: Anamalai, Periyar and Kalakkad-Mundanthurai TR.
ER: Elephant Reserve, TR: Tiger Reserve, WLS: Wildlife Sanctuary, RF: Reserve Forest, FD: Forest Division

their global distributional range. Deviation of all loci from the assumption of HWE at the pan-India scale could be attributed to structured sub-populations across India, giving rise to the Wahlund effect (von Wahlund, 1928).

The free-ranging populations of elephants exhibit some degree of 'structure' due to the social organisation as some individuals are often more closely related than others. Surprisingly, in few cases, the genetic differentiation between elephant populations at a smaller spatial scale (e.g., between NW1 and NW2; NE1 and NE2; SI1 and SI2) was equivalent to or greater than that found between populations at a larger scale (Fig. 6). As a result, the correlation between geographic distance between the populations and pairwise F_{ST} was low, positive and statistically non-significant (Spearman $\rho = 0.34$, p-value=0.13), thereby showing no definitive indications of isolation by distance. This pattern seems to be a common occurrence noted by other studies on Asian elephants (Chakraborty et al., 2014; Goossens et al., 2016; Vidya et al., 2005a), recording moderate or high genetic differentiation with a reduced geographic range in fragmented as well as contiguous landscapes (Fig. 6). However, the presence of a barrier to movement alone can't explain the observed pattern. It can also be attributed to the elephant population being sub-structured into family groups. Further genetic investigations are needed to shed light on the dispersal and gene flow of elephant populations at finer spatial scales.

Though most of the Bayesian and multivariate analyses support the presence of three major genetic clusters (Fig. 3), multiple ecoregions displayed incipient divergence within them.

STRUCTURE produced ecologically relevant clusters with spatial adherence till K= 5 along with a high proportion of (77.5%) assigned individuals. The long generation time and lifespan of Asian elephants may have caused the microsatellite data to reflect their evolutionary history, while comparatively recent population-level structuring was revealed only when analyzing the data at a granular level. GENELAND and GLSI identified the presence of a genetic cline between Rajaji TR and Lansdowne FD-Corbett TR within NW1, where settlements, encroachment, highways and railroad are the major threats. STRUCTURE (K=5) showed partial differentiation between NE1 and NE2, which corroborates the literature identifying Torsa river as a barrier to elephant movement (Sukumar, 1986). Similarly, SI1 and SI2 populations, partially differentiated using STRUCTURE (K=5), are separated by the Palghat Gap, a 30–40 km wide break on the mountain range posing as a weak ecological as well as genetic barrier to multiple species (Joshi et al., 2018; Joshi

Table 5
Comparison of diversity statistics based on microsatellite data of the Asian elephant across its distribution range from published literature.

Study area	Total number of markers used	Number of markers common with this study	Mean number of alleles	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Reference
Borneo	18	11	2.80	0.25	0.34	Goossens et al. (2016)
Thailand	12	7	–	0.74–0.80	0.79–0.83	Thitaram et al. (2015)
China	9	0	3.22–3.66	0.36–0.41	0.32–0.42	Zhang et al. (2015)
Cambodia	9	8		0.58	0.67	Gray et al. (2014)
Nepal	6	0	4.20	0.66	0.61	Flagstad et al. (2012)
Lao PDR	10	9	8.10	0.67	0.75	Ahlering et al. (2011)
Borneo	5	1	1.40	0.01	–	Fernando et al. (2003)
Myanmar	11	3	4.55	0.55	0.59	Kusza et al. (2018)
Alur, Karnataka, India	12	9	4.00	0.62	–	Chakraborty et al. (2014)
Across India:	6	2	–	0.54	0.54	Vidya et al. (2005a)
North-western India						
North-eastern India (NE) -North bank of Brahmaputra river (BR)			–	0.53	0.55	
NE - South-western south-central BR			–	0.39	0.48	
NE – Eastern BR			–	0.57	0.55	
East-central India			–	0.54	0.54	
Nilgiris, Southern India			–	0.52	0.53	
Anamalai, Southern India			–	0.43	0.47	
Periyar, Southern India			–	0.29	0.47	

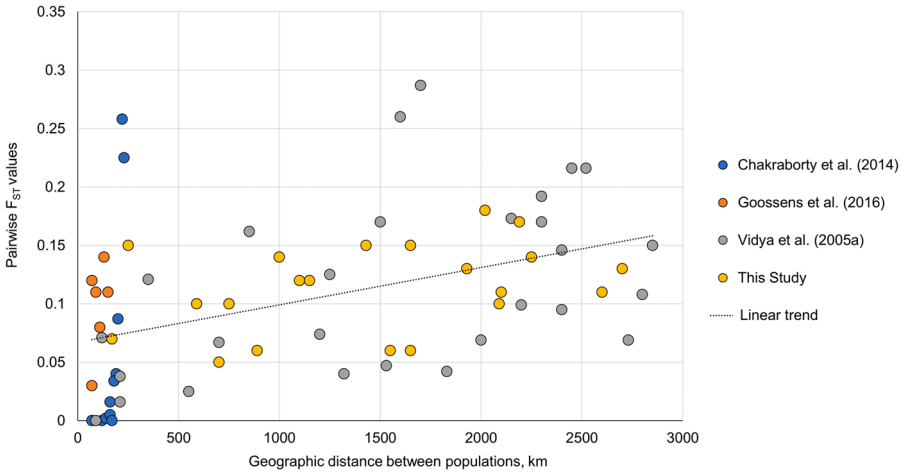

Fig. 6. Comparison of genetic differentiation (F_{ST}) between pairs of Asian elephant populations in relation to the geographic distances between them.

and Karanth, 2013; Robin et al., 2015). Published genetic data on Asian elephants (Vidya et al., 2005a, 2005b) hypothesized that the differentiation between SI1 and SI2 was caused by geological events dated ~0.43 million years ago that led to the emergence of the Palghat gap (Rao et al., 2002). The current study could not differentiate ECI from SI2, a result in line with Vidya et al. (2005a) observing shared mtDNA haplotype (BL) within the β-clade between these two populations. Within SI2, GLSI identified weak differentiation between Anamalai TR and Periyar TR, corroborating the fact that the connectivity has been lost within last 100 years due to agriculture expansion, and the remaining steep slopes are not conducive to large mammal movement (Johnsingh et al., 2009). Similarly, weak segregation between Periyar TR and Kalakkad Mundanthurai TR across the 450 km^2 Shencottah gap – a land-use mosaic of degraded forests, human habitation and linear infrastructure, indicated the need for restoring corridors (Gangadharan

et al., 2011; Johnsingh et al., 2009).

Based on mtDNA data of the D-loop and six microsatellite markers, Vidya et al. (2005a) proposed four genetic landscapes in India – north-western-north-eastern India, east-central India, Nilgiris and Anamalai-Periyar in south India. In comparison, the consensus of our Bayesian and multivariate analyses at the pan-India scale strongly suggest the presence of at least three broad genetically distinct groups or 'management units' viz. NW1, NE1-NE2 and ECI-SI. As these three segments of elephant populations of India represent significantly diverse bioclimatic regimes and preferred food-plants species (Baskaran et al., 2010; Borah and Deka, 2008; Mohapatra et al., 2013; Sukumar, 1990; Williams et al., 2005), we consider the three genetic clusters as separate 'conservation units' henceforth.

4.2. Conservation implications

In addition to genetic diversity and differentiation between the conservation units identified for elephants in India, it is imperative to interpret our results in a unit-specific framework to formulate local-scale, effective conservation strategies. We incorporate genetic data, population history, demography and connectivity for each of the three conservation units and discuss their respective conservation priority.

4.2.1. North-western India (NW)

This eco-region, with the fewest elephants amongst the Indian populations (PED-MoEFCC, 2017), represents the westernmost range of the species with high population genetic divergence and lowest contribution to pan-India genetic diversity (Caballero and Toro, 2002; Petit et al., 1998). This population is primarily confined to the western part of the Terai Arc Landscape, India, a region with high primary productivity (Johnsingh et al., 2004). This population is also vulnerable to climate change-mediated habitat loss of 54–67% (Table 6) (Kanagaraj et al., 2019). The genetic cline observed within NW1, indicative of emerging population differentiation between Rajaji TR and Lansdowne FD-Corbett TR, could be mitigated by maintaining connectivity across the two high-priority corridors identified in this area (Menon et al., 2017).

NW2 has a high genetic similarity with the ECI and SI (Fig. 3). However, based on pairwise comparison of genetic distance reveals that NW2 forms an independent clade compared to the rest of populations (Fig. 5), whereas mtDNA D-loop data (unpublished) for NW2 clusters within α-clade (prevalent in NW and NE) instead of the β-clade (found in ECI and SI). This discordance between the microsatellite and mitochondrial DNA data could be due to small sample size (n = 6) or due to the contribution of male captive elephants from different regions being released in Nepal (Kharel, 2002; Varma and Ganguly, 2011) into the gene pool. There are records of elephants from Nepal moving into the Terai Arc belt of Uttar Pradesh, India, in the 1990 s (Javed, 1996). Probabilistically, these released animals could have mated with the remaining individuals of Bardia National Park (NP), Nepal, thereby retaining the mtDNA haplotype of this landscape while displaying the signature of admixture with nuclear markers. In an earlier study from Bardia NP, the presence of migrant individuals was detected (Flagstad et al., 2012). However, the north-western population could be considered one unit probably severed by relatively recent land-use changes. An assessment of the extent of genetic similarity between NW1 and NW2 populations using multiple genome-wide markers, such as Single Nucleotide Polymorphism (SNPs), could shed new light on this issue. Until then, caution should be exercised to facilitate elephant movement between these populations as hybrids of high-divergence ancestries are known to perform poorly under hyper-local environmental conditions (Bell et al., 2019).

4.2.2. North-eastern India (NE)

The north-eastern population with over 10,000 elephants (PED-MoEFCC, 2017) contributes the most to the aggregate genetic diversity of Indian elephants as it stretches between two biodiversity hotspots, the Eastern Himalayas and the Indo-Burmese region (Pawar et al., 2006). However, up to 26% of habitat loss for elephants in north-eastern India have been predicted due to climate change by 2070 (Kanagaraj et al., 2019). The F_{IS} values observed in the north-east region is the highest (F_{IS}=0.55–0.58) in India, which could be due to habitat fragmentation and inbreeding. However, our limited samples (both NE1 and NE2) were collected from geographic vicinity on one another (<15 km), thereby disproportionately capturing closely related individuals. Out of 91 dyads each from NE1

Table 6
Projected loss of elephant habitat[a] across different eco-regions and the respective population size, number of corridors and their conservation priorities in India.

Eco-regions	Net habitat loss (%) for the Asian elephant under climate change scenarios[a]				Elephant population size[b]	No. of corridors[c]	No. of high priority corridors[c]
	RCP2.6		RCP8.5				
	By 2050	By 2070	By 2050	By 2070			
North-western India (NW)	54.45	31.62	61.5	66.63	2085	11	9
North-eastern India (NE)	25.09	9.52	20.92	25.76	10,139	25	17
East-central India (ECI)	68.33	39.38	72.31	83.01	3128	37	8
Southern India (SI)	23.99	12.29	24.25	38.06	11,960	28	24

Data sources:
[a] Kanagaraj et al. (2019),
[b] PED-MoEFCC (2017),
[c] Menon et al. (2017)

and NE2, there were five dyads of half-sibs and no full-sibs in NE1, while nine dyads of half-sibs and two dyads of full-sibs were present in NE2 (unpublished data). This is also one of the probable reasons that the north-eastern population indicated little admixture (<40% at K=5) amongst individuals. Such a low proportion of admixture at K= 5 may also indicate genetic isolation and homogenization within these two populations. We found a high number of private alleles in NE1 (A_P =12, frequency 0.12) and NE2 (A_P =8, frequency 0.11) compared to other populations. Multiple high priority corridors identified in north-east India (Menon et al., 2017), if functional, would facilitate gene flow and enhance genetic diversity.

4.2.3. East-central India (ECI) and southern India (SI)

We observed high genetic affinity between ECI and SI populations based on both Bayesian and non-Bayesian analyses even though ECI showed relatively lower AR (3.35) and H_O (0.43). The east-central Indian elephant population lags behind the north-eastern and southern populations in terms of contribution to the total genetic diversity at the pan-India scale. On the other hand, this population of ~3100 elephants inhabit one of the most fragmented landscapes and stand to lose up to 83% of the current suitable habitat by 2070 (Kanagaraj et al., 2019). There were four private alleles present in ECI with a low frequency of 0.03. Due to the disjunct configuration and recent loss of structural corridors due to mining and other anthropogenic land-use change (Menon et al., 2017), thorough characterization and subsequent monitoring of genetic diversity and divergence of this insular population should be carried out. In the case of progressive genetic homogenization, adaptive management strategies such as translocation of male individuals from SI2, the genetically closest population, should be relied upon.

Southern India, which holds ~12000 elephants, would lose between 12% and 38% of habitat under the two RCP scenarios (Kanagaraj et al., 2019). This eco-region has the highest heterozygosity (H_O>0.50) in India while being the second-highest contributor to the global diversity of Indian elephants. We recorded the presence of a high number of private alleles in low frequencies in the two populations, SI1 (A_P =6, frequency 0.06) and SI2 (A_P =11, frequency 0.03). We observed moderate differentiation between SI1 and SI2 (F_{ST}=0.06). The presence of admixture individuals in SE1 and two likely immigrants in SI2 across the Palghat gap indicates historical (>12 generations; Blair et al., 2012) connectivity between these two populations. Multivariate analyses also reveal the genetic similarity of SI1 and SI2 populations. Hence, we suggest the proportion of admixture among meta-populations of the SI1 region may be retained and even increased by improving habitat connectivity through corridors. However, as SI1 and SI2 do not share any mtDNA haplotypes (Vidya et al., 2005a, 2005b), elephant movement across the Palghat gap needs to be confirmed with further studies based on satellite telemetry or large-scale molecular tracking.

5. Conclusion

This study provides the first estimate of genetic diversity and delineation of conservation units combining contemporary genetic analyses and relevant ecological information across all four bioclimatic regions of India harbouring elephants. We also identified incipient sub-structuring within NE and SI, indicating potential genetic discontinuity and the need for securing the identified corridors. The current study assesses conservation priorities of the conservation units based on their relative contribution to the global genetic diversity, demography, the current level of connectivity and the risk of climate change-mediated habitat loss.

6. Future perspective

The current study lacks spatial coverage for the north-east Indian elephant populations, especially Kaziranga NP, which is one of the most critical source populations. Therefore, a more comprehensive sampling and a detailed region-specific study in north-eastern India would help uncover local population genetic structure and understand their contribution to the pan-India diversity. Moreover, transboundary sampling of the adjacent elephant populations in Nepal, Sri Lanka, Bhutan and northern reaches of Bangladesh and Myanmar with harmonized markers would provide the complete genetic landscape as these habitats are contiguous with Indian populations in most of the cases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge the academic and administrative authorities of the establishments and organizations involved in this collaborative study for providing the necessary infrastructure. The state forest departments of Uttarakhand, Uttar Pradesh, West Bengal, Assam, Odisha, Karnataka, Kerala and Tamil Nadu are appreciated for granting research permits and providing support in sample collection. We sincerely thank Mr. Suvankar Biswas and Mr. A Madhanraj for their logistical support. This research was partially funded by the Ministry of Environment Forests and Climate Change, Government of India, Operation Eye of the Tiger, India, WWF – Asian Rhino and Elephant Action Strategy, Nepal (Grant ID: WU04), US Fish and Wildlife Service Asian Elephant Program (Grant ID: FWS F12AP01186) and the International Elephant Foundation. The lead author was supported by the Junior Research Fellowship (National Eligibility Test) accorded jointly by the Council of Scientific and Industrial Research and University Grants Commission, India (Grant ID: 2-7/2013 SA-I). We acknowledge the two anonymous reviewers for their helpful critique that

greatly improved this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01888.

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