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Population genetic structure and conservation of Asian elephants (*Elephas maximus*) across India

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Abstract

This study examines the population genetic structure of Asian elephants (*Elephas maximus*) across India, which harbours over half the world's population of this endangered species. Mitochondrial DNA control region sequences and allele frequencies at six nuclear DNA microsatellite markers obtained from the dung of free-ranging elephants reveal low mtDNA and typical microsatellite diversity. Both known divergent clades of mtDNA haplotypes in the Asian elephant are present in India, with southern and central India exhibiting exclusively the β clade of Fernando *et al.* (2000), northern India exhibiting exclusively the α clade and northeastern India exhibiting both, but predominantly the α clade. A nested clade analysis revealed isolation by distance as the principal mechanism responsible for the observed haplotype distributions within the α and β clades. Analyses of molecular variance and pairwise population F_{ST} tests based on both mitochondrial and microsatellite DNA suggest that northern-northeastern India, central India, Nilgiris (in southern India) and Anamalai-Periyar (in southern India) are four demographically autonomous population units and should be managed separately. In addition, evidence for female philopatry, male-mediated gene flow and two possible historical biogeographical barriers is described.

INTRODUCTION

The Asian elephant (*Elephas maximus*) is one of an increasing number of species listed as endangered in the IUCN's *Red Data List*. India is home to approximately 22 700–32 400 free-ranging elephants (Bist, 2002; Sukumar, 2003; unpublished data from the Asian Elephant Research and Conservation Centre), over half the world's total estimated free-ranging Asian elephant population (Sukumar, 2003). In India, as elsewhere in Asia, elephant numbers have declined substantially over the last few millennia due to habitat loss and fragmentation, historical capture in large numbers for domestication and, more recently, poaching of males for ivory. However, the largest Asian elephant populations remain in India, where the elephant is an important flagship species. Despite the large numbers, the level of genetic diversity and population genetic structure of current Indian populations have been little studied. Previous molecular genetic and phylogeographical studies of the species (Nozawa & Shotake, 1990; Hartl *et al.*, 1995, 1996; Fernando *et al.*, 2000, 2003*a*; Fleischer *et al.*, 2001) have lacked sufficient samples from India and, with the exception of Fernando *et al.* (2003*a*), have relied exclusively on samples from a few captive animals, putatively of Indian origin. Thus, evolutionary hypotheses about the species were based on this largely incomplete dataset.

In this study, we examine the genetic variability and population genetic structure of Asian elephant populations across India. We use mitochondrial DNA (mtDNA) and nuclear microsatellite DNA markers to assess genetic diversity and to detect population-level and regional-level genetic differentiation based on F_{ST} values and analyses of molecular variance. We also perform a nested clade analysis (Templeton, 1998) in order to explain the patterns of distribution of mitochondrial haplotypes. This is one of a limited number of population genetic studies of free-ranging large mammals examining a large sample of individuals across a broad spatial scale. We interpret the results from this study to suggest appropriate population units for management and conservation. Insights into elephant social organisation and phylogeography are also obtained from the observed patterns.

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STUDY AREA

Elephants are distributed across northern (northwestern), northeastern, central and southern, India (Fig. 1). The *northern* population is a relict population of approximately 900–1000 animals (Bist, 2002; Sukumar, 2003) distributed along the deciduous forests of the Himalayan foothills. The *northeastern* region of India holds three sizeable populations (totalling 9200–11 300 animals), ranging across a vast expanse of tropical moist deciduous, semi-evergreen and wet evergreen forests and moist grassland along floodplains: (1) the *North Bank* population of over 3000 elephants on the north bank of the Brahmaputra river (Choudhury, 1999), (2) the *Eastern Region* population (corresponding to the southeastern bank population of Choudhury, 1999), consisting of about 1000 elephants in the eastern areas of the south bank of the Brahmaputra and (3) the *Southwest-Southcentral Bank* population comprising approximately 5500 elephants (Sukumar & Santiapillai, 1996; Choudhury, 1999) in the central and western areas of the south bank of the Brahmaputra (Fig. 1). The *central* India population consists of approximately 2400–2700 animals distributed along the Eastern Ghats in several fragmented dry deciduous, moist deciduous and semi-evergreen forest areas. However, extensive movement of elephants across these fragmented forests is known in this region (Datye & Bhagwat, 1995), and we have considered central India as a single population. *Southern* India holds large populations (totalling 10 250–17 000 animals) ranging across varied habitat such as dry thorn, dry deciduous, moist deciduous, semi-evergreen, evergreen and montane evergreen forests, along the Western Ghats and the Eastern Ghats mountain ranges (Fig. 1). The four main populations in southern India listed north to south are: (1) the *North Kanara* population (Vidya *et al.*, 2005) restricted to approximately 250–500 elephants distributed across a few pockets in the Western Ghats, (2) the *Nilgiris–Eastern Ghats* (Nilgiris) population, which is the world's single largest contiguous population of Asian elephants with an estimated 9000 individuals (Asian Elephant Research and Conservation Centre (AERCC), 1998; and unpublished census data for 2002), (3) the *Anamalai* population with approximately 1500–2700 elephants (AERCC, 1998), which is separated from the Nilgiris by the 40 km-wide Palghat Gap and (4) the *Periyar* population, which holds approximately 1500–2500 elephants (AERCC, 1998; Fig. 1).

At present, the Indian government has incorporated over 50% of the elephant range into 'Project Elephant Ranges' as part of its conservation scheme for the species (Bist, 2002). There are 11 such ranges, Project Elephant Range No. 11 covering part of the northern population, five ranges covering the northeastern Indian populations (Range No. 2 in the North Bank, Range No. 3 in the Eastern Region and Range Nos. 4–6 in the Southwest-Southcentral Bank), Range No. 1 in central India and four ranges in southern India (Range Nos. 7 and 8 in the Nilgiri area, Range No. 9 in Anamalai and Range No. 10 in Periyar) (Ministry of Environment and Forests, Government of India, 1993). Less than half these ranges carry the stricter protection designation of wildlife sanctuaries or national parks.

METHODS

Field sampling

As it is logistically extremely difficult to sample tissue or blood from free-ranging elephants, we used dung samples as our source of DNA. We sampled 297 free-ranging elephants and 29 captive elephants, for which reliable wild capture details were available (sampling locations are shown in Fig. 1). Of the samples from free-ranging animals, 53% were collected upon observed defaecation and of the 326 samples collected in total, 92% were collected while they were less than a few hours old. Samples were collected from the outer most layer of dung into 95% ethanol.

Genetic analyses

DNA extraction involved digestion of 0.5 g of dung using Proteinase K, followed by extraction using phenol/chloroform/isoamyl alcohol and purification using QIAGEN gel purification columns (see Fernando *et al.*, 2003*b*). Polymerase chain reactions (PCR) using the primers MDL3 and MDL5 (Fernando & Lande, 2000) were carried out to amplify a 600-base-pair (bp) segment of mtDNA (containing the C-terminal of cytochrome *b* and the hyper-variable left domain of the non-coding control region), which was sequenced from both directions using the internal primers MDLseq-1 and MDLseq-2 (Fernando *et al.*, 2003*b*) in a dye-terminated cycle sequencing reaction. Products of the sequencing reactions were electrophoresed on polyacrylamide gels in an ABI Prism 377 DNA Sequencer. In addition, the tri- and tetra-nucleotide microsatellite loci EMX-1, EMX-2, EMX-3 and EMX-4, isolated from an Asian elephant (Fernando, Vidya & Melnick, 2001) and the dinucleotide loci LafMS02 and LafMS03 isolated from African elephants (Nyakaana & Arctander, 1998) were amplified. PCR products were electrophoresed in an ABI Prism 377 DNA Sequencer along with the internal size standard Tamra-500 (Applied Biosystems, Inc.) and allele sizes were identified using the ABI Gene Scan analysis software v.3.1.2 (Applied Biosystems, Inc.). Since dung is not an optimal source of DNA, precautions were taken to ensure reliable amplification (see Fernando *et al.*, 2003*b*; Vidya *et al.*, 2005). Animals from which dung samples were collected in the absence of observed defaecation were molecularly sexed using a ZFX-ZFY polymorphism (see Fernando & Melnick 2001; Vidya *et al.* 2003). Of the 326 samples collected, we were able to obtain microsatellite data from 317 samples and mitochondrial sequence data from 307 samples, which were not a complete subset of the 317 samples.

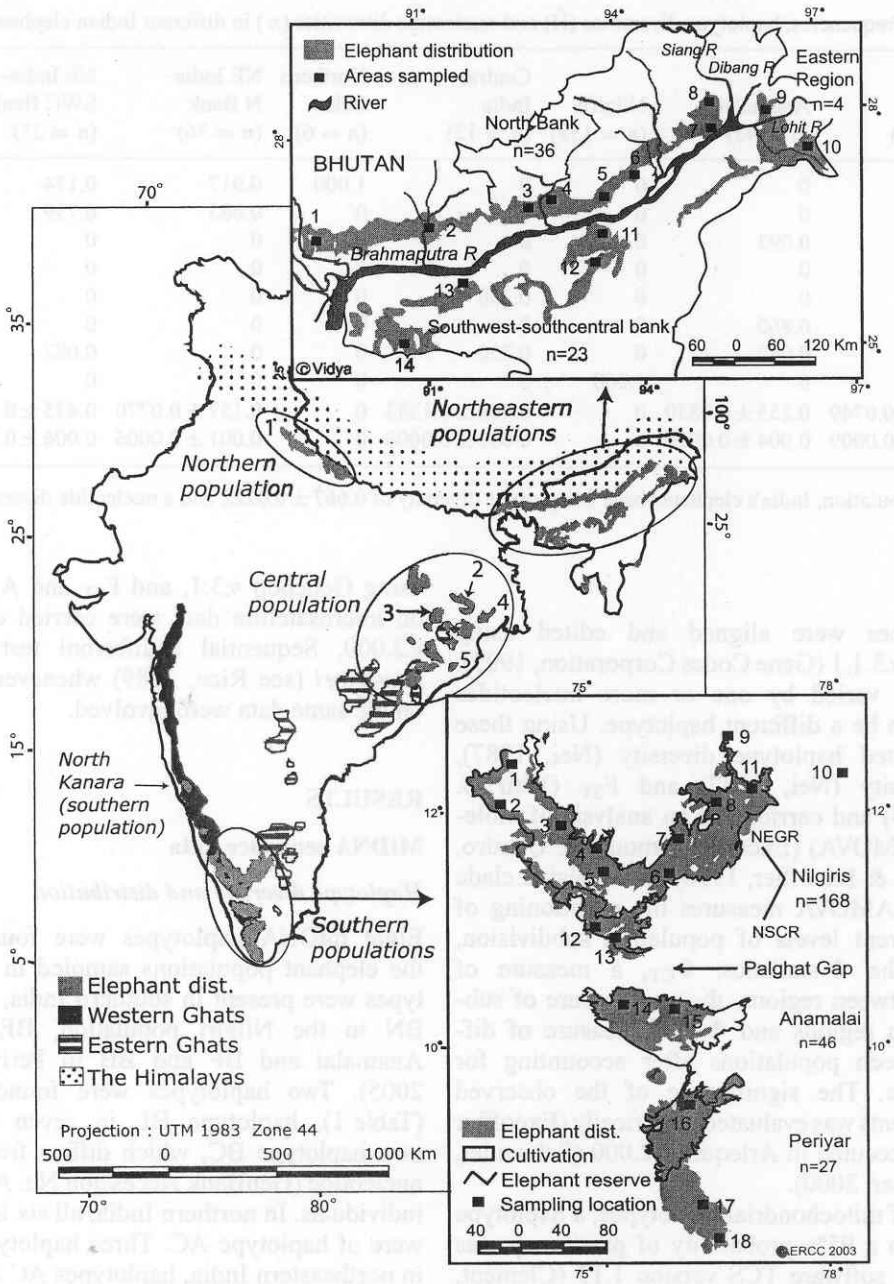

Fig. 1. Elephant distribution in India: distribution in southern India courtesy of Asian Elephant Research and Conservation Centre, northeastern India based on Choudhury (1999), central India based on Sukumar (1989), Datye & Bhagwat (1995), L. A. K. Singh (1995), northern India based on K. N. Singh (1995). Numbers against sampling locations in northern and central India correspond to the following forest divisions: 1, Rajaji National Park; 2, Dalma Wildlife Sanctuary; 3, Saranda Forest Division; 4. Simlipal Tiger Reserve; 5, Chandaka Wildlife Sanctuary. Six and 12 samples were sampled from northern and central India, respectively. More detailed maps are provided for the northeastern and southern Indian regions. The numbers on the map of northeastern India correspond to the following locations: 1, Buxa Tiger Reserve; 2, Manas National Park; 3, Nameri National Park; 4, Pakke Tiger Reserve; 5, Banderdewa forest in Papumpare District; 6, Lakhimpur Division; 7, Jonai Division; 8, Pasighat Forest Division; 9, Lohitpur Division; 10, Namdapha Tiger Reserve; 11, Kaziranga National Park; 12, Parkup Pahar Proposed Reserve Forest; 13, Kamrup East Forest Division; 14, Balphakram National Park. The numbers on the map of southern India correspond to the following locations: 1, Madikeri Forest Division; 2, Virajpet Forest Division; 3, Rajiv Gandhi National Park; 4, Bandipur National Park; 5, Mudumalai Wildlife Sanctuary; 6, Satyamangalam Forest Division; 7, BRT Wildlife Sanctuary; 8, Cauvery Wildlife Sanctuary; 9, Bannerghatta National Park; 10, Thirupattur Division; 11, Hosur Forest Division; 12, Silent Valley National Park; 13, Mannarkkad Forest Division; 14, Parambikulam Wildlife Sanctuary; 15, Indira Gandhi Wildlife Sanctuary; 16, Periyar Tiger Reserve; 17, Kalakkad – Mundanthurai Tiger Reserve; 18, Kanyakumari Division. A sampling location was also present in Dandeli Wildlife Sanctuary in the North Kanara population shown in the main map. The sample sizes obtained from the northeastern Indian populations were 37, 24 and 4, from the Northern Bank, Southwest-Southcentral Bank and Eastern Region, populations, respectively, and from the southern Indian populations they were 2, 168, 46 and 27, from the North Kanara, Nilgiri, Anamalai and Periyar populations, respectively. The Himalayas and the Brahmaputra river system shown in the maps are only a schematic representation and are not accurate.

Table 1. Haplotype frequencies, haplotype diversities ($\hat{H}$) and nucleotide diversities (π) in different Indian elephant populations

Haplotype	Periyar ($n = 24$)	Anamalai ($n = 43$)	Nilgiris ($n = 159$)	Central India ($n = 12$)	Northern India ($n = 6$)	NE India–N Bank ($n = 36$)	NE India–SWC Bank ($n = 23$)	NE India–E Region ($n = 4$)
AC	0	0	0	0	1.000	0.917	0.174	0.500
AH	0	0	0	0	0	0.083	0.739	0.500
BA	0	0.093	0	0	0	0	0	0
BB	0.042	0	0	0	0	0	0	0
BC	0	0	0	0.250	0	0	0	0
BF	0.958	0.860	0	0	0	0	0	0
BL	0	0.047	0	0.750	0	0	0.087	0
BN	0	0	1.000	0	0	0	0	0
$\hat{H}$	0.083 ± 0.0749	0.255 ± 0.0830	0	0.409 ± 0.1333	0	0.157 ± 0.0770	0.435 ± 0.1111	0.667 ± 0.2041
π	0.001 ± 0.0009	0.004 ± 0.0026	0	0.001 ± 0.0008	0	0.001 ± 0.0006	0.006 ± 0.0035	0.002 ± 0.0020

Taken as a single population, India's elephants have a haplotype diversity of 0.667 ± 0.0222 and a nucleotide diversity of 0.012 ± 0.0062.

Data analyses

MtDNA sequences were aligned and edited using SEQUENCHER v.3.1.1 (Gene Codes Corporation, 1999). A sequence that varied by one or more nucleotides was considered to be a different haplotype. Using these data, we calculated haplotype diversity (Nei, 1987), nucleotide diversity (Nei, 1987) and F_{ST} (Weir & Cockerham, 1984) and carried out an analysis of molecular variance (AMOVA) (Excoffier, Smouse & Quattro, 1992; Michalakis & Excoffier, 1996) and a nested clade analysis (NCA). AMOVA measures the partitioning of variance at different levels of population subdivision, giving rise to the Φ-statistics, Φ_{CT}, a measure of differentiation between regions, Φ_{SC}, a measure of substructuring within regions and Φ_{ST}, a measure of differentiation between populations after accounting for regional structure. The significance of the observed variance components was evaluated empirically (Excoffier *et al.*, 1992) as executed in Arlequin v.2.000 (Schneider, Roessli & Excoffier, 2000).

For the NCA of mitochondrial haplotypes, a haplotype network based on a 95% probability of parsimony was created using the software TCS version 1.13 (Clement, Posada & Crandall, 2000) and the network was nested by hand following the guidelines given by Templeton, Crandall & Sing (1992) and Templeton & Sing (1993). The null hypothesis of no geographical association of clades within a nested category was tested by randomly permuting clades among sampling locations using the software GeoDis v2.0 (Posada, Crandall & Templeton, 2000). The inference key of Templeton (2004) was used to distinguish among the alternative hypotheses of restricted gene flow, range fragmentation, range expansion and colonisation.

From the microsatellite data, we calculated the expected and observed heterozygosities using Genepop v.3.1 (Raymond & Rousset, 1995) and allele frequencies and richness, the latter by multiple random sub-sampling (Leberg, 2002), using C programs (written by T. N. C. Vidya, available on request). Linkage disequilibrium between pairs of loci and the Hardy–Weinberg equilibrium test at each locus for each population were carried out using Genepop v.3.1, and F_{ST} and AMOVA tests based on microsatellite data were carried out using Arlequin v.2.000. Sequential Bonferroni tests were applied *a posteriori* (see Rice, 1989) whenever multiple analyses on the same data were involved.

RESULTS

MtDNA sequence data

Haplotype diversity and distribution

Eight mtDNA haplotypes were found in total across the elephant populations sampled in India. Five haplotypes were present in southern India, a single haplotype BN in the Nilgiri population, BF, BL and BA in Anamalai and BF and BB in Periyar (Vidya *et al.*, 2005). Two haplotypes were found in central India (Table 1), haplotype BL in seven individuals and a new haplotype BC, which differs from BL by a single nucleotide (GenBank Accession No. AY589512), in three individuals. In northern India, all six individuals sampled were of haplotype AC. Three haplotypes were observed in northeastern India, haplotypes AC and AH in all three populations and BL only in the Southwest-Southcentral Bank population (Table 1). AC and AH were the predominant haplotypes in the North Bank population and the Southwest-Southcentral Bank population, respectively (Table 1). Within locations on either bank, these two haplotypes were sometimes intermixed. In Dalma Wildlife Sanctuary in central India and Pakke Tiger Reserve in northeastern India, the single individual that had a different haplotype from the others in the location was a male, while in Kaziranga in northeastern India, of the two individuals that had a different haplotype, one was a male and the other a female.

Genetic structure of mtDNA diversity

The AMOVA performed on the different regions of the country showed hierarchical structuring at the level of regions, populations within regions and within

Table 2. Analysis of molecular variance (AMOVA) based on mitochondrial haplotypes

Level of analysis	d.f.	Variance components	Percentage variation	Phi-statistics	*P*-value
Among regions (S, C, N, NE India)	3	5.1688 Va	60.57	$\Phi_{CT} = 0.721$	< 0.001
Among populations within regions	4	1.8131 Vb	36.39	$\Phi_{SC} = 0.907$	< 0.001
Within populations	299	0.1864 Vc	3.04	$\Phi_{ST} = 0.974$	< 0.001
Between regions (S, C India)	1	−0.8416 Va	−68.33	$\Phi_{CT} = -0.586$	1.000
Among populations within regions	2	2.2417 Vb	149.78	$\Phi_{SC} = 0.984$	< 0.001
Within populations	234	0.0374 Vc	18.55	$\Phi_{ST} = 0.974$	< 0.001
Between regions (S, N India)	1	6.1793 Va	32.48	$\Phi_{CT} = 0.731$	< 0.001
Among populations within regions	2	2.2418 Vb	67.10	$\Phi_{SC} = 0.987$	< 0.001
Within populations	228	0.0285 Vc	0.42	$\Phi_{ST} = 0.997$	< 0.001
Between regions (S, NE India)	1	6.1638 Va	58.96	$\Phi_{CT} = 0.755$	< 0.001
Among populations within regions	4	1.8130 Vb	38.19	$\Phi_{SC} = 0.906$	< 0.001
Within populations	283	0.1890 Vc	2.85	$\Phi_{ST} = 0.977$	< 0.001
Between regions (C, N India) (No populations within regions)	1	–	–	$\Phi_{ST} = 0.986$	< 0.001
Between regions (C, NE India)	1	8.3118 Va	83.29	$\Phi_{CT} = 0.876$	< 0.001
Among populations within regions	2	0.4798 Vb	7.99	$\Phi_{SC} = 0.409$	< 0.001
Within populations	71	0.6934 Vc	8.72	$\Phi_{ST} = 0.927$	< 0.001
Between regions (N, NE India)	1	−0.2617 Va	−15.40	$\Phi_{CT} = -0.279$	0.850
Among populations within regions	2	0.4781 Vb	15.17	$\Phi_{SC} = 0.398$	< 0.001
Within populations	65	0.7227 Vc	100.23	$\Phi_{ST} = 0.230$	< 0.001

A locus-by-locus AMOVA was used rather than pairwise differences between haplotypes, so that haplotypes differing by the same number of mutations but different mutational positions would not be considered identical. The percentage of variation, variance components and Φ-statistics, averaged across the variable nucleotide positions, are shown.

Table 3. Pairwise F_{ST} values between elephant populations in India, based on mitochondrial DNA haplotype frequencies (below diagonal) and nuclear DNA microsatellite allele frequencies (above diagonal)

	Nilgiris	Anamalai	Periyar	Central India	Northern India	NE India–N Bank	NE India–SWC Bank	NE India–E Region
Nilgiris		0.071***	0.038***	0.170***	0.069**	0.099***	0.095***	0.069*
Anamalai	0.989***		−0.027	0.260***	0.173***	0.170***	0.216***	0.108*
Periyar	0.998***	0.029		0.287***	0.192***	0.146***	0.216***	0.150***
Central India	0.993***	0.767***	0.845***		0.125**	0.067***	0.162***	0.074
Northern India	1.000***	0.988***	0.996***	0.986***		0.040	0.047	0.042
NE India–N Bank	0.996***	0.985***	0.988***	0.983***	−0.049		0.016	0.025
NE India–SWC Bank	0.968***	0.909***	0.887***	0.855***	0.290*	0.428***		0.121**
NE India–E Region	0.998***	0.981***	0.987***	0.968***	0.442	0.426	−0.043	

The symbols*, ** and *** indicate a statistically significant difference from zero at $P = 0.05$, 0.01 and 0.001, respectively. The significance level for the Bonferroni correction was 0.002, and all the values marked *** were significant after the Bonferroni correction was applied.

populations, with 60.6% of the variation accounted for by differences among regions ($\Phi_{CT} = 0.721$, $P < 0.001$), 36.4% among populations within regions ($\Phi_{SC} = 0.907$, $P < 0.001$) and 3% within populations ($\Phi_{ST} = 0.974$, $P < 0.001$) (Table 2). AMOVAs carried out between pairs of regions revealed significant differentiation between all pairs except for between southern and central India and between northern and northeastern India (Table 2). An absence of differentiation between pairs of regions may result either from true similarity between two regions, or substantial differentiation among one or both regions' constituent populations rendering the comparison between regions non-significant. Pairwise population F_{ST} tests revealed that each of the southern Indian populations was significantly differentiated from central India (Table 3), indicating that the absence of differentiation between the two regions was due to the significant differentiation between southern India's populations and not due to actual similarity between central and southern India. However, each of the northeastern Indian populations was not significantly differentiated from northern India (Table 3), indicating that the two regions are indeed very similar. On the whole, most population pairs showed significant F_{ST} values (Table 3).

Within southern India, the Nilgiris population was significantly differentiated from the more southerly populations of Anamalai and Periyar, from which it is separated by the Palghat Gap (Fig. 1). The latter two

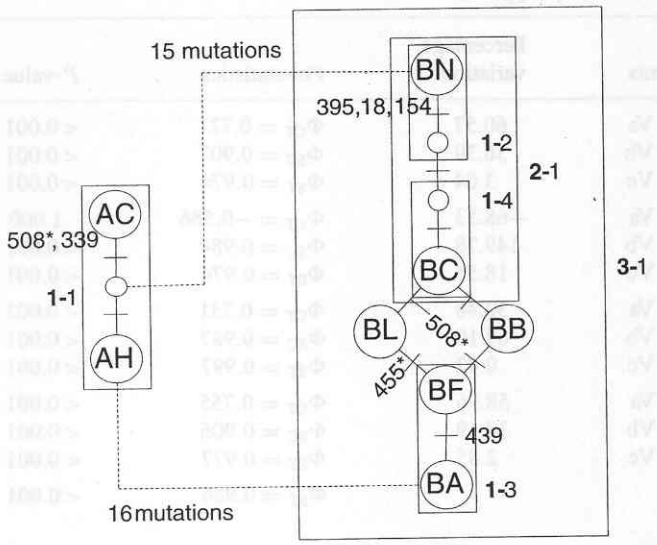

Fig. 2. Network of haplotypes generated by statistical parsimony and hierarchical levels of nesting of the haplotype network. Empty circles are assumed haplotypes. Each cross hatch represents a mutation and the nucleotide position at which the mutation occurred is denoted by the number adjacent to it. Asterisks indicate homoplasies. Dotted lines show non-parsimonious connections. Each clade is defined by a rectangle and the clade is numbered with the level of the clade and the number of the clade at that level. Haplotypes from the α clade are named with the beginning letter A and those of the β clade with a B.

populations were not differentiated from each other (Table 3; also see Vidya *et al.*, 2005). Detailed analysis of the northeastern Indian populations revealed a difference in mitochondrial structuring when data from the two sexes were analysed separately. While significant differentiation between the North Bank and Southwest-southcentral Bank populations was observed based on both sexes ($F_{ST} = 0.428$, $P < 0.001$, $n_{Nbank} = 36$, $n_{SWCBank} = 23$) and on only females ($F_{ST} = 0.434$, $P < 0.001$, $n_{Nbank} = 22$, $n_{SWCBank} = 17$), there was no differentiation (F_{ST} not statistically different from zero) based on males ($n_{Nbank} = 14$, $n_{SWCBank} = 6$). The Eastern Region population was not significantly differentiated (after Bonferroni corrections) from either North Bank or Southwest-Southcentral Bank populations, but these comparisons are based on only four samples from the Eastern Region population.

Nested clade analyses of mtDNA haplotypes

Nesting of the mtDNA haplotype network showed three levels of nesting (Fig. 2). The α and β haplotype clades described previously were present and could not be joined parsimoniously. Clades 1–1, 1–3, 2–1 and 3–1 showed significant geographical associations of haplotypes. This was due to restricted gene flow with isolation by distance in all cases except for clade 2–1, which showed allopatric fragmentation (Fig. 3).

Microsatellite data

Allelic richness, tests for Hardy–Weinberg equilibrium, linkage disequilibrium

Microsatellite data analyses were carried out on 295 samples, which included 212 samples from southern India, 12 from central India, six from northern India and 65 from

Haplotype			1-step clade			2-step clade			3-step clade
ID	D_c	D_n	ID	D_c	D_n	ID	D_c	D_n	ID
AH^T	130.26***	333.37*							
AC^T	562.14*	505.69**							
1-2-3-4-**RGF, IBD**			1-1						
BN^T	--		$1\text{-}2^T$	96.73***	128.03***				
BC^I	--		$1\text{-}4^I$	0.00	1590.94***				
			I-T-	-96.73	1462.91***				
			1-19-**AF**			$2\text{-}1^I$	158.03***	258.37**	
BA^T	0.00*	56.94**							
BF^I	85.63**	83.59**							
I-T	85.63**	26.65**							
1-2-3-4-**RGF, IBD**			$1\text{-}3^I$	-	--		83.00***	285.71	
BB^I	--		--		--		0.00	316.10	
BL^I	--		--		--		744.81*	132227***	
						1-2-11-17-4-**RGF, IBD**			3-1

Fig. 3. Results from the nested clade analysis (NCA) and inferences based on the inference key of Templeton (2004). The different nesting levels are shown on top. The name of the clade is listed, followed by the clade- and nested clade distances (in km). The black lines group the clades into a nesting structure as one moves from left to right. At the bottom of each box is a row showing the interior–tip (I—T) comparison whenever applicable, followed by the steps followed in the inference key and the inference for the particular clade, provided there are significant clade- and nested clade values in the clade. Significant values are marked with asterisks: *, ** and ***, corresponding to statistical significance at $P = 0.05$, 0.01 and 0.001, respectively. Shaded cells indicate a value larger than random, while unshaded cells with asterisk(s) indicate values significantly smaller than random. When a clade was represented by either a single location or a lower-step clade, the test could not be performed and the corresponding cells are left blank. **RGF, IBD** = restricted gene flow with isolation by distance, AF = allopatric fragmentation.

Table 4. Expected/observed percentages of heterozygotes at the six nuclear DNA microsatellite loci used in the different populations

Population	EMX-1[b]	EMX-2[b]	EMX-3[a]	EMX-4[ab]	LafMS02[bc]	LafMS03[c]
S India–Nilgiris[ce]	49.4/52.6	49.4/43.0	17.0/12.7	65.8/58.5	68.6/67.9	68.1/77.9
S India–Anamalai[cd]	55.7/57.8	34.3/29.5	10.6/11.1	58.0/48.8	66.7/65.0	54.9/47.6
S India–Periyar[b]	51.9/33.3	36.2/23.1	15.6/0.0	55.4/43.8	66.8/37.5	53.3/38.1
Central India[bd]	38.5/27.3	51.8/25.0	48.9/8.3	10.0/10.0	55.0/63.6	72.3/54.5
Northern India[c]	53.0/66.7	40.9/50.0	30.3/33.3	75.0/50.0	53.4/40.0	71.2/83.3
NE India–N Bank[bc]	46.2/36.1	50.5/50.0	48.1/60.0	57.1/40.0	53.7/45.9	75.6/86.1
NE India–SWC Bank[abde]	28.0/31.8	47.6/47.8	33.3/27.7	64.1/33.3	40.3/29.2	75.1/63.6
NE India–E Region[ac]	53.3/66.7	53.5/25.0	33.3/33.3	42.9/50.0	73.3/100	73.3/66.7

Results of Wilcoxon matched-pairs tests examining observed heterozygosity between pairs of loci and between pairs of populations are shown in the form of alphabets against the locus or population. Loci/populations that share the same alphabet are not significantly different in observed heterozygosity from each other, while those that do not share alphabets are significantly different, 'a' corresponding to lower heterozygosity than 'b', which is lower than 'c, and so on. (Alphabets superscripted against loci do not correspond to those against populations.)

Table 5. AMOVA based on allele frequencies of six nuclear DNA microsatellite loci: percentage of variation, variance components and Φ-statistics

Level of analysis	d.f.	Variance components	Percentage variation	Phi-statistics	*P*-value
Among regions (S, C, N, NE India)	3	0.1439 Va	9.36	$\Phi_{CT} = 0.094$	0.007
Among populations within regions	4	0.0642 Vb	4.17	$\Phi_{SC} = 0.046$	< 0.001
Within populations	582	1.3289 Vc	86.46	$\Phi_{ST} = 0.135$	< 0.001
Between regions (S, C India)	1	0.3145 Va	18.33	$\Phi_{CT} = 0.183$	0.255
Among populations within regions	2	0.0733 Vb	4.27	$\Phi_{SC} = 0.052$	< 0.001
Within populations	440	1.3275 Vc	77.39	$\Phi_{ST} = 0.226$	< 0.001
Between regions (S, N India)	1	0.0830 Va	5.59	$\Phi_{CT} = 0.056$	0.252
Among populations within regions	2	0.0733 Vb	4.94	$\Phi_{SC} = 0.052$	< 0.001
Within populations	432	1.3274 Vc	89.47	$\Phi_{ST} = 0.105$	< 0.001
Between regions (S, NE India)	1	0.1328 Va	8.68	$\Phi_{CT} = 0.087$	0.098
Among populations within regions	4	0.0641 Vb	4.19	$\Phi_{SC} = 0.046$	< 0.001
Within populations	548	1.3331 Vc	87.13	$\Phi_{ST} = 0.129$	< 0.001
Between regions (C, N India) (No populations within regions)	1	–	–	$\Phi_{ST} = 0.125$	0.003
Between regions (C, NE India)	1	0.1524 Va	9.92	$\Phi_{CT} = 0.099$	0.246
Among populations within regions	2	0.0357 Vb	2.32	$\Phi_{SC} = 0.026$	0.031
Within populations	146	1.3478 Vc	87.76	$\Phi_{ST} = 0.122$	< 0.001
Between regions (N, NE India)	1	0.0235 Va	1.67	$\Phi_{CT} = 0.017$	0.504
Among populations within regions	2	0.0357 Vb	2.53	$\Phi_{SC} = 0.026$	0.031
Within populations	138	1.3487 Vc	95.8	$\Phi_{ST} = 0.042$	0.019

northeastern India. All eight Indian populations showed typical levels of microsatellite diversity (see supplemental information). Small, significant, differences between populations were present at different loci, but Anamalai and Periyar populations in southern India had the highest overall allelic richness. Observed heterozygosity was lowest at locus EMX-3, followed by EMX-4 and the various population pairs showed complex patterns of variation in observed heterozygosity (Table 4).

The loci LafMS02 and EMX-3 in the Periyar population and the locus EMX-3 in Central India deviated from Hardy–Weinberg equilibrium ($P = 0.0006$, 0.0014, 0.0061, respectively; first significant P value for the sequential Bonferroni correction in each population = 0.008). The significant deviation at EMX-3 in Central India is possibly a result of small sample size ($n = 12$) and that in Periyar due to non-random mating rather than selection (see Vidya *et al.*, 2005). All loci were in Hardy–Weinberg equilibrium in the other populations. No significant linkage disequilibrium was observed between any pair of loci in any population ($P > 0.01$, first significant P value for the sequential Bonferroni correction in each population = 0.003).

Genetic structure

An AMOVA of the four regions based on microsatellite data also showed differentiation at the level of the region, within regions and within populations. However, unlike the mtDNA results (Table 2), a large percentage of the total variation (86.5%) was within populations ($\Phi_{ST} = 0.135$, $P < 0.001$), followed by variation among regions (9.4%, $\Phi_{CT} = 0.094$, $P = 0.007$) and variation among populations within regions (4.2%, $\Phi_{SC} = 0.046$, $P < 0.001$) (Table 5).

AMOVAs of pairs of regions failed to discern any regional identity, except between central and northern India. However, regional sub-structuring was significant in all cases (Table 5). In addition, pairwise population F_{ST} tests (based on data from both sexes) were similar to those based on mtDNA (Table 3), with most population pairs being significantly differentiated from one another, suggesting that the absence of differentiation among regions may be due to significant variance within regions rather than similarity between regions. Again, similar to the results based on mtDNA, northern and northeastern Indian regions were similar even when the constituent populations were compared. However, within northeastern India, the North Bank and Southwest-Southcentral Bank populations, which had shown significant differentiation based on mtDNA data (from both sexes together), were indistinguishable based on microsatellite data (Table 3).

DISCUSSION

Population genetic structure

MtDNA diversity is low within the different populations in India with a total of only eight haplotypes across the country. Even if taken as a single unit, the average Indian haplotype diversity (0.667 ± 0.0222) is barely comparable to those of other Asian elephant populations occupying much smaller geographical areas (Fernando *et al.*, 2000; Fleischer *et al.*, 2001) although the average nucleotide diversity (0.012 ± 0.0062) is one of the highest due to the presence of both of the clades recorded for the species. We are not sure why the haplotype diversity is so low; when we constructed a mismatch distribution the observed mismatch did not fit the sudden expansion model ($P < 0.01$, distributions not shown) both when all the haplotypes were included and with only haplotypes of the β clade. Thus, there is little evidence of a population bottleneck. Populations also showed fairly typical levels of nuclear diversity at the dinucleotide loci, comparable to those observed in the African savannah elephant (Nyakaana & Arctander, 1999; Comstock *et al.* 2002, Eggert, Rasner & Woodruff, 2002) again arguing against a recent population bottleneck.

Based on our analyses of population differentiation, we find that northern and northeastern Indian populations are similar in haplotypic composition, with haplotypes largely of the α clade, and so are southern and central Indian populations with haplotypes of the β clade. Our limited sampling in central India precludes the inference that the α clade is competely absent here, but it is unlikely that the α clade, if present, is the predominant clade in this region. The α and β clades are estimated to have diverged approximately 1.2 million years ago (Fleischer *et al.*, 2001). Therefore, elephant populations in central and southern India probably share a different evolutionary history from those in northern and northeastern India. The absence of the α clade in central and southern India may reflect the fact that elephants with α clade haplotypes never colonised the peninsula or went extinct after they did. The existence of α clade haplotypes in Sri Lanka (Fernando *et al.*, 2000) supports the latter hypothesis, but whether the entry of the clade into Sri Lanka itself was completely natural or a consequence of human-induced trade is unclear at present. Trade in elephants, especially adult males for use in war, is known to have occurred between the ancient kingdoms that existed in the subcontinent (Sukumar, 1989: 3). We find that mitochondrial differentiation between populations/regions pre-dates human influence. We are thus unable to detect any influence on wild population genetic structure of elephant trade across India using the present markers. The low probability of once-captive female elephants reverting to the wild, reproducing successfully and establishing a lineage could be a possible reason. Males presumably have a higher chance of reverting to the wild and possibly even breeding, but they cannot be traced through mtDNA, although some degree of possible nuclear homogenisation of populations due to such males cannot be ruled out.

Within peninsular India, differentiation was observed between central and southern India and, further, within southern India. Allopatric fragmentation was observed in the clade 2–1 with haplotypes BC and BN distributed across central and southern India, respectively. Assuming a mutation rate of 3% per million years for the mitochondrial segment examined (Fleischer *et al.*, 2001) these two haplotypes diverged approximately 120 000 years ago and possibly differentiated during a period of climatic aridity when populations were isolated in different refugia. However, this cannot be distinguished at the moment from an alternative scenario of these haplotypes having coexisted in the intervening areas in which elephants are no longer present.

Female philopatry, male dispersal and male-mediated gene flow

Whether Asian elephant males, upon separating from their natal herds, also disperse away from their natal home range (locational dispersal) or remain in their natal territory and move long distances only to breed (social dispersal), has been little studied. In this study, the North Bank and the Southwest-Southcentral Bank populations showed significant mitochondrial differentiation based on females, but not based on males. The former may be explained by female philopatry since Asian elephant females live in a matriarchal society (McKay, 1973; Sukumar, 1989; Fernando & Lande, 2000). The absence of differentiation based on males, along with qualitative data about the presence of adult males of a different haplotype in areas with females of a common haplotype, suggests locational dispersal of males. If male dispersal were purely 'social', given the low probability of sampling males moving outside of their natal range only to mate, significant structuring of mtDNA in both males and females should have been observed. In a recent study in southern India, we have found some evidence for the locational dispersal hypothesis also based on nuclear microsatellite data (Vidya & Sukumar, 2005). However, these data, while suggestive of locational dispersal, do not rule out social dispersal, which may exist in addition, and

more detailed studies on male behaviour and associations and relatedness between males and females are needed to resolve this question.

We also observe contrasting patterns of mitochondrial and nuclear DNA structuring in northeastern India, mtDNA differentiating the North Bank and Southwest-Southcentral Bank populations, with microsatellite DNA showing no differentiation, thus pointing to female philopatry and male-mediated nuclear gene flow. The absence of mtDNA differentiation based on only males corroborates this inference. Insufficient mtDNA diversity precludes any inference regarding male-mediated gene flow in the other regions of India. Genetic evidence of female philopatry and male-mediated gene flow have been reported from populations of other large mammal species such as the North American beluga whale (*Delphinapterus leucas*: Gladden *et al.*, 1999), the African savannah elephant (*Loxodonta africana*) in eastern Africa (Nyakaana & Arctander, 1999), the harbour porpoise (*Phocoena phocoena*) in the northwestern Atlantic ocean (Rosel *et al.*, 1999) and the rhesus monkey (*Macaca mulatta*) across Asia (Melnick & Hoelzer, 1992).

Possible biogeographical barriers

We identified two possible biogeographical barriers within the elephant range in India based on this population genetic study. A barrier possibly exists/existed between the Nilgiri and the more southerly populations in southern India, with both mitochondrial and microsatellite DNA showing significant differentiation across the 40-km wide Palghat Gap, which is the only discontinuity in the Western Ghats mountain range (see Vidya *et al.*, 2005). In northeastern India, the Brahmaputra may have presented an incomplete riverine barrier, as suggested by the gradual increase in the similarity of haplotype compositions towards its upper reaches (Northern India: 100% AC; North Bank population of northeastern India: 92% AC, 8% AH; Eastern Region population: 50% AC, 50% AH; Southwest-Southcentral Bank population: 17% AC, 74% AH; see Table 1, Fig. 1). While occasional dispersers, especially males, may traverse the Brahmaputra, with an average width of 10 km and the greatest volume of water of all the rivers in India, it is possibly a barrier to female herds, with most of the genetic exchange occurring across its upper reaches, in the Eastern Region. It is interesting that the Brahmaputra seems to have been a biogeographical barrier for several species, with the species ranges of golden langur (*Semnopithecus geei*), pygmy hog (*Sus salvanius*) and hispid hare (*Caprolagus hispidus*) restricted to the north bank, while the hoolock gibbon (*Hylobates hoolock*) and stump-tailed macaque (*Macaca arctoides*) are restricted to the south bank (Rodgers & Panwar, 1988). A population genetic study of mtDNA in rhesus monkeys (Melnick *et al.*, 1993) also uncovered the Brahmaputra River as a possible major historical barrier to gene flow. Population genetic studies of other species would be helpful in corroborating whether the Palghat Gap and the Brahmaputra River have served as important biogeographical barriers to a broad range of taxa and thus should be considered in future conservation planning.

Conservation implications

Management Units (MUs) are identified by distinct allele frequencies at nuclear or mtDNA loci and are important for management since they address current population structure (Moritz, 1994). We found high F_{ST} values between most populations/regions based on mtDNA, but a pattern of isolation by distance within the β clade across India, indicating that the haplotypes have evolved through a gradual process of restricted dispersal and not due to allopatry, which would have argued more strongly for populations/regions to be maintained separately. However, concordance of F_{ST} tests based separately on mtDNA and nuclear DNA data (Table 3) suggest four genetically distinct units that may qualify as MUs: northern-northeastern India, central India, Nilgiris and Anamalai-Periyar. In addition, these four MUs are geographically separate at present and, with the exception of the two southern units, geographical distances between MUs are very large.

As mentioned previously, 11 'Project Elephant Ranges' have been designated in the country and each Range encompasses several protected areas: the northern-northeastern Indian population falls under Elephant Ranges 2–6 and 11, central India under Elephant Range 1, Nilgiris under Elephant Ranges 7 and 8 and Anamalai-Periyar under Elephant Ranges 9–10. These elephant ranges and protected areas have traditionally been the units of management. We suggest the use of the above MUs as more objective population units of management and emphasise the need for a concerted effort across administrative boundaries in governance and monitoring populations. This may not be relevant, however, to the northern-northeastern Indian unit as these populations are separated by well over 1000 km. Paetkau (1999) has suggested that if two populations are irrevocably split by anthropogenic habitat alteration, they may be treated as separate MUs. However, if future detailed studies of the northern population indicate the need to introduce animals in order to ensure the health and long-term sustainability of this relatively small population, we would recommend that animals from the northeastern populations be translocated rather than animals from genetically more distinct populations. Unlike the northern-northeastern Indian scenario, the Periyar and Anamalai populations which form a single MU are separated by about 50 km and reconnection of these populations by creating corridors, or translocation of a few males from Anamalai to Periyar, may be beneficial to the Periyar population given the paucity of adult males and the extremely skewed adult sex ratio of 1 male : 100 females here (Ramakrishnan *et al.*, 1998) brought on by ivory poaching in recent decades. Such translocation should, however, be based on clear objectives and include subsequent monitoring of the translocated animals.

The idea of translocating animals across MUs has been subject to debate. Gene flow can constrain local adaptation

and, in the long term, preclude the evolution of new species (Mayr, 1963, 1970; Shields, 1982; Templeton, 1986; Slatkin, 1987) due to the introgression of poorly adapted gene complexes. Such outbreeding depression or indication of pre- and post-zygotic barriers with increased parental divergence has been observed in several studies (Berger & Cunningham, 1995; King & Lawson, 1995; Storfer & Sih, 1998; see also Storfer, 1999; Edmands, 2002). But evidence to the contrary also exists and it is extremely difficult to predict the genetic consequence of translocation between populations based on their degree of divergence; this would depend on the species we are dealing with and its biology (see Edmands, 2002). Therefore, until a better understanding of the consequences of mixing lineages emerges, we do not suggest translocation of elephants across these MUs. Such translocation or connecting MUs, except for the ones within southern India, would also require considerable resources that could perhaps be better spent protecting the existing large populations. For instance, based on the present extent of habitat and trends in population size, it seems unlikely that the Nilgiris and northeastern populations will require immigrants in the near future. But these areas, especially northeastern India, would greatly benefit from better protection of existing elephant habitat. However, translocation across MUs may be appropriate under certain circumstances, if populations show signs of inbreeding depression or obvious deleterious genetic variants (Moritz, 1999; Hedrick, 2001), in which case the advantage of translocation is likely to outweigh any possible swamping of locally adapted genes.

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ALLELIC RICHNESS IN THE DIFFERENT POPULATIONS

Population	EMX-1	EMX-2	EMX-3	EMX-4	LafMS02	LafMS03	All loci (SE)
S India–Nilgiris	2.00 ± .00 (2–2)^{a}	2.00 ± .00 (2–2)^{a}	1.99 ± .02 (1–2)^{bc}	3.00 ± .00 (3–3)^{b}	4.04 ± .10 (3–5)^{c}	4.85 ± .13 (3–6)^{c}	2.78 ^{b} (0.026)
S India–Anamalai	3.53 ± .12 (2–4)^{d}	2.00 ± .00 (2–2)^{a}	1.92 ± .05 (1–2)^{ab}	2.97 ± .03 (2–3)^{ab}	3.97 ± .03 (3–4)^{c}	4.53 ± .12 (3–5)^{b}	2.88^{d} (0.046)
S India–Periyar	2.93 ± .05 (2–3)^{c}	2.00 ± .00 (2–2)^{a}	1.80 ± .08 (1–2)^{a}	2.99 ± .02 (2–3)^{b}	5.38 ± .14 (3–6)^{d}	4.45 ± .12 (3–5)^{b}	2.86^{cd} (0.038)
Central India	3	2	2	2	3	4	2.67
Northern India	3	2	2	3	2	4	2.67
NE India–N Bank	3.39 ± .10 (2–4)^{d}	2.00 ± .00 (2–2)^{a}	2.00 ± .00 (2–2)^{c}	2.91 ± .06 (2–3)^{a}	3.57 ± .12 (2–4)^{b}	4.00 ± .00 (4–4)^{a}	2.82 ^{bc} (0.024)
NE India–SWC Bank	2.61 ± .10 (2–3)^{b}	2.00 ± .00 (2–2)^{a}	2.00 ± .00 (2–2)^{c}	2.99 ± .02 (2–3)^{b}	2.00 ± .00 (2–2)^{a}	3.99 ± .02 (3–4)^{a}	2.47^{a} (0.012)
NE India–E Region	2	2	2	2	3	3	2.33

Mean ± 1.96 S.E. for number of alleles at individual loci obtained by randomly sub-sampling 20 individuals from each population 100 times; range in the total number of alleles from the sets of 20 samples, within parentheses; statistical significance as determined by Mann-Whitney *U* tests between pairs of populations at each locus: 'a' < 'b' < 'c' < 'd', while 'a' is not significantly different from 'ab' since the letter 'a' is shared, and so on. Total numbers of alleles are given in the case of central and northern India and the Eastern Region population of northeastern India since fewer than 20 individuals had been sampled from these populations. The last column shows the allelic richness across all loci combined, with the standard error within parentheses.