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RESEARCH ARTICLE

Identification of conservation priority units in the Asian elephant, *Elephas maximus*

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Abstract

Conservation biologists often deal with species that have small, fragmented populations throughout their range, making it necessary to prioritize populations for management. Genetics provides tools to assist with prioritization according to the levels and distribution of genetic diversity and evolutionary distinctiveness. Many studies have used nuclear microsatellite loci to measure genetic diversity in disparate populations and mitochondrial DNA to assess genetic distinctiveness. However, comparing metrics based on microsatellite genotypes ascertained in different laboratories is complicated by the selection of different loci with distinct nucleotide repeat motifs. This issue may be resolved by comparing metrics to a well-characterized reference population with shared microsatellite markers. The Asian elephant, *Elephas maximus*, is an endangered species with 50–60% of populations in India and Sri Lanka, and small, fragmented populations throughout southeast and insular Asia. We assessed range-wide genetic diversity of the Asian elephant by directly comparing allelic diversity and heterozygosity estimates from 35 populations, overcoming marker selection bias by calibrating metrics to a large population on the Nakai Plateau, Lao PDR, genotyped at 25 loci. We coupled these results with mtDNA analysis to evaluate genetic distinctiveness and identify potential conservation management units. We found the greatest diversity in the populations of southeast Asia and the greatest genetic distinctiveness among the subspecies designations, particularly Borneo and Sumatra, and other southeast Asian populations. The populations of southeast Asia, albeit small, fragmented, and at high risk of extirpation, contain valuable diversity and distinctiveness and are thus of high priority for the preservation of the Asian elephant.

Keywords Microsatellites · Yardstick calibration · Mitochondrial haplotypes · Population Genetics

Introduction

Conservation biologists often deal with species characterized by small, fragmented populations throughout their range. Given limited resources, managers must prioritize populations and determine the appropriate actions required to reduce the rate of further declines and increase the probability of recovery of the species adaptive potential (Hoban 2018). Intraspecific genetic variation increases adaptive potential under changing environmental conditions (Reed and Frankham 2003) and can alleviate the deleterious effects of inbreeding depression (Frankham 2005). Quantifying genetic diversity and differentiation within and between declining populations is therefore a critical component of conservation management.

Here, we evaluate the levels of genetic diversity and genetic distinctiveness among populations of Asian elephants. Despite being icons among the charismatic megafauna, elephant populations continue to decline across their

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native range. Conservation efforts generally focus on the African savanna elephant (*Loxodonta africana*), although populations of the African forest elephant (*L. cyclotis*) and the Asian elephant (*Elephas maximus*) each comprise only 10% the size of the African savanna elephant population (Gobush et al. 2021; Williams et al. 2020). Asian elephant populations are declining at alarming rates in India, Bangladesh, Bhutan, Cambodia, Lao PDR, Thailand, Vietnam, China, Myanmar, Malaysia, Indonesia, and Nepal, largely due to habitat degradation, urbanization, and fragmentation (Williams et al. 2020). While 50–60% of the total Asian elephant population is contained in India and Sri Lanka, smaller populations throughout southeast Asia continue to decline from habitat loss, with some already extirpated and only 16% of suitable habitat under protection (Choudhury et al. 2008; Williams et al. 2020).

Until recently, studies of genetic diversity within populations have been heavily reliant on nuclear microsatellite loci. However, common metrics derived from microsatellites, such as allelic diversity, heterozygosity, and fixation indices (F_{IS}, F_{ST}), are notoriously non-comparable among studies (Skrbinsek et al. 2012) due to non-repeatability of genotype scoring among researchers, inconsistent sample sizes, and differing properties of the microsatellite loci selected for the study. Discrepancies in genetic inference due to the selection of microsatellite loci (marker bias) were clearly demonstrated in studies of isolated Asian elephant (*Elephas maximus*) populations in Borneo, where Fernando et al. (2003) found extremely low levels of nuclear genetic diversity with an allelic diversity (A) of 1.4 and expected heterozygosity (H_E) of 0.041, primarily using loci characterized by Fernando et al. (2001). In contrast, Goossens et al. (2016) found a two-fold increase in A to 2.8 and 30% greater H_E (0.34) in the same populations using a panel of microsatellite loci developed by Kongrit et al. (2008). Although few studies have demonstrated such notable differences in genetic diversity within the same population, large differences in genetic diversity between populations are common. It is unknown whether these differences are biologically important or are artifacts of marker bias. To quantify the levels and distribution of genetic diversity within populations, managers will need methods for direct comparison across independent studies.

The most commonly used microsatellite loci in Asian elephant studies are those characterized by Fernando et al. (2001; the EMX loci) and those by Kongrit et al. (2008; the EMU loci). Diversity estimates differ widely across the species range (Table 1). However, it is unknown whether these differences are biologically important or are artifacts of marker bias as estimates within and between populations may be due to the structure of the microsatellites themselves. While the EMX loci contain complex motifs of tri- and tetra-nucleotide repeats, the EMU loci consist of di-nucleotide repeat regions. Di-nucleotide repeats have been shown to accumulate mutations at a rate 1.5–2.0 times greater than tetra-nucleotides (Chakraborty et al. 1997). Thus, the choice of loci could substantially affect the number of alleles detected per locus and result in substantially different estimates of diversity.

Skrbinsek et al. (2012) developed a computational method to overcome differences in microsatellite marker selection and sample size among studies by calibrating independent estimates of allelic diversity and heterozygosity against a reference "yardstick" population. To utilize their model, the researcher first generates the yardstick population by genotyping a large number of samples on a broad array of loci, ideally representing all loci that have been previously used. For each independent study, the model subsets only loci shared with the yardstick population and randomly resamples corresponding yardstick genotypes to match the study sample size, and this procedure is repeated over a pre-determined number of iterations (Leberg 2002). Allelic diversity and heterozygosity values, along with their standard errors, are calculated for each iteration. Finally, values are averaged over all iterations and the results are compared to the original study estimates using simple ratios. The Skrbinsek et al. (2012) model was validated by the authors with microsatellite data from 10 studies of brown bears that cover 30 populations of varying sizes from across the species range. It was also used to aid in the analysis of genetic diversity in water buffalo (*Bubalis bubalis* and *B. carabanensis*) based on SNP data from 31 populations from across the worldwide range of these species (Colli et al. 2018). It has been used to study diversity patterns in several other mammals, including European brown bears (*Ursus arctos*; Karamanlidis et al. 2018) and has been proposed as a potential tool for conserving black bears (*Ursus americanus*; Murphy et al. 2018) and grey wolves (*Canis lupus*; de Groot et al. 2016). Hindrikson et al. (2016) found that results were in accordance with the recorded demography and population history of 10 wolf (*Canis lupus*) populations of various sizes across Europe.

In addition to within-population genetic diversity, it is important to consider the presence of genetically distinct lineages to identify populations of high conservation priority. Genetically differentiated, locally adapted groups may reflect evolutionary potential and warrant designation as evolutionarily significant units or management units (Moritiz 1994). Although genetic differentiation among populations can be estimated based on microsatellite data, the complexities of microsatellite evolution make interpretation of those measures difficult over any but the most recent timeframes (Ellegren 2004; Eggert et al. 2009). Instead, mitochondrial DNA (mtDNA) control region sequences have been used as they are directly comparable and can reflect ancestral maternal lineages and recent patterns of population divergence.

Table 1 Comparison of genetic diversity between Asian elephant populations using a yardstick population to correct for different panels of loci (N_{loci}) and sample sizes (N_{test}). Allelic diversity (A_{test}), expected heterozygosity (H_{test}) and their corresponding standard errors (ASE_{test}, HSE_{test}) were collected from the compared (test) population study; allelic diversity ($A_{yardstick}$) and expected heterozygosity ($H_{yardstick}$) from the subset-resampled yardstick population with corresponding standard errors ($ASE_{yardstick}$, $HSE_{yardstick}$) were generated; and the ratios of allelic diversity (*AR*) and expected heterozygosity (*HR*) with standard errors (*ARSE*, *HRSE*) between the compared population and the yardstick population were generated

					Compared (test) population		Yardstick population (Subset-resample)		Diversity ratio	
Population		Reference	N_{loci}	N_{test}	A_{test} (ASE_{test})	H_{test} (HSE_{test})	$A_{yardstick}$ ($ASE_{yardstick}$)	$H_{yardstick}$ ($HSE_{yardstick}$)	*AR* (*ARSE*)	*HR* (*HRSE*)
Doi Phamuang	Thailand	Thitaram et al. 2015	7	64	11.571 (0.779)	0.827 (0.020)	6.748 (0.561)	0.702 (0.046)	1.715 (0.183)	1.178 (0.083)
Sublanka	Thailand	Thitaram et al. 2015	7	34	11.571 (0.779)	0.831 (0.019)	7.627 (0.605)	0.713 (0.045)	1.517 (0.158)	1.167 (0.078)
Nakai Plateau	Lao PDR	Ahlering et al. 2011	10	102	8.100 (0.806)	0.748 (0.024)	8.000 (0.494)	0.677 (0.041)	1.013 (0.119)	1.105 (0.075)
Kuldiha	India	Parida et al. 2022	9	33	2.14 (0.122)	0.735 (0.018)	7.220 (0.572)	0.666 (0.042)	0.296 (0.029)	1.103 (0.075)
Phnom Prich	Cambodia	Gray et al. 2014	9	78	6.000 (0.272)	0.668 (0.032)	4.974 (0.512)	0.628 (0.048)	1.206 (0.136)	1.064 (0.095)
Thai Elephant CC	Thailand	Thitaram et al. 2010	6	83	– (–)	0.597 (–)	3.619 (0.446)	0.562 (0.066)	– (–)	1.063 (–)
Taikkyi	Myanmar	Kusza et al. 2018	5	26	4.000 (1.020)	0.612 (0.066)	5.985 (0.727)	0.594 (0.069)	0.668 (0.189)	1.031 (0.164)
Bago Yoma	Myanmar	Budd et al. 2021	8	127	4.725 (0.131)	0.676 (0.031)	8.125 (0.479)	0.675 (0.046)	0.582 (0.038)	1.002 (0.083)
Myaing Hay Wun	Myanmar	Kusza et al. 2018	5	35	3.800 (0.820)	0.586 (0.091)	5.798 (0.751)	0.593 (0.069)	0.655 (0.165)	0.988 (0.192)
Maesa	Thailand	Thitaram et al. 2010	6	53	– (–)	0.595 (–)	6.003 (0.656)	0.617 (0.061)	– (–)	0.965 (–)
Similipal	India	Parida et al. 2022	10	43	2.07 (0.125)	0.640 (0.045)	6.961 (0.569)	0.665 (0.042)	0.297 (0.030)	0.963 (0.091)
Reintro. Found.	Thailand	Thitaram et al. 2010	6	20	– (–)	0.598 (–)	6.953 (0.601)	0.623 (0.061)	– (–)	0.959 (–)
Nilgiris	India	Vidya and Sukumar 2005a	6	140	3.500 (0.612)	0.529 (0.073)	7.333 (1.054)	0.555 (0.068)	0.477 (0.108)	0.953 (0.176)
Wild-sourced Zoo	USA	Lei et al. 2011	7	164	6.143 (0.891)	0.609 (0.048)	8.143 (0.829)	0.639 (0.041)	0.754 (0.134)	0.952 (0.097)
Zoo	USA	Kongrit et al. 2008	16	2	4.563 (0.364)	0.612 (0.038)	7.406 (0.429)	0.648 (0.032)	0.616 (0.061)	0.945 (0.074)
Seima	Cambodia	Eggert and Ruiz-Lopez 2011	11	55	5.250 (0.566)	0.620 (0.048)	6.561 (0.479)	0.661 (0.036)	0.800 (0.104)	0.937 (0.089)
Salak-Phra	Thailand	Kongrit et al. 2008	16	20	4.188 (0.309)	0.598 (0.042)	7.083 (0.436)	0.647 (0.032)	0.591 (0.057)	0.924 (0.079)
Alur	India	Chakraborty et al. 2014	10	29	3.800 (0.276)	0.573 (0.033)	6.824 (0.642)	0.621 (0.047)	0.557 (0.066)	0.922 (0.087)
Satkosia	India	Parida et al. 2022	9	30	1.82 (0.197)	0.614 (0.042)	7.288 (0.580)	0.667 (0.042)	0.250 (0.034)	0.921 (0.086)
Captive-born Zoo	USA	Lei et al. 2011	7	37	4.571 (0.854)	0.568 (0.059)	7.134 (0.739)	0.635 (0.042)	0.641 (0.137)	0.896 (0.110)
Anamalai	India	Vidya et al. 2005b	6	45	3.333 (0.451)	0.465 (0.077)	5.965 (0.912)	0.552 (0.068)	0.559 (0.114)	0.843 (0.174)
Sepon	Lao PDR	Eggert and Ruiz-Lopez 2012a	13	31	3.846 (0.296)	0.563 (0.033)	7.370 (0.436)	0.679 (0.035)	0.522 (0.051)	0.829 (0.064)
Bukit Tigapuluh	Sumatra	Moßbrucker et al. 2015	10	104	3.333 (0.314)	0.551 (0.053)	7.600 (0.562)	0.667 (0.042)	0.439 (0.053)	0.826 (0.095)
Periyar	India	Vidya et al. 2005b	6	27	3.500 (0.612)	0.455 (0.066)	6.592 (0.984)	0.553 (0.068)	0.531 (0.122)	0.822 (0.156)
Cat Tien	Vietnam	Vidya et al. 2007	6	17	2.333 (0.192)	0.437 (0.059)	6.879 (1.001)	0.554 (0.068)	0.339 (0.057)	0.789 (0.144)
Pu'Er-Mengyang	China	He et al. 2020	7	49	3.143 (0.315)	0.488 (0.076)	6.834 (0.645)	0.644 (0.064)	0.460 (0.063)	0.757 (0.140)
Lower Kinabatanagan	Borneo	Goossens et al. 2016	16	43	3.300 (0.321)	0.470 (0.040)	6.521 (0.442)	0.644 (0.032)	0.506 (0.06)	0.730 (0.072)
Nangunhe	China	Zhang et al. 2015	4	24	3.500 (0.750)	0.300 (0.091)	6.797 (0.881)	0.452 (0.069)	0.515 (0.129)	0.664 (0.225)
Deramakot	Borneo	Goossens et al. 2016	16	33	2.500 (0.218)	0.420 (0.053)	6.790 (0.443)	0.645 (0.032)	0.368 (0.040)	0.651 (0.088)
Mengyang	China	Zhang et al. 2015	4	55	4.250 (0.893)	0.284 (0.099)	5.402 (0.869)	0.445 (0.071)	0.787 (0.208)	0.637 (0.244)

Table 1 (continued)

Population		Reference	Compared (test) population				Yardstick population (Subset-resample)		Diversity ratio	
			N_{loci}	N_{test}	A_{test} (ASE_{test})	H_{test} (HSE_{test})	$A_{yardstick}$ ($ASE_{yardstick}$)	$H_{yardstick}$ ($HSE_{yardstick}$)	*AR* (*ARSE*)	*HR* (*HRSE*)
Mengla	China	Zhang et al. 2015	4	32	3.750 (0.65)	0.234 (0.050)	6.483 (0.895)	0.450 (0.070)	0.578 (0.128)	0.520 (0.136)
Simao	China	Zhang et al. 2015	4	8	2.250 (0.217)	0.181 (0.028)	7.286 (0.862)	0.453 (0.068)	0.309 (0.047)	0.401 (0.087)
Central Forest	Borneo	Goossens et al. 2016	16	127	3.600 (0.354)	0.250 (0.042)	7.438 (0.428)	0.648 (0.032)	0.484 (0.055)	0.386 (0.068)
Shangyong	China	Zhang et al. 2015	4	59	3.250 (1.244)	0.168 (0.099)	5.174 (0.869)	0.445 (0.073)	0.628 (0.263)	0.378 (0.230)
Tabin Reserve	Borneo	Goossens et al. 2016	16	21	1.700 (0.202)	0.210 (0.052)	7.060 (0.436)	0.647 (0.032)	0.241 (0.032)	0.325 (0.082)

Nearly every population genetic study on the mtDNA of Asian elephants has used an approximately 630 bp fragment containing a portion of the C terminal of cytochrome *b*, the threonine and proline tRNAs, and the 5' end of the non-coding control region (d-loop), described in Fernando et al. (2000). Using data from this fragment, studies have found deep-rooted mitochondrial clades in Asian elephants, which were first suggested by Fernando et al. (2000) and Fleischer et al. (2001) and further validated across the species' range by Vidya et al. (2009). These mtDNA clades are described as the α-clade, persisting primarily in the north-east areas of the range, and the β-clade, primarily found in the south and south-west (including the Indonesian and Malaysian islands) with the two clades overlapping in Myanmar and Thailand.

Our primary objective was to ascertain populations for conservation priority in the Asian elephant by identifying lineages with high genetic diversity and genetic distinctiveness. We calibrated microsatellite data from previous Asian elephant population genetic studies by analyzing shared microsatellite markers in a large "yardstick" reference population, allowing direct comparison of allelic diversity and heterozygosity despite differing marker selection. Using a GIS framework, we then applied Bayesian kriging prediction to our calibrated allelic diversity and heterozygosity metrics to infer continuous patterns of diversity across the species distribution, including unstudied regions. Finally, because the yardstick model considers only population level genetic diversity estimates based on microsatellite loci, we did not use those data to compute genetic distances among populations. Instead, we evaluated populations using evolutionary distinctiveness (ED, Isaac et al. 2007), an index that measures the relative contribution of a taxon (i.e. population) to the phylogenetic diversity of the species. We calculated scores of compiled mtDNA sequences spanning the species distribution to assess genetic differentiation and subspecies designations utilizing evolutionary significant unit and management unit definitions (Moritiz 1994).

Materials and methods

Yardstick reference population

To generate a reference (i.e., "yardstick") population, we used DNA samples ($N = 91$) that were confirmed to be unique Asian elephants in a previous survey of the Nakai Plateau in Lao PDR (Budd 2021). For all individuals, we amplified 25 microsatellite loci including EMX and EMU loci and four African elephant loci (LA4, Eggert et al. 2000; FH94, Comstock et al. 2000; LafMS02, LafMS03, Nyakaana and Arctander 1998) that have been most often used in previous studies. DNA samples were arranged into 6 multiplexed panels (Supplementary Table 1). We

included negative (no DNA) and positive controls in each panel for contamination detection and genotype standardization.

Polymerase chain reactions (PCRs) were performed in 8 µl volumes comprised of 2µM primer mix, 0.8mM BSA, and Platinum® Multiplex PCR Master Mix (2X Master Mix, GC Enhancer; Applied Biosystems, Foster City, CA). Thermocycler conditions were as follows: 95 °C for 2-min; 40 cycles of 94 °C for 30-sec, multiplex-specific annealing temperature for 90-sec (Supplementary Table 1), 72 °C for 1-min; followed by a final extension at 60 °C for 30-min. Preparations for all PCRs were conducted in a UV–Sterilized hood.

Amplified products were submitted for fragment analysis in an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA) at the University of Missouri DNA Core facility with added 600LIZ size standard. Chromatograms were visualized and individuals were genotyped using GeneMarker v.1.97 (SoftGenetics, State College, PA; Holland and Parson 2010).

Yardstick calibration model

We used the yardstick genotypes as a reference population for comparison of genetic diversity metrics ascertained from multiple Asian elephant populations across independent studies. We compiled data from previous Asian elephant studies (test populations) that shared loci with our yardstick population (avg shared loci 8.5 ± 4.0 SD (range 4–16; Table 1.). From each test population, we used sample size (N_{test}), expected heterozygosity (H_{test}), allelic diversity (A_{test}) and corresponding standard errors (HSE_{test}; ASE_{test}) for shared loci as inputs for our yardstick calibration model.

We developed a yardstick calibration model following Skrbinsek et al. (2012) in *R v.*4.0.3 (R Core Team 2018). For each test population, we first subset the yardstick population to retain only shared loci using the *R* package *genepopedit v.*1.0.0.6 (Stanley et al. 2017). Then, for each of 1,000 iterations, we randomly sampled yardstick genotypes, without replacement, from individuals matching the number of samples in the test population (N_{test}) and calculated mean and standard error values for expected heterozygosity ($H_{yardstick}$, $HSE_{yardstick}$) and allelic diversity ($A_{yardstick}$,$ASE_{yardstick}$) using the *R* package *adegenet v*.2.1.5 (Jombart 2008; Jombart and Ahmend 2011).

We then calculated (1) pairwise heterozygosity ratios (*HR*) of H_{test} and the corresponding yardstick population mean ($H_{yardstick}$) and (2) pairwise allelic diversity ratios (*AR*) of A_{test} and the corresponding yardstick population mean ($A_{yardstick}$) to assess the relative diversity of each test population following Skrbinsek et al. (2012):

$$HR = \frac{H_{test}}{H_{yardstick}} \quad (1)$$

$$AR = \frac{A_{test}}{A_{yardstick}} \quad (2)$$

Diversity ratios (*HR*, *AR*) were therefore relative to the yardstick population, where values of 1.000 indicated equal diversity between the test and yardstick population, and deviations greater or lower than 1.000 indicated higher or lower diversity, respectively. We also calculated the standard error for 3) heterozygosity ratios (*HRSE*) and 4) allelic diversity ratios (*ARSE*) as in Skrbinsek et al. (2012):

$$HRSE = \sqrt{HR^2 \cdot \left(\left(\frac{HSE_{test}}{H_{test}} \right)^2 + \left(\frac{HSE_{yardstick}}{H_{yardstick}} \right)^2 \right)} \quad (3)$$

$$ARSE = \sqrt{AR^2 \cdot \left(\left(\frac{ASE_{test}}{A_{test}} \right)^2 + \left(\frac{ASE_{yardstick}}{A_{yardstick}} \right)^2 \right)} \quad (4)$$

To assess differences in inference that were made possible by using the yardstick method, we ranked the populations according to the values reported in the original studies (H_{test} and AR_{test}) and the diversity ratios (HR and AR) and compared the rankings in a Spearman's rank order correlation test (Zar 2005) in Statziki (https://www.statziki.com/Spearman).

Spatial patterns of genetic diversity

We applied empirical Bayesian kriging prediction in ArcGIS Pro (ESRI) to *HRSE* and *ARSE* values to predict spatial patterns of genetic diversity across Asian elephant populations. For the genetic input, we applied a log-empirical transformation with a K-Bessel semi-variogram model and used standard circular neighborhood parameters. For the geographic input, we generated the centroid of each population based on the source literature indications. The predictive raster was then masked using the IUCN Asian elephant shapefile (Williams et al. 2020) to the current extent of the Asian elephant distribution. We repeated empirical Bayesian kriging using the test population reported values and standardized the scale of the test population and the predicted diversity ratios to 100 to allow for spatial comparisons.

Evaluation of distinct lineages

We compiled mtDNA sequences from published articles, reports, and GENBANK accessions. We continued the naming convention set forth by Vidya et al. (2005a) to the haplotypes described in these additional studies for clarity

and to avoid name duplicates (such as "New Haplotype") that were frequently encountered in the literature; original haplotype names and citations are provided in Supplementary Table 2. We manually trimmed and aligned sequences using ClustalW (Thompson et al. 1994) in Geneious *v.*8.0.5 (Kearse et al. 2012). We then collapsed sequences into haplotypes using FaBox *v.*1.5 (Villesen 2007) and removed duplicate haplotypes. We conducted maximum likelihood analyses in PAUP* *v.*4.0a169 (Swofford 2002) with a heuristic search and 1000 bootstrap replicates to assess support. The DNA substitution model was selected using the automated model selection tool and determined using the optimal AICc.

For each haplotype, we calculated evolutionary distinctiveness scores using fair proportions (Isaac et al. 2007) using Picante (Kembel et al. 2010) in *R*. Visualization into a median joining network (Bandelt et al. 1999) was generated in POPART (Leigh and Bryant 2015) with individual haplotypes colored to signify source country.

Results

Evaluation of the yardstick model

To validate the model, we compared the results to several studies that were done in our lab by different researchers. Genotyping results for these studies were scored both manually and electronically and different sequencing platforms and sizing standards were used over the 10 year period at the University of Missouri DNA Core Facility. The similarities and differences were as expected: the yardstick population at Nakai in 2018/2019 was compared to the Nakai 2011 population (Ahlering et al. 2011) and found to be quite similar (Table 1); a genetic MRC survey (Eggert and Ruiz-Lopez 2012a) found that the Sepon, Lao PDR, population was smaller and the yardstick calibration found lower allelic diversity but similar heterozygosity; a genetic MRC survey (Eggert and Ruiz-Lopez 2011) found that the Seima, Cambodia, population was also smaller and the yardstick model found it to have less allelic diversity but similar heterozygosity; the Bago Yoma, Myanmar, population, while similar in size was more isolated geographically and had much lower allelic diversity but similar heterozygosity (Budd et al. 2021). All these findings were in accordance with expectations based on the demographic and geographic history of the populations.

Population genetic and spatial diversity

We compiled data from 35 test populations from India, Lao PDR, Vietnam, Thailand, China, Myanmar, Cambodia, Sumatra, Borneo, peninsular Malaysia and US Zoos (Table 1). Among the reference populations, *AR* ranged from 0.241 (*ARSE* ± 0.032) to 1.715 (± 0.183) and *HR* ranged from 0.325 (*HRSE* ± 0.082) to 1.178 (± 0.083). Rankings of the values from the original studies and the ratios of diversity generated under the yardstick model differed significantly (Spearman's rank correlation *A*test vs. $AR = 0.991774$, $p < 0.05$; *H*test vs. $HR = 0.91911$, $p < 0.05$). For both allelic diversity and heterozygosity, we found the highest ratios of diversity in populations in Thailand, Lao PDR, Cambodia, and Myanmar while populations from Borneo and India contained the lowest diversity (Table 1). For populations in China, rankings were mixed, with Mengyang ranking 10th in A_{test} and 6th in *AR* but 30th in both H_{test} and *HR*, Shangyong ranking 24th in A_{test} and 11th in *AR* but 35th in H_{test} and 34th in *HR*, and Mengla ranking 16th in A_{test} and 15th in *AR* but 32nd in H_{test} and 31st in *HR*. All other Chinese populations had diversity ratios in the lowest third of the rankings for allelic diversity (A_{test} and *AR*) and heterozygosity (H_{test} and *HR*).

Analysis of genetic diversity based on geographic patterns showed hotspots for expected heterozygosity in southeast Asia (Myanmar, Thailand, Cambodia, Lao PDR) and southern India (Fig. 1A). Based on allelic diversity, mainland southeast Asia was the primary diversity hotspot (Fig. 1B). The lowest relative diversity using each metric was seen in the small populations of Borneo and China (Fig. 1A, B).

Evolutionary distinctiveness

We compiled mtDNA sequences from populations in 12 home range countries and North American zoos (Supplemental Table 2). We found 69 unique Asian elephant haplotypes; 31 from the α-clade and 38 from the β-clade. Of these, only 19 were shared between two or more range countries, allowing for 50 private haplotypes. Additionally, two haplotypes (AO, AP) were found only in zoo populations (Lei et al. 2011).

The optimal substitution model for the maximum likelihood analysis of evolutionary distinctiveness was determined to be HKY + I + G. MtDNA haplotypes with the highest evolutionary distinctiveness scores were found to be AI (0.054; Myanmar), BBG (0.046; peninsular Malaysia), BD (0.036; Borneo), BY (0.031; Lao PDR), BR (0.027; Sumatra), and AP (0.027; North American Zoos), while the lowest scores were AH (0.000; multiple countries), AB (0.001; multiple countries), AD (0.001; multiple countries), AR (0.002; Lao PDR), and BF (0.002; India, North American Zoos; Fig. 2). The most widely distributed haplotypes were BO, found in 5 range countries and North American Zoos, and AD and BP, each of which was found in four range countries and North American Zoos.

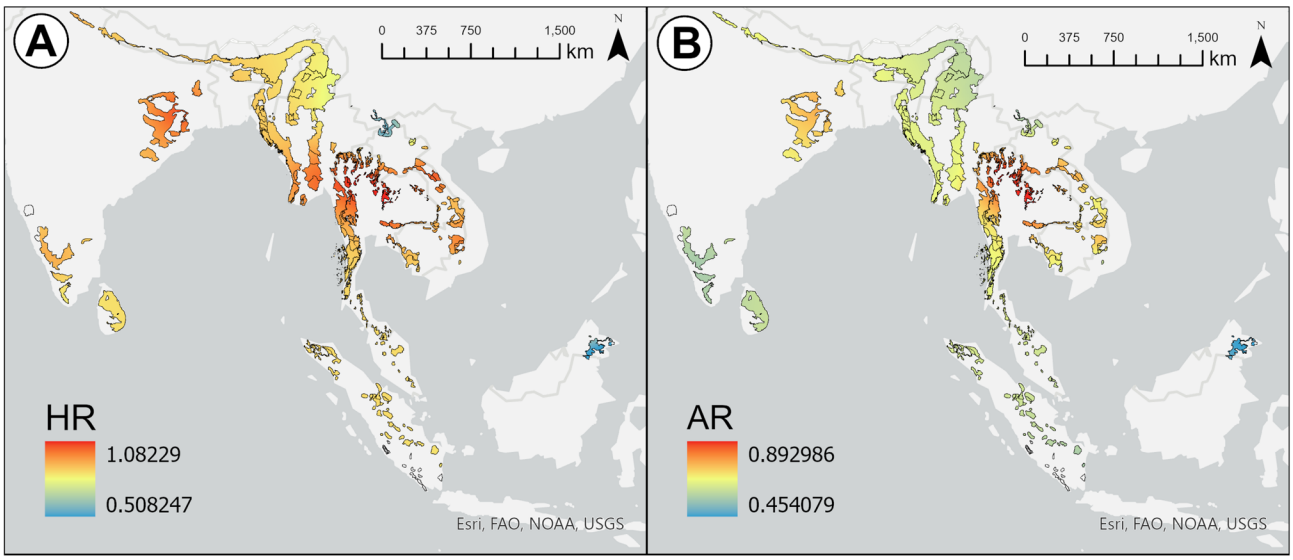

Fig. 1 Spatial distribution of genetic diversity values for Asian elephants following empirical Bayesian kriging of (**A**) pairwise heterozygosity ratios (HR) and (**B**) allelic diversity ratios (AR)

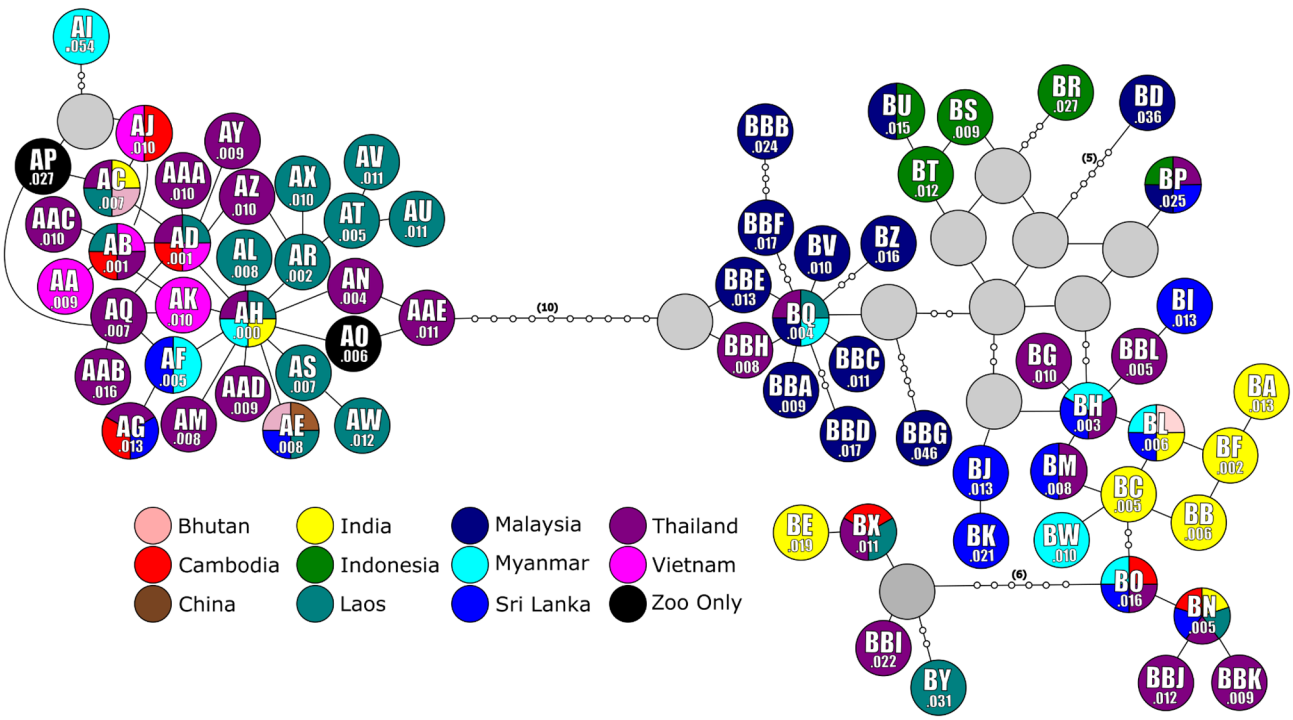

Fig. 2 Haplotype network for 69 Asian elephant mtDNA haplotypes with evolutionary distinctiveness scores shown and colors indicating source country. Grey circles indicate missing haplotypes that are presumed to exist but were not sampled

Discussion

The Asian elephant is experiencing alarming decreases in the number and sizes of populations across its range (Blake and Hedges 2004; Hedges 2006). Evaluating the levels and distribution of genetic diversity is vital to conservation, as it allows managers to infer migratory pathways across fragmented landscapes, estimate census and effective population sizes and demographic parameters, and infer the proximate and ultimate causes of human-elephant conflict. As populations decline in size, a reduction

in allelic diversity due to genetic drift is predicted to occur before the loss of heterozygosity (Nei et al. 1975; Cornuet and Luikart 1996). Thus, monitoring levels of both measures of genetic diversity is essential to allow managers to detect genetic erosion and, to the extent possible, avoid the deleterious effects of inbreeding depression (Hoban et al. 2021).

In their study of isolated populations of elephants on Goossens et al. (2016) suggested that using highly polymorphic markers, such as the microsatellite loci developed by Kongrit et al. (2008), rather than randomly selected markers to estimate genetic diversity likely produced overestimated values. Specifically, they suggested that although both their Bornean values and the values reported by Ahlering et al. (2011) for the Nakai population were similar to values reported for populations of other rare or endangered species, they were likely overestimates. Their study did not suggest a remedy for such over- or underestimation, presumably caused by the use of different loci. The yardstick method we used in this study allows for direct comparison of studies after calibration. While it does not produce "corrected" values for allelic diversity and heterozygosity, it does produce ratios that managers can use to compare populations.

Although the largest Asian elephant populations are found in India, we found that the Southeast Asian populations of Thailand, Lao PDR, Cambodia and Myanmar harbored higher levels of allelic diversity and heterozygosity. The higher levels of diversity are in accordance with the results of Ahlering et al. (2011), who suggested that the high levels of diversity found in the Nakai Plateau, Lao PDR population underscored its importance for the conservation of the species. As has been shown previously, we found that studies of Chinese and Bornean populations consistently reveal low diversity, which is especially notable after the data are corrected for marker bias.

In addition to monitoring levels of diversity, managers must also consider genetic differentiation among populations. Many studies of Asian elephants have used mtDNA to estimate levels of differentiation due the relative ease of amplifying it from non-invasively collected dung samples, its higher mutation rate than nuclear DNA which enables studies within and among populations, and its maternal mode of inheritance in a largely matrilineally structured species. Ballard and Whitlock (2004) argued that mtDNA should not be used for this purpose as it may be subject to natural selection, Galtier et al. (2009) argued that mtDNA mutation rates differ among species and do not exhibit "molecular clock-like" behavior, and Toews and Brelsford (2012) cite instances of mito-nuclear discordance that could be problematic in biogeographic studies. While we do not disagree with these limitations, our study used mtDNA to document and compare levels of genetic differentiation. The action of natural selection on mtDNA would enhance differentiation, which would suggest that managers should use caution when managing highly divergent populations as a single unit. We do not attempt to date population divergences, thus no molecular clock was applied. While mito-nuclear discordance has been documented in African elephants (Roca et al. 2005), we are unaware of such findings in Asian elephants. In any case, our study did not reveal surprising asssociations among populations that might suggest the presence of discordance.

He et al. (2020) suggested that inbreeding in Chinese populations might be alleviated by establishing connectivity among remnant Chinese populations and with regions such as Lao PDR or by initiating further actions such as translocations to facilitate genetic rescue. However, prior to taking such actions managers should evaluate genetic differentiation between potential donor and recipient populations. For example, despite their findings of low diversity in Bornean elephants, Goossens et al. (2016) expressed concerns with the potential for outbreeding depression if gene flow was established between Bornean and mainland elephant populations.

In Asian elephants, three subspecies are currently recognized: the Asian mainland elephant or Indian elephant, *E. m. indicus*, the Sri Lankan elephant, *E. m. maximus*, and the Sumatran elephant, *E. m. sumatranus.* Additionally, the Bornean elephant, *E. m. borneensis*, has been supported by genetic studies (Fernando et al. 2003; Sharma et al. 2018) but awaits further range-wide assessment prior to designation (Williams et al. 2020). Our mtDNA results support the evolutionary distinctiveness of the Sumatran elephant subspecies (*E. m. sumatranus*; 5 haplotypes BP, BR, BS, BT, BU with evolutionary distinctiveness values from 0.009 to 0.027) and the proposed Bornean elephant subspecies (*E. m. borneensis*; single haplotype BD with high evolutionary distinctiveness 0.036). In our haplotype network, the Sumatran haplotypes group together with multiple steps between them and other β clade haplotypes. Thus, they meet the criteria of evolutionary significant units, or lineages of populations that maintain their identity from other such lineages and demonstrate independent historical fates (Wiley 1978); management units that demonstrate reciprocal monophyly at mtDNA (Mortiz 1994); and/or phylogenetic species that possess a combination of derived traits and a unique evolutionary history (Cracraft 1982). Regardless of taxonomic classification, the present study underscores Goossens et al. (2016) concerns for the potential of outbreeding depression if translocations are used to enhance genetic variability, especially for insular populations.

We also found evolutionary distinctiveness among Indian elephant (aka mainland elephant, *E. m. indicus*) populations, particularly among peninsular Malaysia and Myanmar populations. Although the largest populations of Asian elephants are found in India, we found lower

regions of diversity and distinction in these populations than in the small, fragmented populations of southeast and insular Asia. The largest Asian elephant population, Nilgiris, is characterized by a single mtDNA haplotype (BN; Vidya et al. 2005a) that is of low evolutionary distinctiveness. Nilgiris is also among the populations with levels of nuclear diversity lower than those in Thailand, Lao PDR, Cambodia, and Myanmar. The lower diversity found in this large population underscores the importance of conservation efforts focusing not only on the large populations of India, but also on the smaller populations of southeast and insular Asia for the preservation of diversity and distinctive lineages within the species.

Across southeast Asia, surveys have produced varying estimates of population sizes, especially in Lao PDR (ranging from 500 to 1000; Duckworth and Hedges 1998; Choudhury et al. 2008; Khounboline 2011), Vietnam (100–130 elephants; Williams et al. 2020) and Cambodia (250 to 2000 elephants; Kemf and Jackson 1995; Osborn and Vinton 1999; Murdoch 2008). In Myanmar, where more potential elephant habitat exists than in any other country in the species' range, the total population is estimated at approximately 2,000 individuals, but the country's recent and extreme poaching climate may drastically reduce those numbers in coming years (Leimgruber et al. 2011; Sampson et al. 2018). The small populations, such as those in southeast Asia that harbor the highest diversity, are also among those with the highest rates of habitat loss and fragmentation (Williams et al. 2020) and are therefore at high risk for local extirpation. The loss of diversity in southeast Asia is likely to be directly detrimental to the long-term preservation of the species.

Conservation management aims to reduce the rate of population declines and increase the probability of recovery of a species adaptive potential under changing environmental conditions; thus, methods for quantifying genetic diversity and differentiation within and between declining populations are essential tools. The issue of marker selection bias in the comparison of genetic diversity across a species range is not unique to elephants as yardstick calibration models have also been used in studies of brown bears (Skrbinsek et al. 2012) and water buffalo (Colli et al. 2018) and European wolves (Hindrikson et al. 2016). Despite the overwhelming evidence of marker selection bias using microsatellites, few studies address it. One important limitation of yardstick calibration modeling is the need to create a large, well-studied population that is amplified at all microsatellites in the comparison, which may be costly and time-consuming. However, for study species such as Asian elephants, where separate incomparable panels have dominated the literature, yardstick calibration modeling can be an important tool for identifying patterns of diversity to aid in conservation management.

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Author contributions KB designed the study and carried out the labwork, initial data analyses and writing of the first draft of the manuscript. JG and LLS conducted data analyses, data interpretation and contributed substantially to the writing of the final draft of the manuscript. LSE administered the project and contributed to the writing of the final draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

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Data availability Data from the yardstick population (Nakai Plateau, 25 microsatellite loci) and the R code used to calculate the diversity ratios have been deposited in DRYAD: https://doi.org/10.5061/dryad.vdncjsz11. Genetic diversity data from populations used for comparison to the yardstick population are provided in Table 1. Accession numbers for all mtDNA sequences used in the evolutionary distinctiveness analyses are shown in Supplementary Table 2.

Declarations

Competing interests The authors declare no competing interests.

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