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HAPLOTYPE DISTRIBUTION AMONG ENDANGERED ASIAN ELEPHANTS (*Elephas maximus*) IN PENINSULAR MALAYSIA

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ABSTRACT

Asian elephants are classified as an endangered species on the IUCN red list, warranting more research and conservation efforts to protect them. A study of the distribution of haplotypes among Asian elephants in Peninsular Malaysia was performed using a partial DNA sequencing of a D-loop region. In this study, 10 haplotypes (Hap01–Hap10) were detected in Peninsular Malaysian populations with a high haplotype diversity ($\hat{H}$) of 83%. Hap01 was shared by Kelantan (n = 1), Johor (n = 2), Pahang (n = 2), and Perak (n = 2). The other shared haplotype was Hap06, which was evident in the Pahang (n = 1) and Johor (n = 1) samples. DnaSP analysis demonstrated that low genetic diversity (π) was observed in Peninsular Malaysian elephants (0.55%). Conversely, the gene flow was high (Nm = 9.65 migrants per generation). In a test of population subdivision, all pairwise comparisons for Peninsular Malaysia were low (0.00 to 0.13) except for Kelantan–Pahang (0.57). Our results demonstrated that the genetic diversity was low within the different populations of Peninsular Malaysia. The level of genetic differentiation was also low, but the gene flow was high regardless of the geographic distance of the Asian elephant populations in Peninsular Malaysia.

Key words: Asian elephant, *Elephas maximus,* haplotype, D-loop region, Peninsular Malaysia

INTRODUCTION

Elephants is classified under the order of Proboscidea with two extant genera of the family Elephantidae, namely *Elephas* and *Loxodonta*. Asian elephants are widely distributed, covering much of South Asia in the west to Indochina in the east, and a larger part of Southeast Asia, including Peninsular Malaysia, Sumatra, and Borneo (Fernando *et al.,* 2003; Vidya *et al.,* 2005a). With the passing of time, however, the Asian elephant population has become decreased. The most serious threat faced by the population of elephants is habitat destruction resulting from deforestation activities (Sukumar, 2006). The increase in the illegal hunting of wild elephants has also worsened the situation (Stiles, 2004; Sukumar, 2006).

To counter the threat of extinction of Asian elephant populations, many studies have been carried out focusing on aspects of the ecology, behavior, and phylogeography on this species (Vidya *et al.,* 2009). Phylogeography studies are able to identify changes in population size and determine the genetic structure of species, which is also important for conservation management purposes (Fleischer *et al.,* 2001). Molecular ecology studies were conducted by Vidya *et al.* (2005b) focusing on the social organization of Asian elephants in southern India using nuclear microsatellite loci. Moreover, Fernando and Lande (2000) found that there were more complex social groups and discovered differences in the social organization of these groups between Asian elephants and African elephants. In addition, Fickel *et al.* (2007) studied the effect of pressure outbreeding of Asian elephants in relation to species conservation and conflict.

Studies involving genetic characteristics have mostly focused on the population of Asian elephants in India and Sri Lanka. Hartl *et al.* (1996) reported that population differentiation between the mainland and Sri Lanka was small based on Cyt *b* DNA sequences. Conversely, Fernando (1998) reviewed the structure of Asian elephant populations

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on the mainland and Sri Lanka, and showed high genetic deviation between the two populations. Fernando *et al.* (2000) previously reviewed aspects of elephant phylogeography in the vicinity of Sri Lanka, Bhutan, India, Laos and Vietnam. Other researchers, such as Fleischer *et al.* (2001), sequenced mtDNA to assay genetic variation and phylogeography across Asian elephants' range from seven countries, namely Sri Lanka, India, Nepal, Myanmar, Thailand, Malaysia, and Indonesia. Vidya *et al.* (2005a) studied the differential populations within and among populations of Asian elephants in southern India with the sequencing of mtDNA and nuclear microsatellites. They observed low mitochondrial haplotype diversity in southern India, with a total of five haplotypes.

To date, the same comprehensive study has yet to be carried out on Asian elephant populations in Peninsular Malaysia. The objective of this study is to analyze the distribution of haplotypes among Asian elephants in Peninsular Malaysia based on the variation in the highly variable D-loop region of mtDNA. This locus was selected based on the suitability of the genetic analysis of populations (Lim *et al.,* 2010; Abdul-Latiff *et al.,* 2014) and closely related taxa (Ang *et al.,* 2012; Rosli *et al.,* 2014), and because it is ideal for studying the phylogeography in species with a female-based social structure such as elephants (Fernando, 1998). Genetic information is important for operational activities and managing Asian elephant conservation in Peninsular Malaysia.

MATERIALS AND METHODS

Samples and DNA extraction

All samples were provided by the Department of Wildlife and National Parks, Peninsular Malaysia (DWNP). The individuals were from various states of Peninsular Malaysia, namely Terengganu (n = 3), Kelantan (n = 3), Perak (n= 3), Johor (n = 7), and Pahang (n = 5; as shown in Table 1). Genomic DNA was extracted from FTA cards and fresh blood using the Whatman FTA Protocol BD01 and Qiagen QIAamp blood kit, respectively. Extraction without any genetic material was also performed as a negative control in polymerase chain reaction (PCR) amplification. Four sequences of the D-loop were obtained from GenBank, namely BP, BQ, BU, and BV (GenBank Access No: AY245813, AY245816, AY245812 and AY245814 respectively) with *Mammuthus primigenius* (FJ753556) and *Loxodonta africana* (GQ357177, AF219244, DQ316069, and NC_000934) as outgroups. Twenty-one individuals from five regional habitats were collected (Table 1).

PCR amplification

PCRs using MDL3 and MDL5 primers (Fernando & Lande, 2000) were carried out to amplify a 600 bp segment of mtDNA containing the C-terminal of cytochrome b and the hypervariable left domain of the noncoding control region. The forward primer used was 5'-TTA CAT GAA TTG GCA GCC AAC CAG-3' and the reverse primer was 5'-CCC ACA ATT AAT GGG CCC GGA GCG-3'. Amplification

Table 1. Origin and group of the Malay Peninsular Asian elephant samples

Sample No	State	Locality	Group Name
1	Perak	Lenggong	Piah
2	Perak	Lenggong	Piah
3	Perak	Sungai Siput	Piah
4	Johor	Jemaluang	Jemaluang
5	Johor	Segamat	Labis
6	Johor	Segamat	Labis
7	Johor	Kota Tinggi	Panti
8	Johor	Kota Tinggi	Bandar Tenggara
9	Johor	Kota Tinggi	Panti
10	Johor	Kluang	Kg Peta
11	Pahang	Kuantan	Remen Chereh
12	Pahang	Muadzam Shah	Muadzam
13	Pahang	Muadzam Shah	Muadzam
14	Pahang	Muadzam Shah	Muadzam
15	Pahang	Kuala Lipis	Som
16	Kelantan	Jeli	Gunung Basor
17	Kelantan	Chalil	Relai
18	Kelantan	Tanah Merah	Jedok
19	Terengganu	Ulu Terengganu	Ulu Terengganu
20	Terengganu	Setiu	Pelong
21	Terengganu	Setiu	Pelong

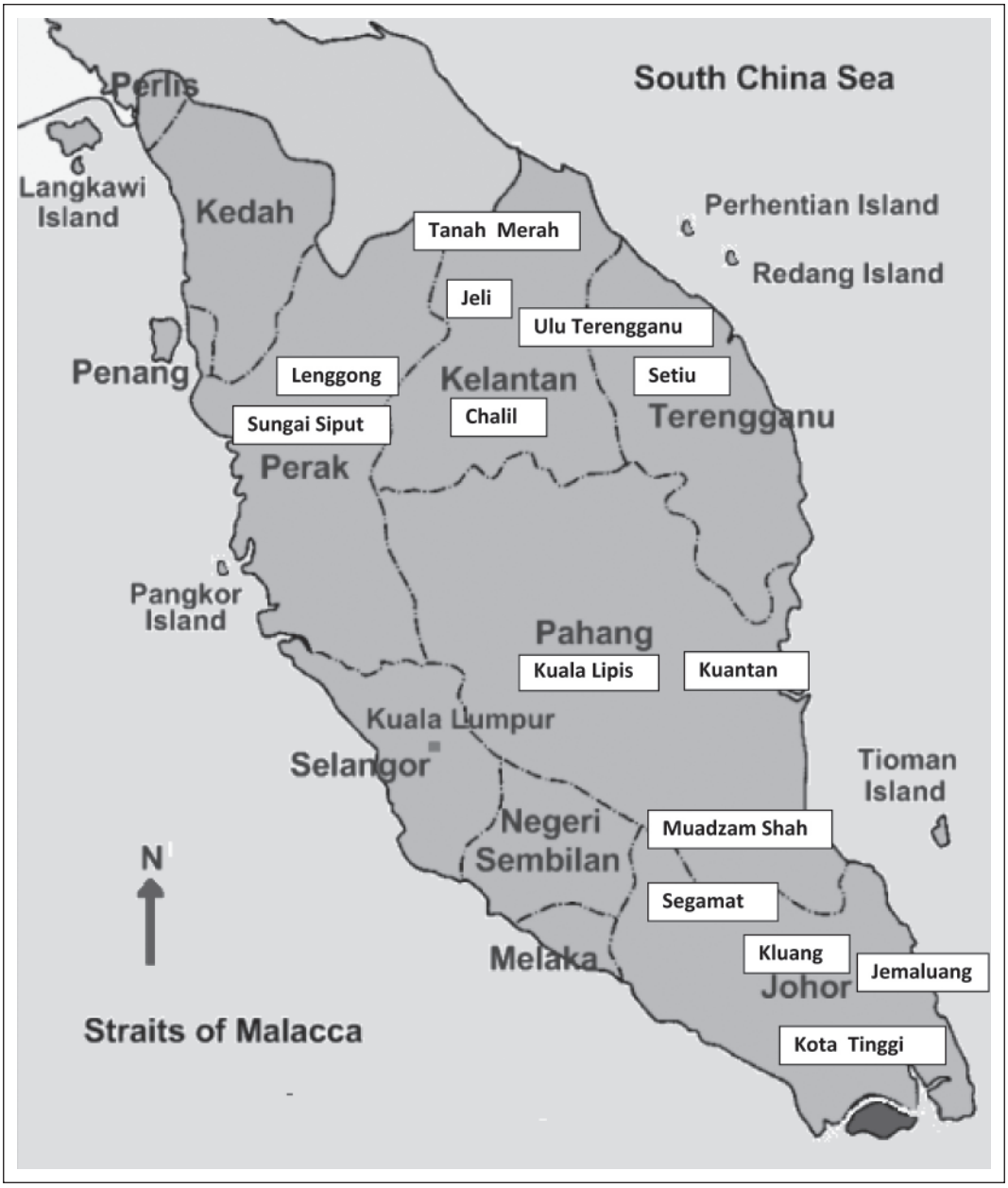

Fig. 1. Sampling location in Peninsular Malaysia.

was performed in 25 µl of a solution containing each dNTP at 0.4 mM, each primer at 0.4 µM, genomic DNA (10–100 ng), and 2.5 units of *Thermus aquaticus* polymerase (Promega). Reactions, which were carried out in a PTC-100™ Programmable Thermal Controller (MJ Research, Inc., USA), were preceded by a 3 min denaturation step at 95°C followed by 25 cycles of 1 min denaturation at 95°C, 45 s annealing at 56°C, and a 1 min extension at 72°C with a final extension at 72°C for 7 min. This cycle was repeated 25 times. PCR products were purified with the Wizard® SV gel purification kit and PCR clean-up system (Promega Corp., USA) following the procedure recommended by the manufacturer. Amplifications were electrophoresed on 1.5% agarose and stained with ethidium bromide; bands were visualized under ultraviolet (UV) and photos were taken with Alphaimager, Alpha Innotech.

Sequences alignments and genetic variation analyses

Sequences were aligned using MEGA software version 4.0. The program DnaSP5 (Librado & Rozas, 2009) was used to calculate the average number of nucleotide differences k, average number of nucleotide substitutions per site using the Jukes and Cantor correction Dxy(JC), nucleotide diversity, and estimates of population subdivision at the nucleotide level using NST. FST between

populations was also calculated using DnaSP5. A sequence that varied by one or more nucleotides was considered a different haplotype.

RESULTS

A variable part of the D-loop region was amplified and used as a marker for species determination. Sequences were obtained from all of the samples analyzed, except for four samples that did not yield amplification products. Most likely, this failure was due to the degradation of the samples during storage. Within the analyzed 544 bp region of the mitochondrial D-loop gene, 25 polymorphic sites defined 10 different haplotypes in the elephant population of Peninsular Malaysia (Figure 2) with a mean sequence of divergence between haplotypes of 0.010. The number of mutations was 25, of which 20% involved transversions and 12% involved indels. Analyzing the sample as one population gave the following result: haplotype diversity value, $\hat{H}$ = 0.830; variance of $\hat{H}$ = 0.007; standard deviation of $\hat{H}$ = 0.085; and nucleotide diversity, π (Jukes-Cantor) = 0.005. The value of the structuring populations (FST) was 0.025 and the level of gene flow (Nm) was 9.65 migrants per generation among population. The average number of nucleotide differences, k, was 2,982. D (Tajima) = -2.20901 ($P < 0.01$).

In this study, DnaSP5 analysis showed that Peninsular Malaysia has 10 haplotypes, namely Hap01 to Hap10 (Table 2), including BQ and BV individuals (Vidya *et al.,* 2009). There were 25 mutations, of which 20% involve transversions and 12% involve indels. No polymorphic positions greater than two different nucleotides were detected (C, T, and G). Hap01 had the highest relative frequency , followed by Hap06. Johor had the highest number of haplotypes (h=6), with haplotype diversity of 0.9524 (Table 3). Perak and Terengganu had the lowest numbers of haplotypes (h=1). Moreover, Johor and Kelantan had the highest genetic diversity (0.006) compared to other states. The low range of genetic diversity among the states (0.000–0.006) demonstrated low divergence among populations.

	Nucleotide's Positions																									
Haplotype	**0 6 1**	**0 8 8**	**0 8 9**	**0 9 5**	**0 9 7**	**1 4 3**	**1 5 6**	**2 1 9**	**2 2 3**	**2 4 6**	**4 0 7**	**4 1 9**	**4 4 5 7**	**4 4 6 0**	**4 6 6 7**	**5 4 1**	**5 4 3**	**5 6 9**	**5 7 0**	**5 7 1**	**5 7 3**	**5 7 4**	**5 7 5**	**5 8 8**	**5 9 0**	**Relative Frequency**
Hap01	T	A	A	C	-	G	G	G	C	T	C	C	G	C	G	T	-	C	C	-	T	A	A	G	G	0.420
Hap02	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	A	-	G	.	.	.	.	0.053
Hap03	.	.	.	.	-	.	.	.	.	.	.	.	.	.	A	.	-	.	.	-	.	.	.	.	.	0.053
Hap04	.	.	.	.	-	A	.	.	.	.	T	T	A	T	.	.	-	T	.	-	.	.	.	.	.	0.053
Hap05	A	.	.	T	-	.	A	.	.	.	.	.	.	.	.	.	-	.	.	-	.	.	.	A	.	0.103
Hap06	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	.	-	.	.	.	A	.	0.053
Hap07	.	.	.	.	-	.	.	.	A	C	.	.	.	.	.	.	-	.	.	-	.	.	.	.	.	0.053
Hap08	.	.	.	.	-	.	.	A	.	.	.	.	.	.	.	.	-	.	.	-	.	.	.	.	.	0.053
Hap09	.	G	G	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	0.053
Hap10	.	.	.	.	-	.	A	.	.	.	.	T	.	.	.	C	-	.	.	-	.	.	C	.	A	0.053
BV	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	.	-	.	C	.	.	.	0.053

Fig. 2. Polymorphic positions between Asian elephant haplotype sequences obtained in this study.

Table 2. Geographical distribution of the Asian elephant mtDNA haplotypes in Peninsular Malaysia

Haplotype	Localities					Total
	Kelantan	Perak	Johor	Pahang	Terengganu	
Hap01	1	2	2	2	0	7
Hap02	1	0	0	0	0	1
Hap03	1	0	0	0	0	1
Hap04	0	0	0	0	1	1
Hap05	0	0	0	1	0	1
Hap06	0	0	1	1	0	2
Hap07	0	0	1	0	0	1
Hap08	0	0	1	0	0	1
Hap09	0	0	1	0	0	1
Hap10	0	0	1	0	0	1

Table 3. Genetic diversity indices of Asian elephants in Peninsular Malaysia

	Johor (n=7)	Kelantan (n-3)	Pahang (n=4)	Perak (n=2)	Terengganu (n=1)
S	7	5	4	0	0
h	6	3	3	1	1
$\hat{H}$	0.9524	1.0000	0.8333	0.0000	0.0000
k	3.3810	3.3333	2.16667	0.0000	0.0000
πJC	0.0063	0.0062	0.0040	0.0000	0.0000

Abbreviations used: S = Polymorphic Site/Indel/Missing, k = Average number of Nucleotides Differentiate, h = Number of Haplotype, πJC = Nucleotides Diversity (Jukes-Cantor), $\hat{H}$ = Haplotype Diversity

Table 4. Genetic differentiation of the Asian elephant in Peninsular Malaysia

	Kelantan	Pahang	Johor	
Pahang	0.5829	(N_{ST})		
	0.5714	(F_{ST})		
	0.0054	(D_{XY})		
Johor	0.0078	0.0216	(N_{ST})	
	0.0070	0.0219	(F_{ST})	
	0.0062	0.0050	(D_{XY})	
Perak	0.0010	0.1334	0.0123	(N_{ST})
	0.0000	0.1333	0.0139	(F_{ST})
	0.0031	0.0023	0.0032	(D_{XY})

In terms of genetic differentiation between the populations, NST ranged from 0.0010 to 0.5829 (Table 4). Kelantan and Pahang had the highest NST value (0.5829), while the lowest value was found between Kelantan and Perak (0.0010). Thus, Kelantan exhibited a significant difference with Pahang compared to Perak. The range of population structure among the states based on the analysis of F-statistics (FST) was 0.0000 to 0.5714, with the highest FST value between Kelantan and Pahang. The average number of nucleotide substitutions per site (DXY) at Kelantan–Johor was the highest (0.0062), while that for Pahang–Perak was the lowest (0.0023; Table 4).

DISCUSSION

Analyzed as one population, low genetic diversity was observed in Peninsular Malaysia (<0.55%), with a total of 10 haplotypes. Genetic diversity for each state was also very low (<0.62%). Genetic divergence among populations was very low or nonexistent (Table 3). Thus, from the standpoint of evolution, elephants have established a population in Peninsular Malaysia. The low genetic diversity for the D-loop also showed a close similarity between the haplotypes. In comparison with the four haplotypes previously used by Vidya *et al.* (2009), namely BQ, BP, BU, and BV, seven individuals were found to correspond to the haplotype BQ (Table 2). However, no sequence resembled the other haplotypes in their study. The frequency of haplotypes ranged from up to 1 (for seven haplotypes that appeared only once) to 8 (for the most common haplotype). This implies that there was a shared haplotype among the populations.

Hap01 had the highest frequency, at 42.1% (n = 8), whilst Hap06 was 10.5% (n = 2; Table 1). Hap01 was shared by Kelantan, Johor, Pahang, Perak, and BQ, while Hap06 was shared by Pahang and Johor. This was supported by the low population structure, the F_{ST}, of Kelantan, Pahang, Johor, and Perak (Table 4). The average number of replacement nucleotides per site for the D-loop was low (<0.006). The rate of evolution of D-loop nucleotide substitution for the elephants was 3.5% per millions of years (Zhang, 2007). Hence, it is possible that the mutations observed in the four states were due to the history of different groups rather than geographical isolation. All individuals that shared the haplotype Hap01 had a close relationship with the distance of sequence divergence of 0.00%, except for BQ (0.33%). Most likely, the BQ individual did not come from Kelantan, Johor, Pahang, or Perak. Our study also found that the gene flow between the five states of Peninsular Malaysia was high (Nm = 9.65) and led to a low estimate of population structure of the state in the F-statistic analysis (FST = 0.025). This indicates that gene flow was occurring among the populations. This is contrary to the geographical position of the said states, which have been divided to the north, south, east, and west coasts; this should prevent gene flow. The geographical isolation of allopatric populations is the main reason for the prevention of gene flow between two populations; this permits the evolution of genomes adapted to local conditions (Hall, 1993). However, humans are also responsible for the high level of gene flow due to the process of translocation, as elephants have been used as transportation medium for trade and war (Sukumar, 2006).

From an ecological perspective, the effective migration barriers are rivers, roads, cities, savannahs, and other geographically isolated open areas (Bachmann *et al.,* 2000). However, our study showed that there are no restrictions on the biogeography of elephant population in Peninsular Malaysia. Significant differences in the frequency haplotype for each state may indicate a new compilation relating to the distribution of the elephants' descendants. This could be due to the genetic effects of habitat fragmentation and the range of place-limited stays (Fernando *et al.,* 2000). In general, mammals are highly mobile and always

move between habitats in search of food. Hence, it is difficult to hold them in only one habitat system (Lim, 2008).

Genetic information plays a direct and important role in conservation efforts (Masstor *et al.,* 2014). In the attempt to decrease the risk of extinction by reducing inbreeding and the loss of genetic diversity, the genetic diversity value (π) can be used to measure the health of the population and evolutionary potential. Species or population risks must be identified in the event of a reduction in genetic diversity. Our study showed that overall, Asian elephant populations have low genetic diversity, but with high gene flow. Trudeau *et al.,* (2004) found that gene flow through migration among colonies that are in the process of recovery can result in recovery of the genetic variability that was lost during the bottleneck. The D value (Tajima) is -2.00 ($P<0.05$). A negative Tajima D indicates the abundance of polymorphism frequencies as a sign of the development of low population size and/or positive selection of successful breeding. The results of this study do not conflict with the findings of the Wildlife Conservation Society (WCS) based in New York with the cooperation of the Department of Wildlife in 2006–2007. This study found that there were a total of 631 elephants in a national park with a size of 4.343 km^2. This large population showed that the elephant population has no extinction problem. They estimated the size of elephant populations in national parks using techniques to calculate the lump of elephant droppings. This technique has been adopted as a scientifically accurate method (Eggert *et al.,* 2003). However, DNA analysis on stool samples for the identification of gender was also able to show endangered animal sex ratios, that is, male and female animals inhabiting certain areas within the habitat (Bhagavatula & Singh, 2006).

Although the Asian elephants in Peninsular Malaysia are not facing extinction, the results of this study will provide the insight into various genetic angles related to Asian elephants. This will enable the parties involved to take preliminary action with a view to conservation management. The pattern of variation involves genetic records and demographic trends such as population size and migration. In fact, the sequence data may show the history of population and conservation-related information, such as the mating system (the interaction between elephants). Data can also be used to assess the genetic relationships between populations, such as gene flow, which is important for monitoring genetic diversity. All of this information will enable authorities to develop planning systems and engage in conservation management of wildlife in general and elephants in particular.

CONCLUSIONS

In conclusion, the relationships among Asian elephants in Peninsular Malaysia are quite close to each other in terms of both intrapopulation and interpopulation characteristics. There are 10 haplotypes in Peninsular Malaysia based on the D-loop found in this study. Four out of five states studied share the same haplotype (Hap01), namely Johor, Perak, Pahang, and Kelantan. On the other hand, Hap06 is only shared by the states of Pahang and Johor. Currently, wild elephants in Peninsular Malaysia are translocated to a safer place to ensure their safety from extinction. The results of this study may be used to evaluate the effectiveness of strategies in the translocation approach to manage Asian elephants in Peninsular Malaysia. Our study has shown that translocation will not result in negative genetic effects. This demonstrates that the translocation carried out by the government was able to save Asian elephants in Peninsular Malaysia from the threat of extinction without any inbreeding effects. A comprehensive study on a greater number of Asian elephants in Peninsular Malaysia and East Malaysia should be carried out using high polymorphic microsatellite markers to give a more accurate picture of the Asian elephant's population genetics.

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REFERENCES

Abdul-Latiff, M.A.B., Ruslin, F., Faiq, H., Hairul, M.H., Rovie-Ryan, J.J., Abdul-Patah, P., Yaakop, S. & Md-Zain, B.M. 2014. Continental monophyly and molecular divergence of Peninsular Malaysia's *Macaca fascicularis fascicularis. BioMed Research International,* **2014**: Article ID 897682.

Ang, A., Srivasthan, A., Md-Zain, B.M., Ismail, M.R.B. & Meier, R. 2012. Low genetic variability in the recovering urban banded leaf monkey population of Singapore. *Raffles Bulletin of Zoology,* **60(2)**: 589-594.

Bachmann, L., Rumpler, Y., Ganzhorn, J.U. & Tomiuk, J. 2000. Genetic differentiation among natural populations of *Lepilemur ruficaudatus. International Journal of Primatology* **21(5)**: 853-864.

Bhagavatula, J. & Singh, L. 2006. Genotyping faecal samples of Bengal tiger *Panthera tigris tigris* for population estimation: A pilot study. *BMC Genetics* **7**: 48.

Eggert, L.S., Eggert, J.A. & Woodruff, D.S. 2003. Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. *Molecular Ecology,* **12**: 1389-1402.

Fernando, P. 1998. Genetics, ecology, and conservation of the Asian Elephant. PhD Thesis, University of Oregon, Eugene, OR.

Fernando, P., Pfrender, M.E., Encalada, S.E. & Lande, R. 2000. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity,* **84**:362-372.

Fernando, P. & Lande, R. 2000. Molecular genetic and behavioural analysis of social organization in the Asian elephant (*Elephas maximus*). *Behavioral Ecology and Sociobiology,* **48(1)**: 84-91.

Fernando, P., Vidya, T.N.C., Payne, J., Stuewe, M., Davison, G., Alfred, R.J., Andau, P., Bosi, E., Kilbourn, A. & Melnick, D.J. 2003. DNA analysis indicates that Asian elephants are native to Borneo and are therefore a high priority for conservation. *PLoS Biology,* **1(1)**: 110-115.

Fickel, J., Lieckfeldt, D., Ratanakorn, P. & Pitra, C. 2007. Distribution of haplotypes and microsatellites alleles among Asian elephants (*Elephas maximus*) in Thailand. *European Journal of Wildlife Research,* **53(4)**: 298-303.

Fleischer, R.C., Perry, E.A., Muralidharan, K., Stevens, E.E. & Wemmer, C.M. 2001. Phylogeography of the Asian elephant (*Elephas maximus*) based on mitochondrial DNA. *Evolution,* **55(9)**: 1882-1892.

Hall, M. 1993. Species, speciation and extinction. In Skelton, P. (eds) *Evolution: A Biological and Palaeontological Approach*. Addion-Weley Publihing Company: United Kingdom. 370-444 pp.

Hartl, G.B., Kurt, F., Tiedeman, R., Gmeiner, C., Nadlinger, K., Mar, K.U. & Rubel, A. 1996. Population genetics and systematic of Asian elephants (*Elephas maximus*): A study based on sequence variation at the Cyt *b* gene of PCRamplified mitochondrial DNA from hair bulbs. *Zeitschrift fur Saugetierkde,* **61**: 285-294.

Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics,* **25(11)**: 1451-1452.

Lim, B.L. 2008. Critical habitats for the survival of Malayan mammals in Peninsular Malaysia. *Journal of Science and Technology in the Tropics,* **4**: 27-37.

Lim, L.S., Ang, K.C., Mahani, M.C., Shahrom, A.W. & Md-Zain, B.M. 2010. Mitochondrial DNA polymorphism and phylogenetic relationships of Proto Malays in Peninsular Malaysia. *Journal of Biological Sciences,* **10(2)**: 71-83.

Masstor, N.H., Samat, A., Shukor, M.N. & Md-Zain, B.M. 2014. Molecular phylogeny of the Bamboo sharks (*Chiloscyllium* spp.). *BioMed Research International,* **2014**: Article ID 213896.

Rosli, M.K.A., Syed-Shabthar, S.M.F., Abdul-Patah, P., Abdul-Samad, Z., Abdul, S.N., Burhanuddin, M.N., Zulkifli, N.A., Shukor, M.N., Budsabong, K., Changtragoon, S., Sekiguchi, T., Sasaki, H. & Md-Zain, B.M. (2014). A new subspecies identification and population study of the Asian small-clawed otter (*Aonyx cinereus*) in Malay Peninsula and Southern Thailand based on fecal DNA method. *The Scientific World Journal,* **2014**: Article ID 457350.

Stiles, D. 2004. The ivory trade and elephant conservation. *Environmental Conservation,* **31(4)**: 309-321.

Sukumar, R. 2006. A brief review of the status, distribution and biology of wild Asian elephants (*Elephas maximus*). *International Zoo Yearbook,* **40**: 1-8.

Trudeau, K.M., Britten, H.B. & Restani, M. 2004. Sylvatic plague reduces genetic variability in black-tailed prairie dogs. *Journal of Wildlife Diseases,* **40(2)**: 205-211.

Vidya, T.N.C., Fernando, P., Melnick, D.J. & Sukumar, R. 2005a. Population differentiation within and among Asian elephant (*Elephas maximus*) populations in southern India. *Heredity,* **94(1)**: 71-80.

Vidya, T.N.C., Fernando, P., Melnick, D.J. & Sukumar, R. 2005b. Population genetic structure and conservation of Asian elephants (*Elephas maximus*) across India. *Animal Conservation,* **8**: 377-388.

Vidya, T.N.C., Raman, S. & Melnick, D.J. 2009. Range-wide mtDNA phylogeography yields insights into the origins of Asian elephants. *Proceedings of the Royal Society Series B Biological Sciences,* **276(1658)**: 893-902.

Zhang, L. 2007. Current conservation status and research progress in Asian elephants in China. *Gajah,* **27**: 35-41.