GENETIC DIVERSITY OF WILD ELEPHANT (*Elephas maximus* Linnaeus, 1758) IN THE NORTHEASTERN PART OF THAILAND

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ABSTRACT

The phylogeography and genetic diversity of wild elephants in the northeastern part of Thailand was studied at Phuwua Wildlife Sanctuary (PW), Nong Khai Province; Dongyai Wildlife Sanctuary (DY), Buri Rum Province; Phuluang Wildlife Sanctuary (PL), Loei Province and Phukhieo Wildlife Sanctuary (PK), Chaiyaphum Province. The study was procedured from September 2009 through August 2010. The objectives were to investigate the levels of genetic diversity within populations of wild elephants in northeastern Thailand and also to study the evolutionary relationship among population clusters and with other areas within Thailand. PCR technique and base sequences from cytochrome b to the heading of control region in mitochondrial DNA were used. The results from 39, 57, 10, and 8 dung samples from PW, PK, PL and DY wildlife sanctuaries, respectively, showed 2 halplotypes (A and B) in PW, 2 haplotypes in PK (A and C), 2 haplotypes in PL (A and B), and only one haplotype in DY (A). Genetic analysis of the tip of cytochrome b to the base of the control region found variation at 27 sites of the 601 base pairs sampled which can be categorized into 3 haplotypes (AH, BQ, and NewB) for PW, 3 haplotypes (AH, NewA1, and BH) for PK, 3 haplotypes (AH, AB, and BO) for PL, and only 2 haplotypes (AH and AD) for DY. It can thus be concluded that there are 8 haplotypes of wild elephant genetic diversity and 2 population clusters, clade A and clade B. In Northeast Thailand, clade A was found more than clade B. The elephants in groups AB, AD, AH, and newA1 share more common traits than any other groups. Distinctively, elephants in group newB have a close relation with elephant group BO. AD is regarded as the ancestor of the other haplotypes.

Key words: genetic diversity, wild elephant, protected area, Northeastern part of Thailand

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INTRODUCTION

In Thailand elephant is the nation symptom species. They also play an important role in the ecosystem. Thasod (2007) reported the proboscidean fossil from Tha Chang, Nakornratchasima province, northeastern part of Thailand were the most abundant in Thailand, eight genera and 18 species were classified. DNP (2003) reported approximately 2,500 wild elaphants inhabit in 69 protected areas throughtout Thailand and also reported that about 600 wild elephants live in the 6 forest complex areas, 13 protected areas, of the northeastern part. Dejchaisri *et al.* (2009) studied genetic diversity of wild elephant in the southern, western, and northern parts of Thailand. The wild elephant genetic data were little known in the northeastern part althougth this region has unique habitat for wild elephant and was suggested to be the most important source of the species in Thailand, assumed from the most abundant of proboscidian fossil. The results of this study and also the result from Dejchaisri *et al.* (2009), Fernando *et al.* (2002, 2003) will provide more understand genetic diversity of the species in their distribution range. This study focus on the variation in the mitrocondrial DNA in the northeastern part of Thailand wild elephants using sequences of the control regions of the cytochrome b gene of mitochondrial DNA (Lertrit, 2006; Brown *et al.* 1979).

MATERIAL AND METHODS

Sample of elephants'dung

Elephant dung, defecated within 24-48 hours, is the preferred material for considering genetic hereditary characteristics (Sripiboon *et al.*, 2010). We therefore used fresh dung samples as the source of DNA. In total, 114 samples were obtained from free-ranging elephants. The researcher collected dung to cover most populations as much as possible as follow from salt earth and from areas where elephants seek food. The researcher collected 39 samples in Phuwua Wildlife Sanctuary, 57 samples in

Phukhieo Wildlife Sanctuary, 10 samples in Phuluang Wildlife Sanctuary, and 8 samples in Dongyai Wildlife Sanctuary.

Samples were collected from the outer most layer of dung by a clean and new fork was used to scrape off the outer skin of the dung, which normally contains mitochondrial DNA. Samples were placed in a 50 cc test tube containing 15 cc DETs buffer liquid reagent (Frantzen *et al.*, 1998). Test tubes were marked with code, date, and location. The largest three dungs were measured, and their geographical coordinates were recorded. Distances between dungs had to be more than 1 meter approximatlly to avoid collecting duplicate dungs. Dungs were then stored in a refrigerator at 4°C until DNA extraction.

DNA extraction

DNA extraction was processed by using a DNA extraction kit (Nucleospin® Plant II MACHEREY- NAGAL GmbH & Co. KG, Germany) similar to the process done by Dejchaisri *et al* . (2009). The extracted DNA was kept at a temperature of -20°C to observe the increase of DNA.

The increase of DNA by polymerase chain reaction (PCR) method

Two pairs of primers were used in this study. In the first pair, DNA increased in cytochrome b (*cyt b*) 450 bp annealing 53° C [adjusted from Kocher *et al*. (1989)] CB0 5'-CAT GAC TAA TGA TAT GAA AAA CC-3' and CB2 5' - CTC AGA ATG ATA TTT GTC CTC A-3'. In the second pair, DNA increased from the tip of *cyt b* until the basis of control region 630 bp annealing 63°C (Fernando *et al.*, 2000, 2003) MDL5fw 5′-TTA CAT GAA TTG GCA GCC AAC CAG-3′ and MDL3re 5′ CCC ACA ATT AAT GGG CCC GGA GCC-3′. Reaction steps consisted of predenature at 95°C for 5 minutes followed by increasing DNA for 40 cycles, denature at 94°C for 30 seconds, annealing at 63°C for 30 seconds (temperature used depended on primers), extension at 72°C for 30 seconds, and final extension at 72°C for 5 minutes. The consequence of PCR products was tested by 1.5% agarose gel electrophoresis using a constant electrical voltage of 100 volts for 30 minutes (Chokanakul, 2009).

DNA sequencing

The output PCR product was tested to search for base sequencing by using automated base sequencing series A-373 automated sequencer (Applied Biosystems Inc., Foster City, CA), and using the same primer as PCR reaction from First Base Laboratories SDN BH, Malaysia. Subsequently, the DNA sequence was compared with the genetic database from National Center for Biotechnology Information (NCBI) or GenBank.

Genetic diversity

Different DNA sequences (base sequences for cytochrome b are about 450 bp and 630 bp for the control region) were also analyzed by a program called BioEdit Sequence Alignment Editor (Hall,1999) to observe differences, catalog the dungs, and create a phylogenetic tree to compare base sequences of mammoth and African elephants as reported by GenBank (http://www.ncbi.nlm.nih.gov/), using sea cows as an outgroup animal processed by MEGA 4.1 program using neighbor-joining (NJ) and figuring the reliability of the phylogenetic tree by bootstrap 1000 times. The next step was analyzing nucleotides using DNAsp5.10 program to find haplotype diversity (h)(Nei, 1987)andnucleotide diversity(π) (Tajima, 1989), then building a genetic network to evaluate relationships among the samples and differences in the nucleotides of the haplotypes using program TCS1.21 (Clement *et al.*, 2000). The results were set in motion to consider the genetic differences and to segregate genetic diversity and evolutionary relativity in each area.

RESULTS AND DISCUSSION

Wild elephant genetic diversity (the basis of cytochrome b)

Base sequences of elephants in Phuwua Wildlife Sanctuary from the basis of cytochrome b were compared with GenBank records. These indicated that haplotypeA (n=18) and haplotypeB (n=21) were same as the research done by Lertwatcharasarakul *et al.* (2003) as shown in Table 1. Similarly, Maikeaw (2007) studied the blood data from 11 elephants. Maikeaw (2007) study mentioned 3

haplotypes that were put in the categories (haplotypeA, haplotypeB, and haplotypeC) in which haplotypeA and haplotypeB were mostly found. Also, Lertwatcharasarakul *et al.* (2003) reported that haplotypeA and haplotypeB were more common in the Thai elephant population than in any other elephant population.

This study found that wild elephant genetic diversity in Phukieo Wildlife Sanctuary have 2 haplotypes which are haplotypeA (n=49) and haplotypeC (n=8). This study also found that they have 2 haplotypes in Phuluang Wildlife Sanctuary which are haplotypeA (n=6) and haplotypeB (n=4). There is also found 1 haplotype in Dongyai Wildlife Sanctuary which is haplotypeA (n=8) (Table 1). A total of 114 dung samples collected from 4 different locations yielded 81 haplotypeA samples, 25 haplotypeB samples, and 8 haplotypeC samples. However they might have many haplotypes in the study areas that should be futher investigation. Nevertheless, the present results match the findings of Lertwatcharasarakul *et al.* (2003) that haplotypeA and haplotypeB are more common in Thai elephants than in any other elephants. This shows a close relative relation in both wild and domestic elephants, which agrees with Maikeaw (2007) study of blood data from 11 elephants. Umaporn's study found three haplotypes, with haplotypeA and haplotypeB being much more common than haplotype C. In our study, less haplotype C was found from Phukhieo Wildlife Sanctuary (n=8).

Wild elephant genetic diversity considered from the tip of cytochrome b to the basis of the control region

Samples from Phuwua Wildlife Sanctuary were analyzed from the tip of cytochrome bto the base of the control region, and compared with GenBank records from Fernando *et al.* (2000, 2003). This study found 3 haplotypes. HaplotypeAH (n=18) and haplotype BQ (n=1) are the same as those recorded by Fernando *et al.* (2000, 2003) while haplotypeNewB (n=20) is a new haplotype found in Phu Wua Wildlife Sanctuary with no previous record in GenBank. These findings are shown in Table 1.

Table 1. Haplotypes and samples of wild elephants from Phuwua, PhuKhieo, Phuluang and Dongyai Wildlife Sanctuaries

Haplotype		Wildlife Sanctuary								
Cyt-B	D-loop	Phuwua		Phukhieo		Phuluang		Dongyai		Total
		(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	
A	AH	18	46.15	44	77.19	5	50	7	87.5	74
A	AB	0	0	4	7.02	1	10	0	0	5
A	AD	0	0	0	0	0	0	1	12.5	1
A	NewA1	0	0	1	1.75	0	0	0	0	1
B	BQ	1	2.56	0	0	0	0	0	0	1
B	BO	0	0	0	0	4	40	0	0	4
C	BH	0	0	8	14.04	0	0	0	0	8
B	NewB	20	51.28	0	0	0	0	0	0	20
	Total (n)	39	100	57	100	10	100	8	100	114

The study of the Asian elephant habitat by Vidya *et al.* (2009) reported that in Laos,haplotypeAB (n=2), haplotypeAD (n=4), haplotypeAE (n=7), and haplotypeBQ (n=1) were found in 14 samples. This study found 1 matching sample out of 39 samples from Phuwua Wildlife Sanctuary. So, it can be possibly said that those wild elephants derived maternal inheritance from Laos because some elephants from Phuwua Wildlife Sanctuary were moved from Thailand to Laos and from Laos to Thailand several times in the past. However, haplotypeBQ is not more common because of natural disasters, epidemics, secluded areas, or human invasions reducing the numbers of surviving elephants. Also, insufficient samples were collected in both Phu Wua Wildlife Sanctuary and Laos.

HaplotypeNew B is a new haplotype which does not appear in GenBank records and which has been found only in Phuwua Wildlife Sanctuary. It can be inferred that those elephants in Phuwua Wildlife Sanctuary inherited the haplotype from nearby areas such as Laos. Some elephants from

Phuwua Wildlife Sanctuary were moved between Thailand and Laos several times over the past few decades. Furthermore, these elephants were never moved to other areas in Thailand, which is why they were not also influenced by other genes from other areas.

Three haplotypes were found in Phukieo Wildlife Sanctuary: haplotypeAH (n=44), haplotypeNewA1 (n=1), and haplotypeBH (n=8). Three haplotypes were found in Phuluang Wildlife Sanctuary: haplotype AH (n=5), haplotype AB (n=1), and haplotype BO (n=4). In Dongyai Wildlife Sanctuary, two haplotypes were found: haplotype AH (n=7) andhaplotype AD (n=1). Table 1 shows the diversity from a total of 114 dung samples from 4 different areas. Eight haplotypes were found: 74 samples of haplotypeAH, 5 samples of haplotypeAB, 1 sample of haplotype AD, 1 sample of haplotype NewA1, 1 sample of haplotype BQ, 4 samples of haplotypeBO, 8 samples of haplotype BH, and 20 samples of haplotypeNewB. The test of cytochrome b to the basis of control region can be categorized into 3 haplotypes which have more genetic diversity. This finding agrees with report of Brown *et al.* (1979) which stated that the control region has a higher rate of mitochondrial DNA mutation than other areas.

HaplotypeAH was found in 4 different areas (n=74), which indicates that elephants from Phuwua Wildlife Sanctuary have a genetic exchange which has been influenced by nearby areas for a long time

Genetic relationship

A total of 114 samples were analyzed to locate haplotype diversity (h)and nucleotide diversity (π)from base sequences. As shown in Table 2, haplotype diversity (h)of elephants in Phuluang Wildlife Sanctuary is high, at h=0.644. In Phuwua Wildlife Sanctuary the rate is h=0.537.

Table2.Haplotype frequency and genetic diversity range of elephant excrement from research location

Wildlife Sanctuaries	Haplotype frequency								Sample size	Nucleotide diversity(π)	Haplotype diversity(h)
	AH	AB	AD	NewA1	BQ	BH	BO	NewB			
Phuwua	0.462				0.036			0.512	39	0.0181	0.537
Phukhieo	0.772	0.070		0.018		0.140			57	0.0083	0.386
Phuluang	0.500	0.100					0.400		10	0.0158	0.644
Dongyai	0.875		0.125						8	0.0004	0.250

These two areas have 3 haplotypes, because the frequency distance of each haplotype has led to a lower rate of haplotype diversity. Although 4 haplotypes were found in Phukhieo Wildlife Sanctuary, the rate of haplotype diversity (h=0.386) is less than that of Phuwua Wildlife Sanctuary because of different haplotype frequencies. Also, the haplotype diversityfrom the elephant samples in Phuwua Wildlife Sanctuary is h=0.250.

Phuwua Wildlife Sanctuary has a higher nucleotide diversity(π=0.0181) than Phuluang Wildlife Sanctuary (π=0.0158). It can be said that the elephants in Phuwua Wildlife Sanctuary have greater genetic diversity in their base sequence because of gene transfer from nearby areas. Meanwhile, this area is small (only 186 km^2), which does not match with the elephant population at present.

Using the base sequence (tip of cytochrome b to the base of the control region) to study the diversity shows a relationship of genetic diversity on the phylogenetic tree by the neighbor-joining (NJ) process. The reliability of the phylogenetic tree was determined by bootstrap 1,000 times. It was used to consider the relationship of gene evaluation by comparing the base sequences of Asian elephants (examined from dungs), mammoths (accession number DQ188829), and African elephants (accession number NC_000934) reported from GenBank using sea cows (accession numbers AJ421723and AY075116) as an outgroup by MEGA version 4.1program (Fig. 2).

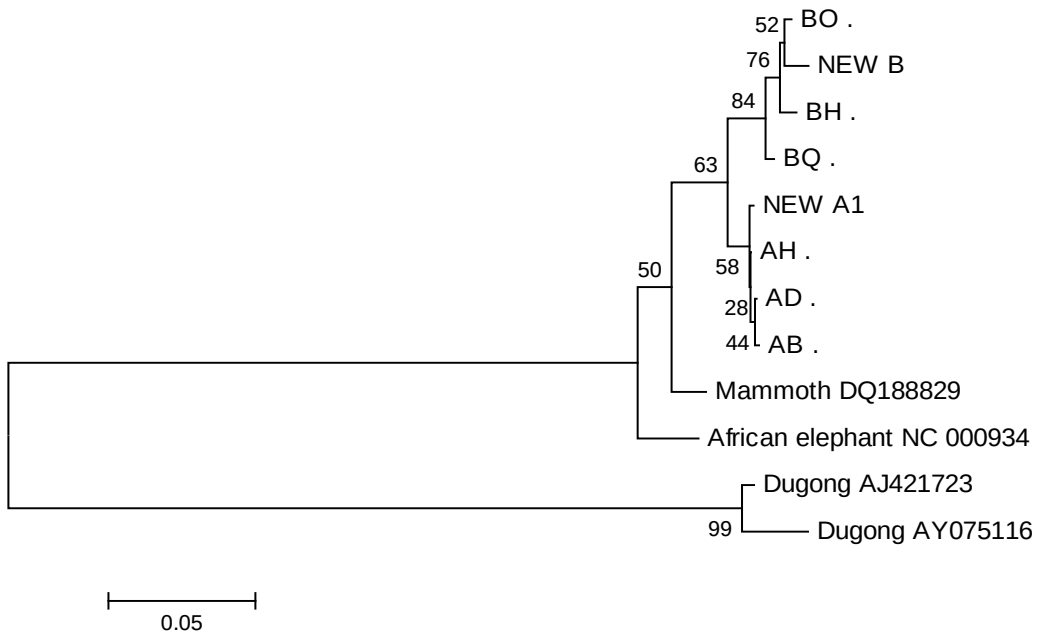

Figure 2. Phytogenetic tree of base sequence from the tip of cytochrome b to the base of the control region (601 bp) compared with the base sequence of the mammoth (accession number DQ 188829) and the African elephant (accession number NC_000934) reported by GenBank using sea cows (accession numbers AJ421723 and AY075116) as an outgroup animal.

From the test, it was found that the Thai elephant which is regarded as an Asian elephant (haplotypeAH, haplotypeAB, haplotypeAD,haplotypeNewA1, haplotypeBQ, haplotypeBH, haplotypeBO, and haplotypeNewB) has a closer relationship to the mammoth than to the African elephant, which agrees with Maikeaw (2007), Yang *et al.* (1996), and Krause *et al.* (2006). It can be inferred from the phylogenetic tree that the haplotypeNewB which was found in Phuwua Wildlife Sanctary evolved from haplotypeBH (found in Phukhieo Wildlife Sanctaury) and shared the same evolution as haplotypeBO (from Phuluang Wildlife Sanctuary). Considering the similarities of the haplotypes, it was found that haplotype NewA1 from Phukhieo Wildlife Sanctuary agreeing with the report of Dejhaisri *et al.* (2009) is similar to haplotype AH which is 0.998. Also, haplotypeNewB is similar to haplotypeBO which is 0.99 (Table 3).

Table 3. Sequence identity matrix analyzed from the tip of cytochrome b until the basis of control region (601 bp) of each haplotype from Phuwua, Phukhieo, Phuluang and Dongyai Wildlife Sanctuaries.

	AH	AD	AB	NewA1	BQ	BO	BH	NewB
AH	ID	0.998	0.996	0.998	0.976	0.971	0.968	0.965
AD		ID	0.998	0.996	0.975	0.97	0.966	0.963
AB			ID	0.995	0.973	0.968	0.965	0.965
NewA1				ID	0.975	0.97	0.966	0.963
BQ					ID	0.986	0.99	0.98
BO						ID	0.993	0.99
BH							ID	0.983
NewB								ID

Phuwua Wildlife Sanctuary showed haplotypeAH, haplotypeBQ, and haplotypeNewB. Phukhieo Wildlife Sanctuary showed haplotypeAH, haplotypeBH, and haplotypeNewA1. Phuluang Wildlife Sanctuary showed haplotypeAH, haplotypeAB, and haplotypeBO. Dongyai Wildlife Sanctuary showed haplotypeAH and haplotypeAD. Elephants can be divided into 2 groups using the results from both parts (Table 4). Haplotype A, haplotype AH, haplotypeAB, haplotypeAD, and haplotypeNewA1 are related to clade A haplotype B, haplotype C, haplotypeBQ, haplotypeBH, haplotypeBO, and haplotype NewB match clade B (Fig. 3).

Table 4. Numerical position of elephant excrement's nucleotide sequence from 4 different areas from the tip of cytochrome b to the base of the control region (601 bp) and cytochrome b (250 bp), compared with the nucleotide sequence of Asian elephants reported by GenBank (accession number NC_005129)

Haplotype	Numerical position of nucleotide sequence																											No.	CB0-CB2
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5		
	1	1	1	2	3	3	3	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7		
	8	9	9	5	2	3	9	2	1	6	7	7	7	8	8	9	9	0	1	1	2	3	8	8	0	1	4		
	0	0	7	1	0	3	6	7	8	5	2	3	4	4	5	5	6	4	2	8	2	4	2	7	9	8	5		
AH	C	T	C	C	G	A	A	C	T	T	T	A	G	C	A	T	G	C	T	C	T	G	A	G	T	T	T	74	A
AB	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	5	A
AD	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	A
NewA1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	A
BQ	T	C	.	T	.	G	G	T	.	C	.	.	A	.	.	C	C	T	.	T	.	.	.	.	C	.	C	1	B
BH	T	C	T	T	.	G	G	T	.	C	.	.	A	T	G	C	T	T	.	T	C	A	.	.	C	.	C	8	C
BO	T	C	.	T	.	G	G	T	.	C	.	.	.	T	G	C	T	T	.	T	C	A	.	A	C	.	.	4	B
NewB	T	C	.	T	A	G	G	T	.	C	C	.	.	T	.	C	C	T	C	T	C	A	G	A	C	C	.	20	B

(.) means the same sequence as upper sequence.

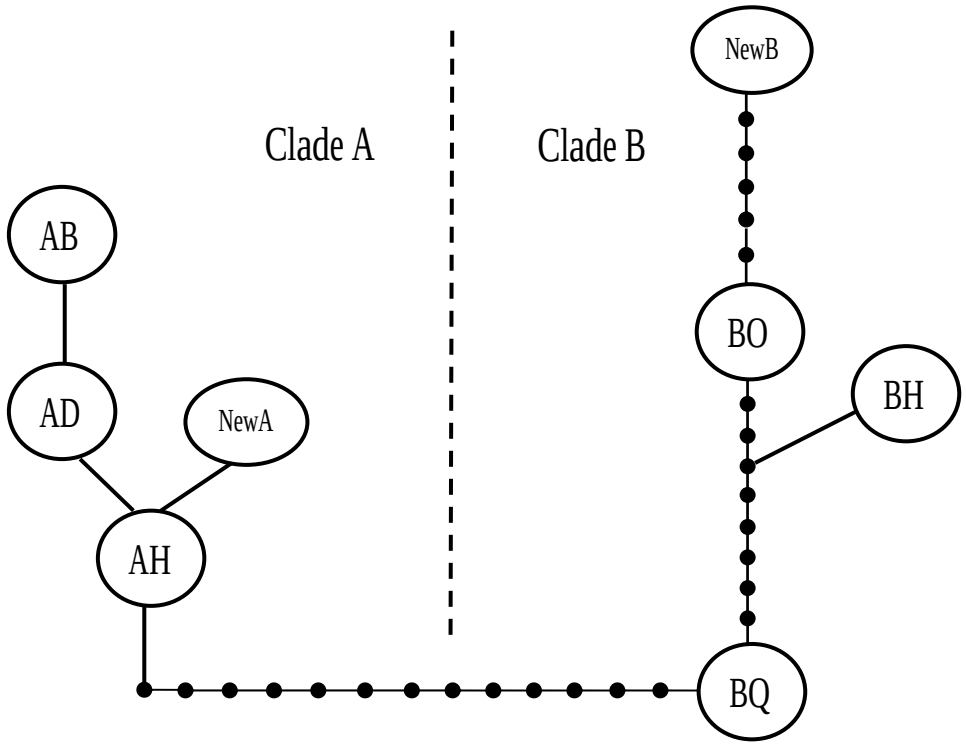

Fig.3 Data network of haplotypes from elephant excrement in 4 locations specified from the tip of cytochrome b to the base of the control region. Phuwua Wildlife Sanctuary: haplotypeAH, haplotypeBQ, and haplotypeNewB. Phukhieo Wildlife Sanctuary: haplotypeAH, haplotypeBH, and NewA1. Phuluang Wildlife Sanctuary: haplotypeAH, haplotypeAB, and haplotypeBO. Dongyai Wildlife Sanctuary: haplotypeAH and haplotypeAD

The eight elephant haplotypes were created as a network (Fig. 3). The network shows that groups haplotypeAB, haplotypeAD, haplotypeAH, and haplotypeNewA1 have a closer relationship than other groups. Additionally, elephants in the haplotypeNewB group share a similarity with the haplotypeBO group with a different base sequence for 5 levels. HaplotypeBO can be found only in Phuluang Wildlife Sanctuary, so it can be said that haplotypeNewB elephants have moved from Phuluang Wildlife Sanctuary to Phuwua Wildlife Sanctuary. Maps of the area show that the mountain lines of these two sanctuaries are connected. Thus, it can be assumed that haplotypeAD was the ancestor of the other haplotypes.

From the study of the 4 locations above, it can be concluded that clade B was found more than clade A in Phuwua Wildlife Sanctuary. However, clade A was found more than clade B in Phukhieo and Phuluang Wildlife Sanctuaries. Only clade A was found in Dongyai Wildlife Sanctuary (Fig.4). This study matches up with Fleischer *et al.* (2001) & Fernando *et al.* (2003), who reported that clade A and clade B were found in northeast Thailand and most of clade A was found in the eastern part of Thailand up to Cambodia.

This study shows that, from all 4 locations, both clade A and clade B were found in Phuwua, Phukhieo, and Phuluang Wildlife Sanctuaries, but only clade A was found in Dongyai Wildlife Sanctuary in Buri Ram province which is near the Cambodia border, so it is possible for clade A to be especially found in this area. Clade B was mostly found in the Southern part of Thailand (Dejchaisri *et al.*, 2009).

Dejchaisri *et al.* (2009) mentioned that Kangkrachan National Park and Tai Rom Yen National Park, a part of southern part of Thailand, have more clade B than clade A, and that only clade B was found in Malaysia and Indonesia (Fleischer *et al.*, 2001; Fernando *et al.*, 2003).

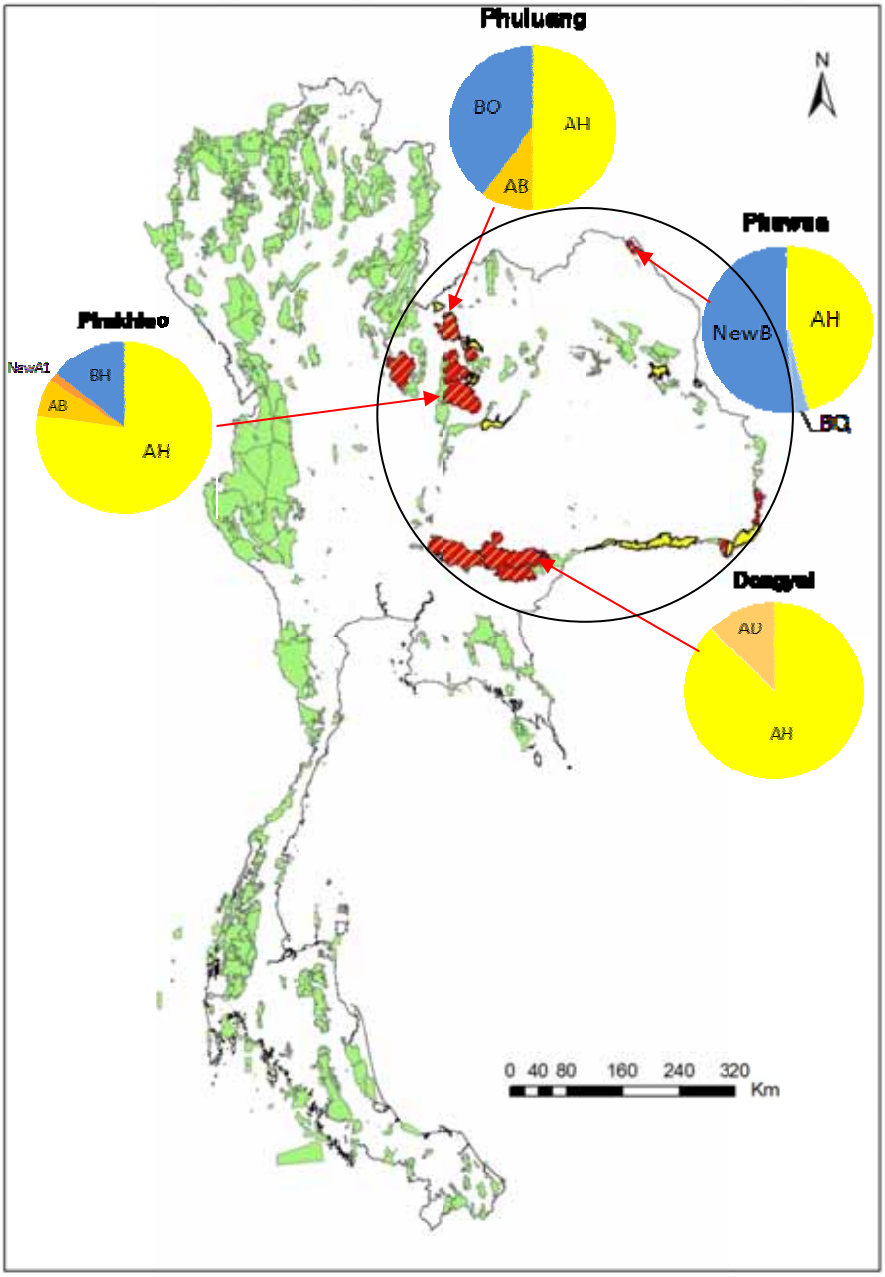

Fig. 4 Study areas Phuwua, Phuluang, Dongyai and Phukhieo Wildlife Sanctuaries, the red area mean protected areas has wild elephants' sign in the northeastern part, the yellow area mean protected areas has no wild elephants' sign in the northeastern part and the green area mean protected areas. Each clade of elephant dung for each area (4 areas). Clade A is indicated by yellow, pale orange, and light orange. Clade B is indicated by dark blue and blue.

CONCLUSION

Base sequences (haplotypes) were determined for 114 samples of elephant dung from 4 different locations. Phuwua Wildlife Sanctuary yielded 39 samples, Phukhieo Wildlife Sanctuary yielded 57 samples, Phuluang yielded 10 samples, and Dongyai yielded 8 samples. Using first primer CBOfw-CB2re to consider Cytochrome b found that elephants in Phuwua and Phuluang Wildlife Sanctuary yielded 2 haplotypes (A and B), elephants in Phukhieo Wildlife Sanctuary yielded 2 haplotypes (A and C), and all elephants in Dongyai Wildlife Sanctuary yielded the A haplotype.

Second primer MDL5fw-MDL3re examination from the tip of cytochrome b to the basis of the control region found the differences of 27 base 3 haplotypes (AH, BQ, and NewB) from elephants living in Phuwua Wildlife Sanctuary, 3 haplotypes (AH, NewA1, and BH) from Phukhieo Wildlife Sanctuary, 3 haplotypes (AH, AB, and BO) from Phuluang Wildlife Sanctuary, and only 2 haplotypes from Dongyai Wildlife Sanctuary (AH and AD).

Haplotype diversity (h)of elephants inPhuwua Wildlife Sanctuaryis 0.537, which is less than Phuluang Wildlife Sanctuary (h=0.644) but more than Phukhieo Wildlife Sanctuary (h =0.386) and Dongyai Wildlife Sanctuary (h =0.25).

It can be concluded that wild elephant genetic diversity consists of 2 population clusters, which are clade A and clade B.

RECOMMENDATIONS

1. This study conveyed some samples in particular areas. Additional studies should be done order to produce additional supporting data.

2. The study of genetic diversity of wild elephants should be conducted in extreme detail and the results should be used to produce a database that preserves this information about the forest.

3. Knowledge about the management and movement of wild animals should be developed practically to improve genes and avoid excessive elephant population.

4. Building up the connecting area between Phuwua Wildlife Sanctuary and the nearby area in Laos should be considered to maintain elephant genetic diversity.

5. Phuluang Wildlife Sanctuary should be developed as a designated long-term conservation area for wild elephants in the northeastern part of Thailand. A wildlife corridor

should be built to connect nearby protected areas such as Phukhieo Wildlife Sanctuary, Numnoa, and Phukradueng National Parks.

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