

High diversity of mitochondrial DNA of the Thai swamp buffalo indicates a domestication in Southeast Asia

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Summary

Sequencing of the mitochondrial cytochrome *b* gene and control regions from swamp buffaloes sampled in different regions in Thailand reveals a considerable diversity. From the 31 control region haplotypes, only two were shared with previously described samples of Chinese swamp buffaloes. The relative occurrence of the A and B haplogroups in Thailand is about the same as in south China, suggesting that these haplogroups do not represent separate domestication events. Mismatch distributions, median networks and values for haplotype diversity indicate that maternal ancestry is more complex in Thailand than in China. The present data are consistent with a scenario in which domestication took place in Indochina and domestic animals migrated into China along the coast.

Introduction

The water buffalo (*Bubalus bubalis*) has been divided into two distinct subspecies, the swamp type (*Bubalus bubalis carabensis*; 2n=48), and the river type (*Bubalus bubalis bubalis*; 2n=50). Both types have been evolved from ancestral buffalo-like animals approximately 1 million year ago (Kikkawa et al., 2003) and are supposed to have been domesticated separately in the Indus valley and China, respectively (Cockrill, 1981; Kumar et al., 1994; Lau et al., 1998; Lei et al., 2007; Yindee et al., 2010). A clear separation of the river and swamp mtDNA sequences was confirmed by a divergence of protein encoding genes (Barker et al., 1997), autosomal microsatellites (Zhang et al., 2007) and, more recently, Y-chromosomal sequences (Yindee et al., 2010). In the Chinese swamp buffalo population, two mtDNA haplogroups A and B were observed. These were estimated to have diverged about 18000 yr ago, but do not have distinct geographical ranges. Lineage A has undergone a population expansion and is predominant north of the Yangtze river. The largest haplotype diversity was found near the coast south of the Yangtze river.

The data available so far (Groeneveld et al., 2010) do not allow a comparison of the mtDNA diversity patterns of different countries. This precludes a more definite localization of the site of domestication. Here we report the mtDNA sequences of Thai swamp buffaloes from the northern, northeastern, middle and southern regions. Our data reveals a complex haplotype pattern in Thailand with a diversity higher than the found region in China (Lei et al., 2007). This is most consistent with a domestication of swamp buffalo in Southeast Asia.

Materials and methods

Sample collection and DNA preparation

Sixty-four blood samples were obtained from domestic buffaloes throughout Thailand (Table 1) and extracted following phenol-chloroform extraction and ethanol precipitation standard procedures.

Table 1: Origin and number of Thai swamp buffalo blood samples.

Origin	Number of samples	
	cytochrome <i>b</i>	coding region
<i>Northern part of Thailand</i>		
- Chiang Rai (CR)	2	2
- Lampang (LP)	9	10
- Phitsanulok (PS)	1	1
<i>North-Eastern part of Thailand</i>		
- Buri Ram (BR)	10	9
- Surin (SR)	5	5
- DLD ¹ (DL)	6	6
<i>Middle part of Thailand</i>		
- Nakhon Pathom (NP)	4	4
- Sa Kaew (SK)	11	12
- Chon Buri (CB)	1	1
- Karnchanaburi ¹ (KC)	7	7
<i>Southern part of Thailand</i>		
- Phang Nga (PN)	2	2
- Prachuap Khiri Khan (PK)	3	3
- Surat Thani (ST)	1	1
	62	63

¹ From buffalo bulls of Department of Livestock Development, Surin province, Thailand.

PCR and DNA sequencing

Primers used for PCR amplification and sequencing of the mtDNA *cytb* gene and control region were designed on the basis of the complete water buffalo mtDNA sequence 2, Genbank AY702618 entry.

Table 2: Oligonucleotide primers for amplification and sequencing of segments of the mtDNA cytochrome *b* and control region regions. Numbering is based on the Genbank entry NC_006295.

Target	Position	Primers
Forward-1	14478-14500	5'-CATGACTAATGATATGAAAAACC-3'
Reverse-1	15532-15554	5'-GTTGKCCTCCRATTCATGTRAG-3'
Forward-2	15355-15377	5'-TTYGCATACGCAATCYTACGATC-3'
Reverse-2	16285-16305	5'-CCTGAAGAAAGAACCAGATGC-3'
Forward-3	16178-16202	5'-GCTTAACCACCATGCCGCGTGAA-3'
Reverse-3	418-399	5'-AGGCATTTTCAGTGCCTTGC-3'

Data analysis

Sequences were aligned and checked using the Seqman program from DNASTar 5.0 package. Insertion/deletions in aligned sequences were excluded in the analyses. Haplotype diversity for the control region segment 15792-363 in Thailand (this study) and China (Lei et al., 2007) was calculated using the Arlequin 3.1 program (Excoffier et al., 2005). Reduced median networks and the pairwise mismatch distributions (cytochrome *b* segment 14530-15690, 1160 bp, and control regions segment 15811-16721, 910 bp) were constructed via the program Network 4.5.1.6 (Bandelt et al., 1999).

Results and discussion

We collected 62 cytochrome *b* sequences and 63 control region sequences (Tables 1, 3) using primers indicated in Table 2.

Table 3: Haplotypes from cytochrome b segment and control region segment of Thai swamp buffaloes. The first letter of the haplotype code indicates the haplogroup A or B. Most control region haplotypes were observed only once. Haplotypes AH1 to AH4 are shared by Chinese swamp buffaloes (Excoffier et al., 2005).

Cytochrome <i>b</i> haplotype	Control region haplotype	Number	Region
ACT1	AH1	16	DL(2x), BR(3x), PS, ST, LP(4x), SK(5x)
ACT1	AH2	1	NP
ACT1	AH3	2	BR, CR
ACT1	AT1	2	SR, KC
ACT1	AT3	1	NP
ACT1	AT4	1	SK
ACT1	AT6	1	BR
ACT1	AT8	1	CB
ACT1	AT9	1	NP
ACT1	AT15	1	DL
ACT1	AT17	1	SK
ACT1	AT18	1	SK
ACT1	AT19	1	SK
ACT1	No data	1	BR
ACT2	AH1	1	BR
ACT3	AH4	1	SR
ACT3	AT2	1	LP
ACT3	AT7	1	DL
ACT3	AT10	1	KC
ACT3	AT11	4	DL(2x), NP, KC
ACT3	AT13	2	SK, KC
ACT3	AT14	1	LP
ACT3	AT16	1	KC
ACT4	BT1	1	SK
ACT5	BT11	1	PK
ACT6	BT10	1	PK
ACT6	BT11	1	BR
ACT7	AT12	1	LP
ACT8	AH4	2	PN(2x)
ACT9	AH1	1	KC
ACT9	AT5	1	LP
BCT1	BT3	1	BR
BCT2	BT5	2	BR, KC
BCT2	BT6	1	PK
BCT3	BT7	1	SK
BCT3	BT8	1	SR
BCT3	BT9	1	CR
BCT4	BT4	1	SR
BCT5	BT2	1	LP
No data	BT5	1	SK
No data	IT1	1	LP

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The alignment (Table 4) shows that haplotypes are divided in two haplogroups. These correspond to the A and B lineages defined by Lei et al. (2007) and are represented by 19 and 11 control region haplotypes and 8 and 6 cytochrome *b* haplotypes, respectively. Only four control region haplotypes (AH1-AH4) were also found in the 119 Chinese swamp buffaloes (Lei et al., 2007). The ratio of A and B

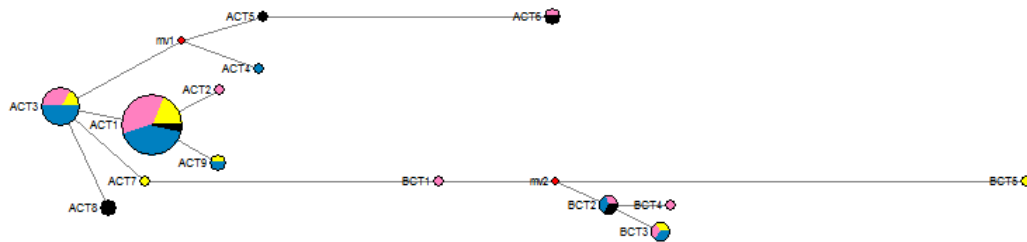
a diversity across Thailand that is higher than that calculated previously for Chinese populations (Lei et al., 2007).

Table 5: Source and genetic diversity index of Thai swamp buffaloes compared with Chinese swamp buffaloes (Lei et al., 2007): *n*, number of animals; *k*, number of haplotypes; *h*, haplotype diversity.

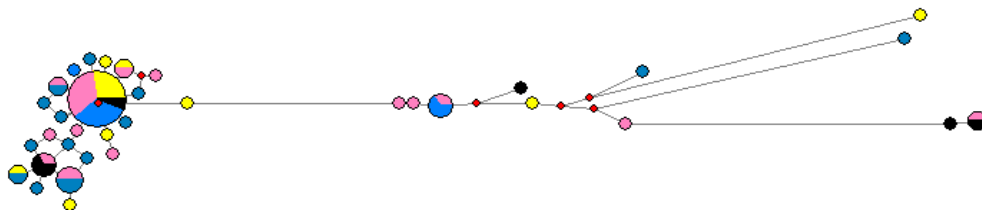
Region	Lineage A		Lineage B		<i>h</i> ± SD, control region	<i>h</i> ± SD cytochrome <i>b</i>
	<i>n</i>	<i>k</i>	<i>n</i>	<i>k</i>		
North Thailand	10	6	2	2	0.872 ± 0.091	0.758 ± 0.122
Northeast Thailand	15	9	5	5	0.916 ± 0.055	0.710 ± 0.097
Middle Thailand	20	14	4	3	0.938 ± 0.039	0.632 ± 0.090
Southern Thailand	3	2	3	3	0.933 ± 0.122	0.933 ± 0.122
Xinlong (China, Hainan island at southern coast)	26	14	3	2	0.833 ± 0.070	
Fu'an (China, at coast 630 km south of Yangtze)	10	3	7	5	0.853 ± 0.066	
Wenzhou (China, at coast 400 km south of Yangtze)	23	8	7	7	0.837 ± 0.064	
Hanzhong (China, north of Yangtze, 1260 km inland)	6	3	-	-	0.600 ± 0.215	
Xinyang (China, north of Yangtze 1000 km inland)	26	9	3	3	0.663 ± 0.101	
Binhu (China, at Yangtze river, 850 km inland)	5	2	1	1	0.600 ± 0.215	
Jiangnan (China, at Yangtze river, 950 km inland)	2	2	-	-	not defined	

Fig 1 shows median networks as visualization of haplotypes relationships, with the cytochrome *b* haplotypes allowing a clustering of D-loop haplotypes, most of which were observed only in one animal. Both the cytochrome *b* and the D-loop networks illustrate the separation of the A and B haplogroups with haplotypes IT1 in an intermediate position. The Thai network is more complex than the Chinese pattern, in which high frequencies of AH1 and BT5 suggest a population expansion (Lei et al., 2007). The network does not indicate a differential geographic distribution of haplotypes within Thailand, this in contrast to the Y-chromosomal haplotypes.

a.



b.



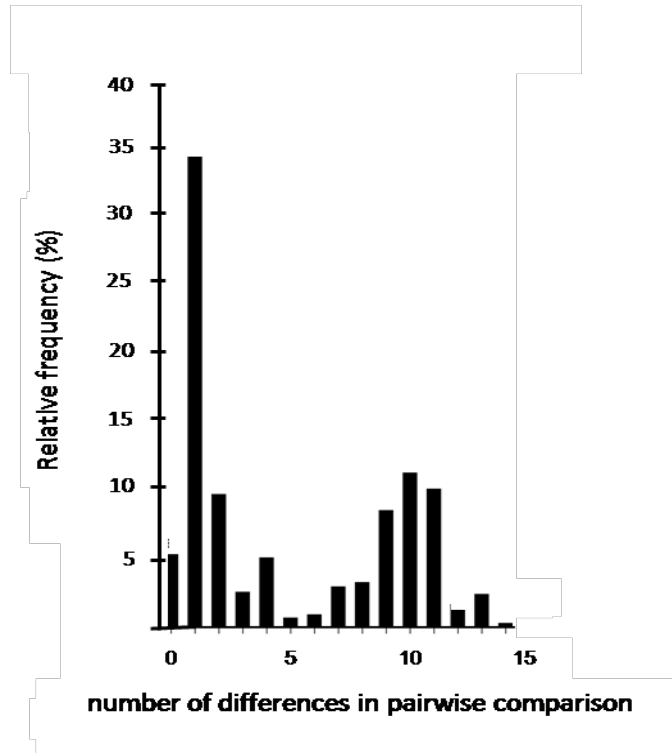
c.



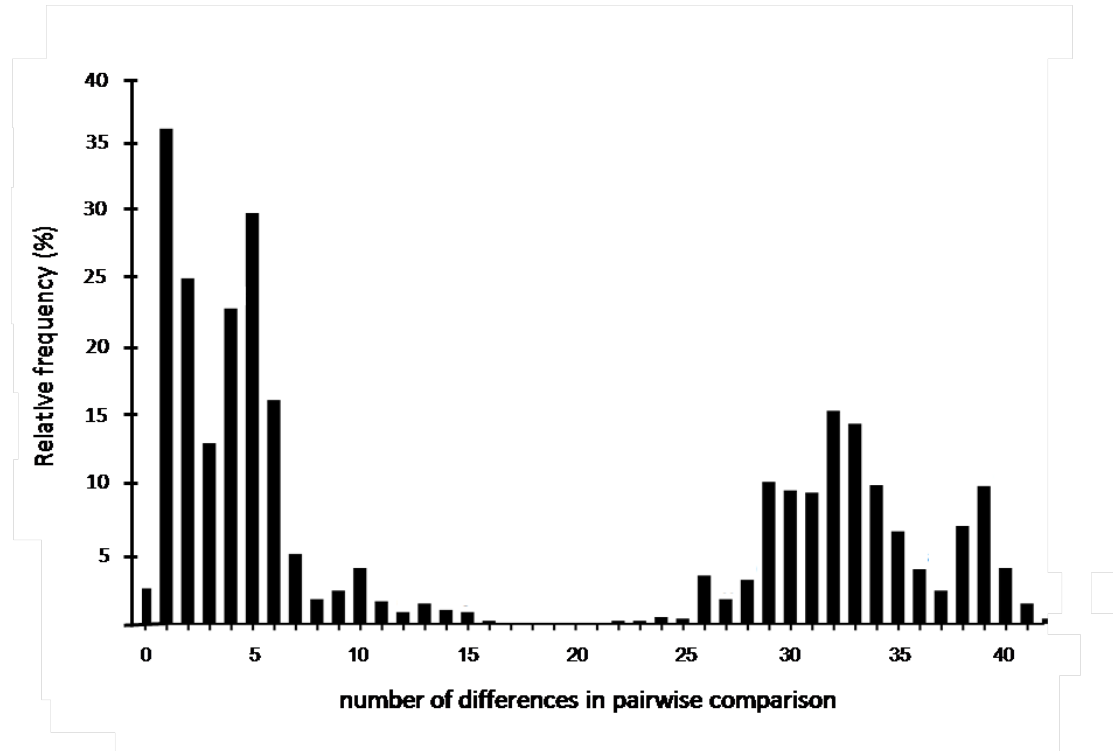
Fig 1: Reduced median networks of segments from (a) cytochrome *b* of Thai swamp buffalo, (b) Control region of Thai swamp buffalo, and (c) Control region of Chinese swamp buffalo. In (a) and (b) colors indicate the region in Thailand: yellow, north; pink, northeast; blue, middle; black, south.

The difference between the Thai and Chinese populations is also apparent from mismatch distributions (Excoffier et al., 2005; Schneider and Excoffier, 1999) (Fig 2). Again the Chinese population has a less complex pattern with a more complete separation of the A and B haplotypes, which may indicate their descent from relatively few animals.

a.



b.



c.

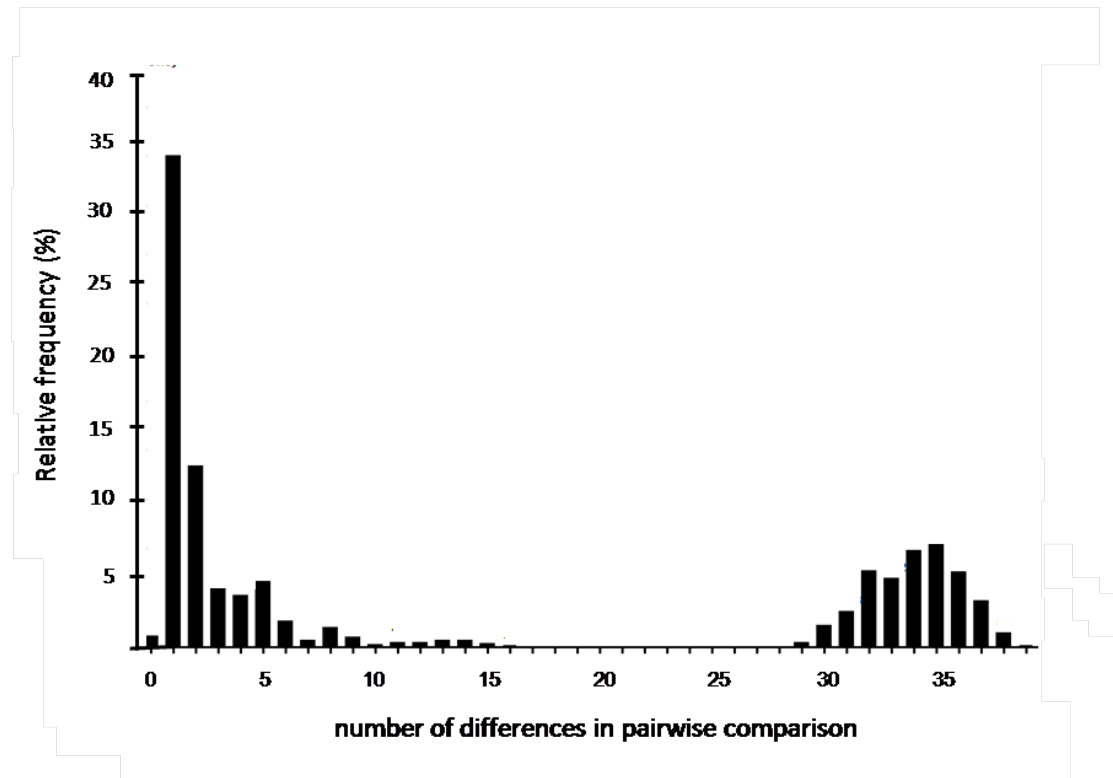


Fig 2: Mismatch distributions of segments from (a) cytochrome *b* of Thai swamp buffalo, (b) Control region of Thai swamp buffalo, and (c) Control region of Chinese swamp buffalo.

These observations do not support China as primary site of domestication. Although more definite conclusions would require a more comprehensive sampling, the presently available data suggest a relatively recent origin of the Chinese

population. Especially the predominance of the AH1 haplotype indicates a smaller base population than that for the Thai animals. This is most outspoken north of Yangtze river and in the western regions south of the Yangtze, suggesting that gene flow along the Chinese coast was more intensive than from the coast to the west or across the Yangtze river. However, the high diversity value in Thailand points to Southeast Asia as site of domestication. This is consistent the finding in the Bang-Chiang excavations of fossil bones from 2300 to 500 yr BCE, which that revealed the use of water buffalo for traction (Higham, 2002).(Fig 3).



Fig 3: Prehistoric cave painting (2000-1000 B.C.) at Khoa-Pra-Raa, Uthai Thani province, Thailand. Depicting someone seems used the animal (buffalo?) to plow a paddy field.

Our data may warrant further molecular investigations, which are to be correlated with archaeological evidence. Molecular evidence would include not only swamp buffaloes mtDNA from other countries, but also Y-chromosomal variation, so far only studied for Thai buffaloes (Yindee et al., 2010), as well as autosomal genetic markers. This information is likely to generate new insights in the genetic history of the swamp type buffalo and will also be essential for the management of the genetic resources of a livestock species that is well adapted to the Southeast Asian conditions of agriculture.

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