

## **Genetic Diversity and Variation in Captive Asian Elephants (*Elephas maximus*) in Thailand**

Authors: Kriangwanich, Wannapimol, Nganvongpanit, Korakot, Buddhachat, Kittisak, Brown, Janine L., Siengdee, Puntita, et al.

Source: Tropical Conservation Science, 11(1)

Published By: SAGE Publishing

URL: <https://doi.org/10.1177/1940082918816871>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Genetic Diversity and Variation in Captive Asian Elephants (*Elephas maximus*) in Thailand

Tropical Conservation Science  
Volume 11: 1–10  
© The Author(s) 2018  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1940082918816871  
journals.sagepub.com/home/trc  


Wannapimol Kriangwanich<sup>1</sup>, Korakot Nganvongpanit<sup>1,2</sup> , Kittisak Buddhachat<sup>2,3</sup>, Janine L. Brown<sup>4</sup>, Puntita Siengdee<sup>2</sup>, Siriwadee Chomdej<sup>2,5</sup>, Pakkanut Bansiddhi<sup>6</sup>, and Chatchote Thitaram<sup>6</sup>

## Abstract

Numbers of wild Asian elephants (*Elephas maximus*) have been decreasing gradually throughout Asia due primarily to human activities, such as poaching, and habitat encroachment and destruction that lead to human–elephant conflict. Sustainability problems exist in captive populations as well, where morbidity and mortality rates are high and reproduction is low. Determining the genetic diversity of these populations is essential for conservation and sustainable utilization efforts. Intersimple sequence repeat markers were used to assess the genetic variation and differentiation in 97 captive Asian elephants from seven elephant camps in Chiang Mai, Thailand. The nine primers chosen for the analysis revealed 88 bands in male and 115 bands in female elephants, of which 37 (42.05%) and 83 (63.64%) were polymorphic, respectively. Shannon's index information ( $I = 2.415 \pm 0.054$ ) and expected heterozygosity ( $H_e = 0.892 \pm 0.008$ ) indicated high species-level genetic diversity. The fixation index ( $F_{st}$ ) was  $-0.130 \pm 0.016$ , demonstrating there was no genetic subdivision between populations. A cluster analysis was performed using Unweight Pair-Group Method with Arithmetic Mean and dendrograms, which illustrated genetic relationships among captive Asian elephants that included 2 main clusters across the seven camps and 27 clusters for the 97 individual elephants. This high variability may be due to the different origins of these individuals, including originating from other Asian countries. Thus, this study showed that intersimple sequence repeat marker analysis was effective in demonstrating high genetic diversity among captive Asian elephants in Chiang Mai province and found cluster differences that could be used to guide breeding management to decrease the risk of inbreeding among Asian elephant groups.

## Keywords

Asian elephant, breeding, genetic diversity, captive, population

## Introduction

The Asian elephant (*Elephas maximus*) has three recognized subspecies: *Elephas maximus maximus* from Sri Lanka, *Elephas maximus indicus* from the Asian mainland, and *Elephas maximus sumatranus* from the Sumatran island of Indonesia (Vandebona, Goonesekere, Tiedemann, Ratnasooriyac, & Gunasekeraa, 2002). However, molecular genetic studies provide little evidence to support differentiation between the Sri Lankan and Asian mainland elephants because they both share mitochondrial DNA haplotypes and so should not be considered as distinct subspecies

<sup>1</sup>Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

<sup>2</sup>Excellence Center in Veterinary Bioscience, Chiang Mai University, Thailand

<sup>3</sup>Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand

<sup>4</sup>USA Center for Species Survival, Smithsonian Conservation Biology Institute, Front Royal, VA, USA

<sup>5</sup>Department of Biology, Faculty of Science, Chiang Mai University, Thailand

<sup>6</sup>Center of Excellence in Elephant and Wildlife Research, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

Received 9 October 2018; Accepted 12 November 2018

### Corresponding Authors:

Chatchote Thitaram, Department of Small Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.

Email: chatchote.thitaram@cmu.ac.th

Korakot Nganvongpanit, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.

E-mail: korakot.n@cmu.ac.th



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<http://www.creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>)

(Vandebona et al., 2002). An estimated 40,000 to 50,000 Asian elephants are distributed across 13 Asian countries (Nijman, 2014; Sukumar, 2006), including Thailand, which has the second largest elephant habitat (47,864 km<sup>2</sup>) and a healthy wild population of 3,000 to 3,500 elephants. Thailand also has a large number of captive elephants; about 3,000 to 4,000 individuals used primarily in the tourism industry (Nijman, 2014). A major conservation problem relates to the capture and trade of wild elephants from Myanmar to Thailand. These smuggled elephants are sold to tourist camps throughout Thailand but particularly those in Chiang Mai, Phuket, and Surin provinces. Origin and habitat differences might affect genetic diversity in elephants. Thus, Asian elephants coming from other countries might have genetic differences that could be used to help determine the country of origin (Ahlering et al., 2011; Kemf & Santiapillai, 2000; Nijman, 2014; Nijman & Shepherd, 2012; Shepherd & Nijman, 2008; Stiles, 2009) and thus slow illegal capture and trade of this endangered species.

For genetic variation studies, a good marker is one with high genetic variability and the ability to generate multilocus data from the genome under study (Anne, 2006). Intersimple sequence repeat (ISSR) are segments of DNA that are flanked at both ends by microsatellite sequences. Dominant markers usually produce multiple DNA fragments in a single reaction, yielding a large number of loci across the genome with unknown DNA sequences of the target regions. ISSR markers have a high reproducibility capability compared with random-amplified polymorphic DNA (RAPD), where DNA fragments are amplified by polymerase chain reaction (PCR) using short synthetic primers. It is possible that DNA amplification from ISSR with longer primers and at higher annealing temperatures may be better than those of RAPD, which can result in mismatches between RAPD primer and template, and a total absence of PCR product. Thus, the RAPD results can be more difficult to interpret than ISSR (Askari, Abadi, & Baghizadeh, 2011; Zietkiewicz, Rafalski, & Labuda, 1994). Moreover, ISSR markers are more economical and less time-consuming compared with Amplified Fragment Length Polymorphism, a technique based on selective PCR amplification of restriction fragments from a total digestion of genomic DNA which makes developing locus-specific markers from individual fragments difficult and requires the use of different kits adapted to the size of the genome being analyzed. To date, ISSR marker analyses have been applied primarily to plant genetics (Bentley, Barker, & Dold, 2015; Li, Zhong, Sui, Ji, & Ying, 2013; Ng & Tan, 2015; Tiwari et al., 2015) but are beginning to be used to estimate the genetic diversity and for individual identification in animals, particularly in domestic species like fish, cattle, buffalo, goats, and sheep (Askari et al., 2011; Aytekin, Ozdil,

Zulkadir, Boztepe, & Sariyel, 2011; Maltagliati, Lai, Casu, Valdesalici, & Castelli, 2006; Moradi, Rostamzadeh, Rashidi, Vahabi, & Ferahmand, 2014; Nesteruk, Makarova, Svishcheva, & Stolpovsky, 2015; Srinulgray & Piyapattanakorn, 2009; Stolpovsky et al., 2011). Genetic diversity or genetic structure in Asian elephants from different regions of India, Bhutan, Sri Lanka, Laos, Vietnam, and Malaysia has been evaluated based on mitochondrial DNA or nuclear microsatellites (Ahlering et al., 2011; Elliza, Shukor, Othman, & Md-Zain, 2015; Fernando, Pfreder, Encalada, & Lande, 2000; Vidya, Fernando, Melnick, & Sukumar, 2005a, 2005b; Vidya & Sukumar, 2005; Vidya, Varma, Dang, Van Thanh, & Sukumar, 2007). Likewise, genetic diversity in both captive and wild Asian elephants in Thailand has been evaluated on the basis of mitochondrial DNA or nuclear microsatellites (Fickel, Lieckfeldt, Ratanakorn, & Pitra, 2007; Thitaram et al., 2010; Thongchai et al., 2011). Genetic diversity was found to be high for captive elephants reintroduced into protected areas in northern Thailand (Thitaram et al., 2015), and among tourist elephants where there can be significant movement of animals between camps (Thitaram et al., 2010). Moreover, a historical report of captive elephant transportation found considerable movement of elephants among South East Asian countries (Lair, 1997; Thitaram et al., 2010) and selection of tame bulls and cows for tourist purposes.

Today, many captive bulls are subfertile; thus, only one or a few bulls are used for breeding in each camp, with occasional transfer of elephants among camps for breeding (Thitaram, 2012; Thongtip et al., 2004). This type of breeding selection bias might eventually lead to inbreeding in some camps. Although genetic diversity based on microsatellite analysis indicated good diversity among camp elephants in northern Thailand (Thitaram et al., 2010), it has been over 8 years since the study was conducted. It has been further suggested that alternative sources of nuclear genes could be used to enhance the characterization of population structure in Asian elephants. To date, no genetic studies utilizing ISSR analyses have been conducted in this species. Thus, the goal was to conduct a follow-up study to reassess genetic diversity of elephants residing in several camps in Chiang Mai province using ISSR markers to provide more information on elephant genetic health and prevent future inbreeding problems that might occur within individual elephant camps in the region.

## Materials and Methods

### Sample Collection

Blood samples (2 ml) from 97 captive Asian elephants (78 females and 19 males) maintained at seven elephant

**Table 1.** Number of Samples in Each Elephant Camps Which Separated Male and Female.

Elephant camps	No. of samples	Male	Female
A	8	1	7
B	11	1	10
C	11	2	9
D	13	4	9
E	12	5	7
F	37	6	31
G	5	0	5
Total	97	19	78

camps in Chiang Mai province (Table 1; Figure 1) were collected from an ear vein into 5-ml ethylenediaminetetraacetate tubes. This study was approved by the Animal Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University, Thailand in 2018 (S32/2561).

### DNA Extraction

Whole blood samples were used for extraction of genomic DNA using the RBC Bioscience™ Real genomics DNA extraction kit for Blood/Bacteria/Cultured cells (RBC Bioscience Corp., New Taipei, Taiwan) following manufacturer instructions. First, fresh blood in

**Figure 1.** Distribution of seven elephant camps in the study area by province.

ethylenediaminetetraacetate was mixed with RBC lysis buffer to lyse the cells, and a cell pellet was obtained. Next, proteinase K (10 mg/ml) was added and mixed with the cells by vortexing and then incubation at 65°C overnight until the sample lysate was clear. After incubation, absolute ethanol was added to the sample lysate. Finally, the standard elution volume was reduced from 100 µl to 50 µl to increase the DNA concentration. The concentration, yield, and purity of the DNA sample were determined using a Beckman Coulter DU® 730 spectrophotometer (Beckman Coulter, CA, USA). Once extracted and checked for purity, genomic DNA from the blood cells was kept at -20°C for further analysis.

### ISSR Amplification

Twenty one ISSR primers from the University of British Columbia (Microsatellite UBC primer set 9, University of British Columbia, Vancouver, Canada) were screened, resulting in the selection of nine that produced reproducible bands for ISSR amplification in this study (Table 1). All samples were amplified individually by PCR using 25 µl of deionized water, 19.3 µl of 10 mM dNTP mix solution, which contained dATP, dCTP, dGTP, and dTTP, 0.5 µl (Vivantis, Selangor Darul Ehsan, Malaysia), 2.5 µl of 10× ViBuffer S (160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 500 mM Tris-HCl [pH 9.2 at 22°C], 17.5 mM MgCl<sub>2</sub>, and 0.1% Triton™ X-100), 0.2 µl of 5u Taq DNA polymerase (Vivantis, Selangor Darul Ehsan, Malaysia), 0.5 µl of ISSR primer, and 2 µl of 10 ng template DNA. For every PCR reaction, deionized water was used instead of DNA templates as a negative control. PCR amplifications were performed in PTC-200 The DNA Engine® Thermal Cycler (Bio-Rad Laboratories, Inc., CA, USA) under the following conditions: predenaturation at 95°C for 5 min, followed by 38 cycles consisting of a denaturation step at 94°C for 45s, annealing step at 57°C for 45 s, and extension step at 72°C for 2 min. The last step involved a final extension at 72°C for 10 min. The PCR products were stained by RedSafe™ Nucleic acid staining solution (iNtRON Biotechnology, Gyeonggi-do, South Korea) and then separated electrophoretically on 1.5% agarose gel (PanReac AppliChem ITW companies, Darmstadt, Germany) by MINI GEL II tank electrophoresis (Select BioProducts, NJ, USA) containing 1× Tris-acetate-ethylenediaminetetraacetate (TAE) buffer at 130 V for 40 min and visualized by UV light in a UV Transilluminator gel dock (Major Science, CA, USA) to detect the amplification and record results by digital photography. A 100 bp DNA Ladder RTU (GenedireX, Inc., Miaoli, Taiwan) was used as a DNA marker for measuring DNA size or molecular weight of the PCR products. Bands that were observable after gel

electrophoresis were scored independently by two persons at least 2 times for greater precision and confirmed the results from interrater repeatability.

### Statistical Analysis

We analyzed results from ISSR amplification products from male and female elephants separately and also from the overall population. Only bands or loci that were clearly observed and unambiguous from the ISSR-amplified fragments were scored in a binary symbol for band *presence* (1) or *absence* (0), and a binary matrix was generated to determine the level of polymorphism for each primer represented by the percentage of polymorphic bands calculated using the formula of Ng and Tan (2015).

$$\text{Percentage of polymorphic bands} = \frac{\text{No. of polymorphic bands}}{\text{Total no. of bands}} \times 100\%$$

Interpopulation genetic variation in this study was determined by the observed number of alleles ( $N_a$ ), effective number of alleles ( $N_e$ ), expected level of heterozygosity in the population ( $H_e$ ), and Shannon's information index or indices ( $I$ )—a diversity index that is a quantitative measurement commonly used to characterize different species within a community. For measuring population differentiation from the genetic structure,  $F$ -statistics or a fixation index ( $F_{st}$ ) was used. Genetic similarity of captive Asian elephants in each camp was calculated as described by Takezaki and Nei (2007). All calculations were performed using GenAIEx Version 6.5 program with Microsoft Excel. The genetic similarity and genetic differentiation were calculated using pairwise comparisons of elephant camps. Finally, DARwin software program version 6.0 and interactive tree of life web-based programs were used to create individual elephant and elephant camp dendrograms using hierarchical clustering.

## Results

### ISSR Polymorphism

Preliminarily, all 21 primers were screened at three different annealing temperatures (55°C, 57°C, and 59°C), twice in each condition, which found that at an annealing temperature of 57°C, 9 primers in the microsatellite UBC primer set 9 of the University of British Columbia produced informative results that were reproducible. This made them suitable ISSR primers to use as genetic markers to assess genetic variation and differentiation, or fingerprinting, of the 97 captive Asian elephants in

**Table 2.** The Nucleotide Sequences of Intersimple Sequence Repeat Primers, Number of Total Bands and Polymorphic Bands, and Percentage of Polymorphic Bands in Both Male and Female Captive Asian Elephant.

Markers	Primer sequence	No. of total bands		No. of polymorphic bands		PPB (%)	
		M	F	M	F	M	F
UBC807	(AG) <sub>8</sub> T	10	9	4	4	40.00	44.44
UBC808	(AG) <sub>8</sub> C	11	12	7	9	63.64	75.00
UBC809	(AG) <sub>8</sub> G	4	12	2	11	50.00	91.67
UBC823	(TC) <sub>8</sub> C	9	11	3	9	33.33	81.82
UBC825	(AC) <sub>8</sub> T	11	14	4	7	36.36	50.00
UBC827	(AC) <sub>8</sub> G	10	13	6	10	60.00	76.92
UBC835	(AG) <sub>8</sub> YC	16	13	5	11	31.25	84.62
UBC847	(CA) <sub>8</sub> RC	8	15	3	10	37.50	66.67
UBC848	(CA) <sub>8</sub> RG	9	16	3	12	33.33	75.00
Mean						42.05	72.17

Note. PPB = percentage of polymorphic bands.

**Table 3.** Diversity Indices for Intersimple Sequence Repeat Markers Evaluated on Captive Asian Elephant.

Populations	$N_a \pm SE$	$N_e \pm SE$	$I \pm SE$	$He \pm SE$	$F_{st} \pm SE$
A	11.000 ± 0.898	9.637 ± 0.848	2.301 ± 0.094	0.888 ± 0.012	-0.128 ± 0.016
B	12.667 ± 1.462	10.549 ± 1.204	2.330 ± 0.210	0.866 ± 0.046	-0.197 ± 0.101
C	13.000 ± 0.972	10.938 ± 0.860	2.450 ± 0.085	0.903 ± 0.009	-0.108 ± 0.012
D	15.556 ± 1.444	12.990 ± 1.305	2.607 ± 0.113	0.915 ± 0.011	-0.095 ± 0.014
E	14.222 ± 1.267	11.313 ± 1.119	2.500 ± 0.106	0.903 ± 0.012	-0.109 ± 0.015
F	19.556 ± 1.168	14.781 ± 0.917	2.791 ± 0.059	0.930 ± 0.004	-0.075 ± 0.005
G	7.444 ± 0.784	7.046 ± 0.844	1.926 ± 0.118	0.840 ± 0.019	-0.196 ± 0.028
Mean	13.349 ± 0.610	11.036 ± 0.469	2.415 ± 0.054	0.892 ± 0.008	-0.130 ± 0.016

Note.  $N_a$  = observed number of alleles;  $N_e$  = effective number of alleles;  $I$ : Shannon's index;  $He$  = expected heterozygosity;  $F_{st}$  = fixation index;  $SE$  = standard error.

this study. Another 12 ISSR markers did not provide informative results due to some optimization problems.

As shown in Table 2, using nine ISSR markers, 88 DNA fragments were produced in male captive Asian elephants, of which 37 (42.05%) were polymorphic; the UBC808 primer produced the most polymorphism (63.64%). In female elephants, 115 DNA fragments were obtained, of which 83 (72.17%) were polymorphic. Again, the UBC 809 primer produced the most polymorphism (91.67%), indicating substantial genetic diversity at the population level.

### Genetic Variation in Captive Asian Elephants

Genetic variation of male and female captive Asian elephants from each camp is shown in Table 3. The mean observed number of alleles ( $N_a$ ) in males ranged from 2.000 at Camp A to 8.778 at Camp F, and in females from 7.444 at Camp G to 13.111 at Camp F. Values of effective number of alleles ( $N_e$ ) were lesser than  $N_a$  in almost every population, except in males at Camps A and B, which had the same  $N_a$  and  $N_e$ . The Shannon's information index of elephants from camp F had the highest value, while Camp G had the lowest value

(Table 3). For male elephants, the lowest Shannon's information index value was from Camps A and B, while the highest value was from Camp F. For females, the Shannon's information index values were  $1.844 \pm 0.294$  to  $2.491 \pm 0.043$ , with the lowest at Camp E and the highest at Camp F, similar to the male elephants. Mean expected heterozygosity ( $He$ ) of overall populations of both male and female elephants was highest in Camp F and lowest in Camp G. Male captive Asian elephants from Camps A and B had the lowest  $He$  value, 0.500, while Camp F had the highest  $He$  value of  $0.852 \pm 0.019$ . For female captive Asian elephants, the  $He$  value results showed that Camp E had the lowest and Camps A and C had the highest values. By contrast, for the entire population, the results showed that the lowest value was from Camp G and the highest was from Camp F, which was similar to  $N_a$ ,  $N_e$ , and the Shannon's information index.

### Genetic Differentiation Among Elephant Camps

$F_{st}$  tests were used to estimate the genetic differentiation of captive Asian elephants in this study. The maximum fixation index ( $F_{st}$ ) value among elephants was found in

**Table 4.** Pairwise Population Matrix of Nei's Genetic Distance Are Below the Diagonal and Pairwise Population  $F_{st}$  Values, Above the Diagonal, in Captive Male Asian Elephant.

	A	B	C	D	E	F
A	0	0.267	0.196	0.140	0.121	0.126
B	1.504	0	0.199	0.156	0.160	0.154
C	1.280	1.280	0	0.099	0.089	0.087
D	0.869	1.105	0.987	0	0.053	0.046
E	0.681	1.240	0.819	0.646	0	0.041
F	0.778	1.301	0.905	0.615	0.543	0

**Table 5.** Pairwise Population Matrix of Nei's Genetic Distance Are Below the Diagonal and Pairwise Population  $F_{st}$  Values, Above the Diagonal, in Captive Female Asian Elephant.

	A	B	C	D	E	F	G
A	0	0.081	0.025	0.081	0.096	0.014	0.032
B	0.469	0	0.084	0.124	0.143	0.068	0.091
C	0.419	0.519	0	0.082	0.094	0.017	0.040
D	0.455	0.307	0.456	0	0.145	0.068	0.087
E	0.685	0.537	0.622	0.590	0	0.082	0.107
F	0.237	0.247	0.304	0.252	0.447	0	0.025
G	0.483	0.589	0.662	0.479	0.789	0.402	0

Camp F, and the minimum value was found in Camp B (Table 3). In addition, pairwise Nei's genetic distance analysis in males indicated that the closest distance was obtained between Camps E and F, and the greatest value was between Camps A and B. Conversely, in female elephants, pairwise Nei's genetic distance analysis showed that the closest distance was between Camps A and F, and the highest was between Camps E and G (Tables 4 and 5). For pairwise  $F_{st}$  values analysis, the closest value in male elephants was between Camps E and F, and the greatest value was between Camps A and B. For female elephants, the results showed that the closest value was between Camps A and F, and the greatest value was between Camps D and E. For the overall population, the least genetic distance was between Camps A and F, while the greatest distance was between Camps E and G. The greatest pairwise  $F_{st}$  value was between Camps B and G, while the closest value was between Camps D and F (Table 6).

### Phylogenetic Dendrograms

Phylogenetic dendrograms of individual Asian elephants identified 27 clusters based on allelic frequency (Figure 2(a)) and showed that each elephant camp differed, with 4, 2, 6, 8, 7, 11, and 3 main clusters observed in Camps A, B, C, D, E, F, and G, respectively (Figure 3). Moreover, the camps divided into two main

**Table 6.** Pairwise Population Matrix of Nei's Genetic Distance Are Below the Diagonal and Pairwise Population  $F_{st}$  Values, Above the Diagonal, in Overall Population of Captive Asian Elephant.

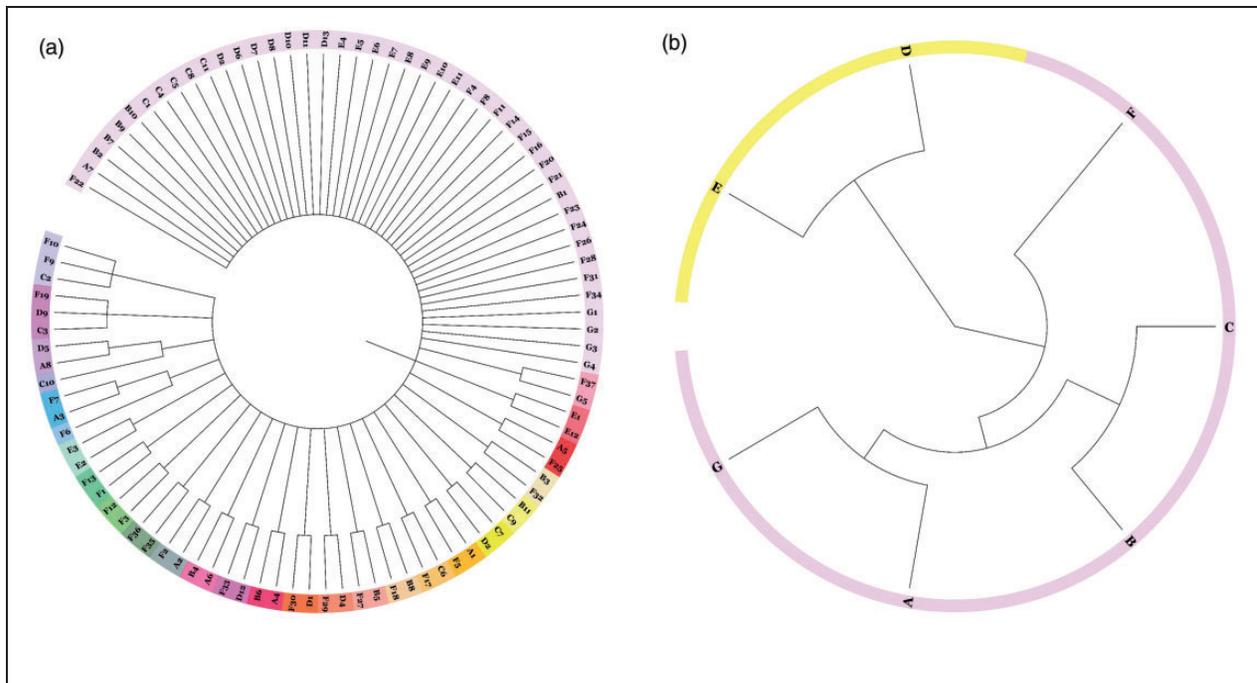
	A	B	C	D	E	F	G
A	0	0.034	0.021	0.022	0.027	0.013	0.034
B	0.647	0	0.029	0.035	0.038	0.024	0.050
C	0.448	0.531	0	0.019	0.024	0.013	0.038
D	0.513	0.779	0.469	0	0.021	0.012	0.035
E	0.636	0.866	0.624	0.557	0	0.017	0.044
F	0.287	0.479	0.335	0.339	0.464	0	0.027
G	0.559	0.881	0.735	0.680	0.917	0.455	0

clusters: Camps D and E and Camps F, C, B, A, and G (Figure 2(b)).

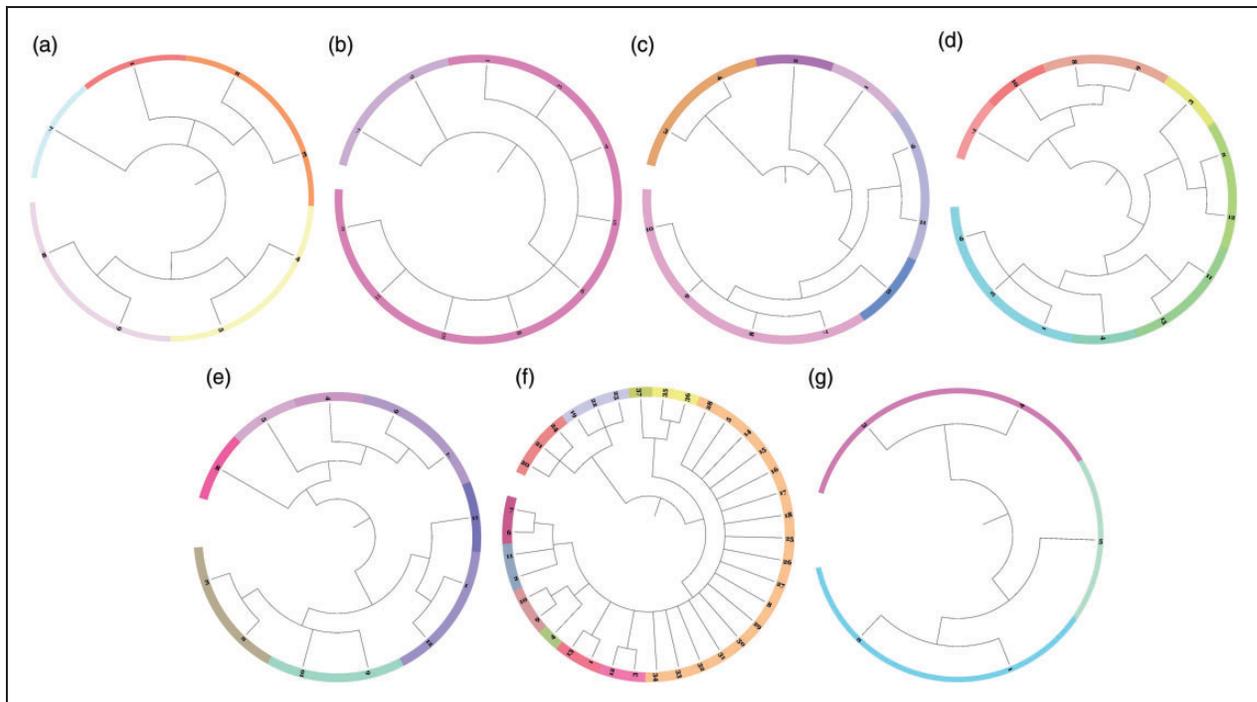
### Discussion

This is the first study to apply ISSR marker analysis to assess genetic variation of captive Asian elephants based on the mean allelic frequency and found good genetic variability within and between tourist camps in Chiang Mai, Thailand. The results showed that the expected heterozygosity ( $H_e$ ) was high in every elephant camp. This high heterozygosity likely reflects the varied origins of elephants brought into the camps and possibly also the fairly routine transfer of elephants between camps (Luan, Yuh Chiang, & Gong, 2006; Wang, Zheng, Toure, Dandekar, & Kafatos, 2001; Wisely, Buskirk, Fleming, McDonald, & Ostrander, 2002). The Shannon's information index values, which were 2.126 in females, 1.366 in males, and 2.145 for the entire population, were higher than other studies in domestic animals, such as cattle, sheep, buffaloes, and goat, which range from 0.18 to 0.75 (Askari et al., 2011; Aytekin et al., 2011; Moradi et al., 2014). The percentage of polymorphic bands also showed a high level of variability in female elephants (72.17%), with a lower value (42.05%) for males, but both indicated high genetic diversity across elephant camps in Chiang Mai, similar to a previous genetic study using microsatellite analyses (Thitaram et al., 2010). The genetic variance within captive Asian elephants further showed high levels of genetic variation (percentage of polymorphic bands = 57.08%,  $I = 2.145$ ,  $H_e = 0.892$ ) again because Asian elephants are a geographically widespread species that may help to maintain genetic variation (Luan et al., 2006; Wang et al., 2001; Wisely et al., 2002).

Genetic diversity reflects the total number of genetic characteristics and is related to the number of genes and their alleles within individuals and can influence adaptability and distribution of a species in diverse habitats. Genetic differentiation is the accumulation of differences in allelic frequencies between completely or partially isolated populations due to evolutionary forces and is



**Figure 2.** The phylogenetic dendrogram of individual captive Asian elephants (a) and the phylogenetic dendrogram of elephant camps (b).



**Figure 3.** The phylogenetic dendrogram of individual captive Asian elephants in Camp A, B, C, D, E, F, and G (a–g, respectively).

important for understanding selection or genetic drift in species like the Asian elephant. Fixation index is a classical statistic for summarizing population differentiation, which measures the difference in heterozygosity among populations (Yamamichi & Innan, 2012).

A fixation index value above 0.25 indicates high genetic differentiation among a population. The fixation index values in our population were negative, so no genetic subdivision was apparent between the populations. Pairwise  $F_{st}$  value was also low, which implies that

there was no differentiation among these camps; that is, there is only one species present in this population. Female elephants had more identical allelic frequencies and shared more genetic alleles compared with male elephants, which also indicates that based on genetic distance, female elephants were more related than males according to Nei's genetic diversity theory (Wang et al., 2001). Elephant society is matriarchal; core groups comprised 5 to 15 related adult female elephants and their male and female offspring, while male elephants live apart from the herd, and interact only for breeding. Thus, the elephant society structure might explain how female elephants are more related to each other than are male elephants. Our results based on the Shannon's information index showed that genetic diversity in males and females differed, which might be for the same reason. Results indicate that from each elephant camp, if the number of samples did not have a distinct value for the Shannon's information index, it would not distinguish as well. Pairwise genetic distance across the population was moderate to high, which might indicate that at present, inbreeding is not a problem among these elephant camps. Genetic variation is a basic requirement for animal breeding as well as for genetic improvement. Results of this study provide baseline information on the genetic status of elephants between and within tourist camps in Thailand that will be important for future conservation of the species. For example, Camps E and G had the greatest genetic distance, so breeding of elephants between those two facilities would help to improve genetic variation and diversity and prevent inbreeding within each camp.

An individual dendrogram was generated using the clustering method of Unweight Pair-Group Method with Arithmetic Mean. This dendrogram indicated that all captive Asian elephants fell into 27 clusters using the nine ISSR markers. In some clusters, only one captive Asian elephant was assigned, which could be due to captive Thai elephants being transported between provinces and also between the countries of Thailand and Myanmar. The finding of many different clusters within camps suggests good breeding management. The elephant camps separated into two clusters: Camps F, C, B, A, and G and Camps D and E, which may also be useful for breeding management and suggests that camps in different clusters should cooperate and transfer genetically valuable bulls or cows for breeding.

The value of genetic diversity in this study was higher compared with studies using other dominant markers, such as RAPD in the Moxoto goat breed population (0.22–0.33) and Amplified Fragment Length Polymorphism for Italian goats (0.21–0.24; Ajmone-Marsan et al., 2001; Olivera et al., 2005). Further studies of genetic variation and differentiation of Asian

elephants should be performed with a greater number of individuals and in other areas of the country. We conclude that ISSR markers are an efficient method for discriminating genotype at the molecular level and can successfully be used to estimate the population level polymorphism and genetic diversity. The markers also provided a good comprehension of genetic variability but showed no genetic differentiation among these captive elephants. In this study, of the 21 primers initially tested, only 9 produced clear reproducible fragments or bands. Thus, the suggestion is that a greater number of biologically relevant ISSR primers that produce reproducible unambiguous bands should be applied in future studies to obtain more accurate results. In conclusion, this study showed the potential of ISSR markers to evaluate genetic diversity in captive Asian elephants and proved an attractive alternative to other molecular markers.

### Implications for Conservation

Inbreeding and its effects on lowering genetic diversity could negatively impact successive generations of captive Asian elephants. Therefore, information about genetic diversity and variation has become more important in the past decade. This is especially true for elephants in Thailand because there is no documentation of pedigrees similar to the studbooks used in western zoos to guide breeding recommendations. Thus, although this study revealed a high diversity among captive Asian elephants within and between the study camps today, attention will need to be paid in the future to ensure this variability is maintained. This is especially important given the limited availability of breeding bulls. The results of this and future studies can be useful for better management of breeding across camps to further conserve the genetics of these valuable populations.

### Acknowledgments

The authors would like to thank all of the owners, managers, and mahouts at the Baan Chang elephant camp, Mae-Taeng elephant camp, Mae-Sa elephant camp, Maetaman elephant camp, Baan Chang Thai elephant camp, Chokchai elephant camp, and Elephant Nature Park for their cooperation. The authors also thank the anonymous reviewers for their suggestion for improving this manuscript and many who colleagues participated in field research and data collection.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The authors disclosed the receipt of the following financial support for research, authorship and/or publication of this article: This work was supported by The Royal Jubilee PhD program, Thailand Research Fund, The Excellence Center in Veterinary Bioscience, and the Center of Excellence in Elephant and Wildlife Research, Chiang Mai University, Thailand.

## ORCID iD

Korakot Nganvongpanit  <http://orcid.org/0000-0002-7617-5507>

## References

- Ahlering, M. A., Hedges, S., Johnson, A., Tyson, M., Schuttler, S. G., & Eggert, L. S. (2011). Genetic diversity, social structure, and conservation value of the elephants of the Nakai Plateau, Lao PDR, based on non-invasive sampling. *Conservation Genetics*, *12*, 413–422.
- Ajmone-Marsan, P., Negirni, R., Crepaldi, P., Milanesi, E., Gorni, C., Valentini, A., & Cicogna, M. (2001). Assessing genetic diversity in Italian goat populations using AFLP markers. *Animal Genetics*, *32*(5), 281–288.
- Anne, C. (2006). Choosing the right molecular genetic markers for studying biodiversity: From molecular evolution to practical aspects. *Genetica*, *127*, 101–120.
- Askari, N., Abadi, M. M., & Baghizadeh, A. (2011). ISSR markers for assessing DNA polymorphism and genetic characterization of cattle, goat and sheep populations. *Iranian Journal of Biotechnology*, *9*(3), 222–229.
- Aytekin, I., Ozdil, F., Zulkadir, U., Boztepe, S., & Sariyel, V. (2011). Evaluation of ISSR markers for genetic diversity analysis in Anatolian water buffaloes. *Journal of the Science of Food and Agriculture*, *91*, 1957–1962.
- Bentley, L., Barker, N. P., & Dold, A. P. (2015). Genetic diversity of the endangered *Faucaria tigrina* (Aizoaceae) through ISSR “fingerprinting” using automated fragment detection. *Biochemical Systematics and Ecology*, *58*, 156–161.
- Elliza, M. N., Shukor, M. N., Othman, N., & Md-Zain, B. M. (2015). Haplotype distribution among endangered Asian elephants (*Elephas maximus*) in Peninular Malaysia. *Malaysian Applied Biology*, *44*(2), 129–135.
- 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.
- Fickel, J., Lieckfeldt, D., Ratanakorn, P., & Pitra, C. (2007). Distribution of haplotypes and microsatellite alleles among Asian elephants (*Elephas maximus*) in Thailand. *European Journal of Wildlife Research*, *53*, 298–303.
- Kemf, E., & Santiapillai, C. (2000). *Asian elephants in the wild*. Gland, Switzerland: WWF International.
- Lair, R. C. (1997). *Gone astray: The care and management of the Asian elephant in domesticity*. Bangkok, Thailand: FAO Regional Office for Asia and the Pacific.
- Li, L., Zhong, Y. H., Sui, N., Ji, Y. L., & Ying, X. Q. (2013). Genetic diversity of *Camellia japonica* (Theaceae), a species endangered to East Asia, detected by inter-simple sequence repeat (ISSR). *Biochemical Systematics and Ecology*, *50*, 199–206.
- Luan, S., Yuh Chiang, T., & Gong, X. (2006). High genetic diversity vs. low genetic differentiation in *Nouelia insignis* (Asteraceae), a narrowly distributed and endemic species in China, revealed by ISSR fingerprinting. *Annals of Botany*, *98*, 583–589.
- Maltagliati, F., Lai, T., Casu, M., Valdesalici, S., & Castelli, A. (2006). Identification of endangered Mediterranean cyprinodontiform fish by means of DNA inter-simple sequence repeats (ISSRs). *Biochemical Systematics and Ecology*, *34*, 626–634.
- Moradi, M. H., Rostamzadeh, J., Rashidi, A., Vahabi, K., & Ferahmand, H. (2014). Analysis of genetic diversity in Iranian mohair goat and its color types using inter simple sequence repeat (ISSR) markers. *Agricultural Communications*, *2*(1), 55–62.
- Nesteruk, L. V., Makarova, N. N., Svishcheva, G. R., & Stolpovsky, Y. A. (2015). Estimation of genetic diversity of Romanov sheep by the coefficient of genetic originality based on ISSR-fingerprinting data. *Russian Journal of Genetics*, *51*(7), 725–729.
- Ng, W. L., & Tan, S. G. (2015). Inter-simple sequence repeat (ISSR) markers: Are we doing it right? *ASM Science Journal*, *9*(1), 30–39.
- Nijman, V. (2014). *An assessment of the live elephant trade in Thailand*. Cambridge, England: TRAFFIC International.
- Nijman, V., & Shepherd, C. R. (2012). The role of Lao PDR in the ivory trade. *TRAFFIC Bulletin*, *24*, 35–40.
- Olivera, R. R., Egito, A. A., Riberio, M. N., Palva, S. R., Albuquerque, M. S. M., Castro, S. R., ... Adriano, M. (2005). Genetic characterization of the Moxoto goat breeds using RAPD markers. *Pesquisa Agropecuária Brasileira*, *40*, 233–239.
- Shepherd, C. R., & Nijman, V. (2008). *Elephant and ivory trade in Myanmar*. Petaling Jaya, Malaysia: TRAFFIC Southeast Asia.
- Srinulgray, T., & Piyapattanakorn, S. (2009, March 17–20). *Genetic diversity of short mackerel *Rastrelliger brachysoma* in the gulf of Thailand and Andaman Sea revealed by ISSR marker*. Paper presented at the Proceedings of 47th Kasetsart University Annual Conference, Science, Bangkok.
- Stiles, D. (2009). *The elephant and ivory trade in Thailand*. Petaling Jaya, Malaysia: TRAFFIC Southeast Asia.
- Stolpovsky, Y. A., Azari, M. A., Evsukov, A. N., Kol, N. V., Ruzina, M. N., Voronkova, V. N., & Sulimova, G. E. (2011). Comparison of ISSR polymorphism among cattle breeds. *Russian Journal of Genetics*, *47*(2), 189–200.
- Sukumar, R. (2006). A brief review of the status, distribution and biology of wild Asian elephants. *International Zoo Yearbook*, *40*, 1–8.
- Takezaki, N., & Nei, M. (2007). Empirical tests of the reliability of phylogenetic trees constructed with microsatellite DNA. *Genetics*, *178*, 385–392.
- Thitaram, C. (2012). Breeding management of captive Asian elephant (*Elephas maximus*) in range countries and zoos. *Japanese Journal of Zoo and Wildlife Medicine*, *7*, 91–96.
- Thitaram, C., Dejchaisri, S., Somgird, C., Angkawanish, T., Brown, J., Phumphuay, R., ... Kangwanpong, D. (2015).

- Social group formation and genetic relatedness in reintroduced Asian elephants (*Elephas maximus*) in Thailand. *Applied Animal Behavior Science*, 172, 52–57.
- Thitaram, C., Somgird, C., Mahasawangkul, S., Angkavanich, T., Roongsri, R., Thongtip, N., Colenbrander, B., ... Lenstra, J. (2010). Genetic assessment of captive elephant (*Elephas maximus*) populations in Thailand. *Conservation Genetics*, 11, 325–330.
- Thongchai, S., Dejchaisri, S., Sukmasuang, S., Thongtipsiridech, S., Wajjwalku, W., & Kaolim, N. (2011). Genetic diversity of wild elephant (*Elephas maximus* Linnaeus, 1758) in the northeastern part of Thailand. *Journal of Wildlife in Thailand*, 18, 16–33.
- Thongtip, N., Kornkaewrat, K., Mahasawangkul, S., Thitaram, C., Saikhun, J., Kalmapichit, A., ... Pinyopummin, A. (2004, October 29). The importance of semen cryopreservation for Asian elephant breeding programmes in Thailand. In: *Proceedings of the 15th Symposium Tropical Animal Health and Production: Management of Elephant Reproduction, Faculty of Veterinary Medicine, Utrecht University, The Netherlands* (pp. 33–37). Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
- Tiwari, V., Mahar, K. S., Singh, N., Meena, B., Nair, K. N., Datt, B., ... Rana, T. S. (2015). Genetic variability and population structure of *Bergenia ciliata* (Saxifragaceae) in the Western Himalaya inferred from DAMD and ISSR markers. *Biochemical Systematics and Ecology*, 60, 165–170.
- Vandebona, H., Goonesekere, N. C. W., Tiedemann, R., Ratnasooriyac, W. D., & Gunasekeraa, M. B. (2002). Sequence variation at two mitochondrial genes in the Asian elephant (*Elephas maximus*) population of Sri Lanka. *Mammalian Biology-Zeitschrift für Säugetierkunde*, 67, 193–205.
- 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, 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., & Sukumar, R. (2005). Social organization of the Asian elephant (*Elephas maximus*) in southern India inferred from microsatellite DNA. *Journal of Ethology*, 23, 205–210.
- Vidya, T. N. C., Varma, S., Dang, N. X., Van Thanh, T., & Sukumar, R. (2007). Minimum population size, genetic diversity, and social structure of the Asian elephant in Cat Tien National Park and its adjoining areas, Vietnam, based on molecular genetic analyses. *Conservation Genetics*, 8, 1471–1478.
- Wang, R., Zheng, L., Toure, Y. T., Dandekar, T., & Kafatos, F. C. (2001). When genetic distance matters: Measuring genetic differentiation at microsatellite loci in whole genome scans of recent and incipient mosquito species. *Proceedings of the National Academy of Sciences*, 98(19), 10769–10774.
- Wisely, S. M., Buskirk, S. W., Fleming, M. A., McDonald, D. B., & Ostrander, E. A. (2002). Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. *Journal of Heredity*, 93(4), 231–237.
- Yamamichi, M., & Innan, H. (2012). Estimating the migration rate from genetic variation data. *Heredity*, 108, 362–363.
- Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20(2), 176–183.