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Research article

Genetic diversity and inbreeding situation of Korat and Siamese cats based on microsatellite markers

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Abstract

Korat cats and Siamese cats are the famous ancient Thai cat breeds that were originated in Thailand. Currently, there are no genetic studies on Thai native cats that can be used for genetic selection, breeding management and prevent inbreeding in the populations. This study aims to investigate genetic diversity in 2 Thai native cat breeds. Thirty seven Korat cats and 30 Siamese cats were determined by using 30 microsatellite markers. Zn-finger and Amelogenin markers were used as sex determination that was 100 percent accurate. Four markers had one allele that was uninformative in both cat breeds. Nineteen and 22 markers were high to moderate informative markers in Korat and Siamese cats, respectively. Therefore, these sets of markers were suitable for genetic diversity evaluation in Thai cats. Average polymorphic information content (*PIC*), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and inbreeding coefficient (*f*) from 28 microsatellite markers without sex identified markers were 0.471, 0.413, 0.510 and 0.215 in Korat cats, 0.551, 0.417, 0.587 and 0.261 in Siamese cats, respectively. Results revealed the moderate level of genetic diversity in both Thai native cats that correlated with the inbreeding coefficient. Population structure analysis by the STRUCTURE program was assigned individual cat to the right cat breed that shown uniform and genetically distinct of Korat and Siamese cats. This study was the beginning of genetic diversity study in only two Thai native cat breeds that helps to understand the genetic background within breeds and will be used to investigate the population structure in these cat breeds in the future.

Keywords: Genetic diversity, Korat cat, Microsatellite marker, Siamese cat

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INTRODUCTION

The domestic cats (*Felis catus*) are now the most numerous companion animal in the United State of America, Europe, Asia including Thailand. Thailand's Department of Livestock Development reported that the number of cat population in 2016 was about 3,035,645 (www.http://dcontrol.dld.go.th/dcontrol/index.php/rabies/747-dogpop2016). All of them were categorized by the life of living, 83.71% of cats with owners and 16.29% of cats without owners often called feral or stray cats. However, there were no official records between numbers of Thai native cats, foreign cats, and feral cats. The mating behavior of feral cat is independently variable and mating choice is upon cat's desire. Breeding of Thai native cats are managed by human, so their genetic diversity and inbreeding situation are affected by humans that differ from feral cats. Nowadays, genetic diversity in the Thai cat population still needs further studies.

DNA is the genetic material of organisms. The study of DNA variations reflects the genetic difference between individuals since the discovery of Polymerase chain reaction (PCR) technique (Saiki et al., 1988). Currently, the advancement and discovery of DNA-based genetic markers provide a new evidence for investigating genetic variability in many populations, especially at microsatellite (short tandem repeat, STR) loci. STR marker typing had become very popular by biotechnologist. Microsatellite markers are short segments of DNA which accumulate high levels of variation within populations or species (Litt and Luty, 1989; Weber and May 1989; Ellegren, 2004). They are widely distributed throughout the genome (Perez-Jimenez et al., 2013; Phumichai et al., 2015). The main types of microsatellite are mono-, di-, tri or tetranucleotide repeats. The common repeats of microsatellite in the cat genome (2N=38) were (CA)n or (GT)n which are distributed approximately every 40 kb (Menotti-Raymond et al., 1999). Although tetranucleotide repeat is less frequent than dinucleotide repeat, they are more accurate for genotyping score. Microsatellite allele sizes are differentiated by a number of repeats which can be detected by capillary gel electrophoresis. Microsatellite markers have many polymorphism alleles and usually used to examine genetic variation in the Felidae population (Menotti-Raymond et al., 1999). These markers are widely used for the study of genetic diversity, individual identification and parentage testing in many cat breeds (Menotti-Raymond et al., 1999, 2008, 2009; Wiseman et al., 2000).

For many decades, foreigners have known that Thai native cats were originated in Thailand. The ancient Thai manuscripts known as the "Tamra Maew" that kept in the National Library of Thailand had mentioned that there were 23 kinds of Thai native cat. They were divided into 17 kinds of good-luck cat and 6 kinds of unfortunate cat. To date, only 5 Thai native cat breeds remain including 4 good-luck cat breeds (Korat cat, Siamese cat, Suphalak Cat and Konja cat) and the Khao Manee cat. The Khao Manee cat is the Thai native cat with no record in the ancient books. This cat breed has a pure white color with yellow or blue eyes. Korat cat, Siamese cat, Suphalak cat and Konja cat are nature-made breed cats and well known as good-luck Thai native cats. Korat cat, Silver Blue cat or Srisawad cat, the origin came from Nakhon Ratchasima province or Korat city. Their characters are Thai-style short hair with

silver color, including three coat color patterns such as silver gray, black gray and red gray. Siamese cat or namely in Thai word Wichian Mat, the origin was undocumented, but the breed was found in the central part of Thailand. The old-style Siamese cat is characterized by blue almond-shaped eyes, oval head shape, small ears, slender, and slim body. The 9 positions of dark coloring points are their characteristic as a signature. Their pointed pattern is a form of partial albinism, resulting from a mutation in tyrosinase, an enzyme involved in melanin production (Imes et al., 2006). Suphalak and Konja cats are the solid brown (chocolate) and black cats, respectively. Nowadays, Thai cat breeds are rare in Thailand. All Thai native cats have heart-shaped face, muscles, long tail and slim body.

To evaluate the genetic diversity based on microsatellite markers, the genetic diversity parameters are analyzed such as a number of alleles, major allele frequency (MAF), observed heterozygosity (*Ho*), expected heterozygosity (*He*), polymorphic information content (*PIC*) and inbreeding coefficient (*f*). Heterozygosity is a major interest in the genetic variation of a natural population. It can identify a great deal about the population. High heterozygosity means lots of genetic variabilities. Genetic variation in populations can be described by genotype and allele frequencies, not the gene frequencies. Expected heterozygosity is the level of heterozygosity of population under Hardy-Weinberg equilibrium (HWE) as Mendel's genetic law. Observed heterozygosity is the level of heterozygosity in the observed population under several factors such as natural selection related with allele frequencies, mutation, gene flow as the movement of alleles between populations, population structure together with inbreeding and non-random reproduction (Halliburton, 2004). Comparisons between the *Ho* value and *He* value can evaluate genetic diversity situation of the observed population (Wiseman et al., 2000). Normally, *Ho* value is not more than *He* value. Naturally, in a population without distress factors, *Ho* value is nearby *He* value. If the *Ho* value is lower than *He*, it means low or moderate heterozygosity or increasing of homozygote or increase of inbreeding (Halliburton, 2004). MAF is the highest frequency of genotype in each microsatellite marker. *PIC* at the molecular level is genotypic variation within microsatellite loci that show low, moderate or high-efficiency level of microsatellite markers (Mateescu et al., 2005). The *PIC* value would be close to zero if there is less allelic variation. For *f* value meant the degree of alleles which are more likely to be homozygous rather than heterozygous in an individual. This situation occurs because the parents are closely related or has low genetic variability in the population.

Among the remaining 5 Thai native cat breeds, Korat cat and Siamese cat were well known and more popular among Thai and foreign cat breeders. This study was chosen Korat cat and Siamese cat for the preliminary study to overview their genetics before further study in Suphalak, Konja and Khao Manee cats. Therefore, the aim of this study is to investigate the genetic diversity and inbreeding situation in 76 Korat cat and Siamese cat breeds.

MATERIALS and METHODS

Sample collection and DNA extraction

The sample comprised of 67 individuals representing the 2 cat breeds in Thailand: 37 Korat cats (K) and 30 Siamese cats (S). The samples from Korat cats (n=37) were obtained from 5 conservative catteries; cattery 1 from Bangkok (n=8) and cattery 2 (n=13), 3 (n=5), 4 (n=4) and 5 (n=7) from the Nakhon Ratchasima province. In Siamese cats, sample group came from 3 conservative catteries and 1 private owner. The samples of 3 conservative catteries were from Bangkok (n=21), Nakhon Ratchasima province (n=2), and Samut Songkhram province (n=6) and one cat from private owner in Nakhon Pathom province (n=1). This animal use protocol has been approved by the Kasetsart University Institutional Animal Care and Use Committee with approved protocol number ACKU61-VET-053 and found to be in accordance to the guidelines of animal care and use under the Ethical Review Board of the Office of National Research Council of Thailand (NRCT) for the conduct of the scientific research. Genomic DNA was extracted from whole blood in EDTA by the standard phenol-chloroform (Sambrook and Russell, 2001). The quality and concentration of DNA were examined by UV spectrophotometer, Nanodrop 2000 (Themo Scientific, USA). Then the concentration was adjusted to 50 ng/µl of elution buffer and kept on -20°C until the test.

Microsatellite analysis

Thirty microsatellite markers were chosen from these selection criteria such as distributed in different chromosomes, dinucleotide or tetranucleotide repeated markers and *PIC* value of marker more than 0.5. The selected markers code were FCA726, FCA733, FCA739, FCA747, FCA045, FCA077, FCA008, FCA096, FCA441, FCA124, F53, FCA391, F42, FCA665, FCA126, FCA132, F124, FCA105, FCA075, FCA220, FCA229, FCA310, FCA586, FCA201, FCA290, FCA596, FCA223, FCA298, Zn-finger and Amelogenin. The primer and microsatellite sequence information was available at GENBANK and previous literature (Menotti-Raymond et al., 1999, 2005). The PCR reaction carried out using a thermal cycler (DNA Engine PTC-2000, BIO-RAD, USA). The PCR conditions were modified from those described earlier (Menotti-Raymond et al., 1999). PCR product fragments were evaluated by capillary gel electrophoresis (QIAGEN®, Germany). The band patterns were visualized by automatic silver staining.

Data analysis

The polymorphisms per locus were analyzed as a number of the alleles MAF, *Ho*, *He*, *PIC*, and *f* values of each locus were performed using the biocalculator software Cervus V3.0.7 (Tristan and Marshall, 1998-2014). Genetic and population structure were evaluated by STRUTURE software (Pritchard and Falus, 2009) in Bayesian clustering method.

RESULTS

Genetic diversity and heterozygosity

All 30 selected microsatellite markers in a domestic cat could amplify the DNA samples from 2 Thai native cat populations. The number of alleles per locus ranged from 1-14 alleles in Korat cat and 1-15 alleles in Siamese Cat. Among these 30 microsatellite markers, 2 markers (Zn-finger and Amelogenin) were used as sex determination. Zn-finger showed a heterozygous genotype (178, 184) for male and homozygous genotype (178, 178) for female. Amelogenin showed a heterozygous genotype (211, 231) for male and homozygous genotype (231, 231) for female. In this study, the sex identification was 100 percent accurate in Korat and Siamese cats when confirmed by the cat pedigree record, 2 alleles for a male cat and 1 allele for a female cat. In order to decrease analysis error, these 2 markers were not used to calculate an average genetic diversity parameter. There were 4 microsatellite markers that showed only one allele; allele 254, 208, 162 and 234 at marker FCA665, FCA105, FCA075, and FCA220, respectively. The result demonstrated that Korat and Siamese cats shared the same allele sizes in these 4 markers.

The average number of alleles from 28 microsatellite markers was 5.68 in Korat cat and 6.96 in Siamese Cat. Marker FCA045, FCA096, FCA441, FCA310, and FCA201 had an extremely high number of alleles ($\geq$ 5 alleles) in Siamese cat than Korat cat. The allele sizes of markers ranged from 124-284 bp. Most of the alleles were in the same interval between 2 Thai cat breeds demonstrating that they shared most of the allele sizes. FCA008 and FCA310 exhibited the highest number of alleles in Korat (14 alleles) and Siamese (15 alleles) cats, respectively. Therefore, it was the most polymorphic maker in these Thai native cats. The results of the number of alleles, allele sizes of each microsatellite marker, *Ho*, *He*, *PIC*, and *f* values of each locus were performed using the biocalculator software Cervus V3.0.7 as shown in Table 1.

Most of the microsatellite markers of either breed had *PIC* value higher than 0.50 except in markers FCA665, FCA105, FCA075, FCA220, FCA126, FCA290, and FCA298. The *PIC* values of each microsatellite locus ranges from 0.00-0.880 in Korat and 0.00-0.862 in Siamese cats (Table 1). The *PIC* value higher than 0.6 indicated high polymorphism, 0.30- 0.59 was moderate polymorphism and *PIC* < 0.3 was low polymorphism (Mateescu et al., 2005). In this study, the *PIC* value was very widely ranged. Four markers (FCA665, FCA105, FCA075, FCA220) had zero *PIC* values in both cat breeds.

Table 1 Marker name, allele sizes, number of the allele, observed heterozygosity (*Ho*), expected heterozygosity (*He*), polymorphic information content (*PIC*) and inbreeding coefficient (*f*) from 28 microsatellite markers in 37 Korat (K) and 30 Siamese cats (S).

Markers	Breed	Allele sizes (bp)	No. of allele	*Ho*	*He*	*PIC*	*f*
FCA008 [a*]	K	146-176	14	0.730	0.902	0.880	0.097
	S	146-172	13	0.567	0.718	0.690	0.119
FCA739 [b*]	K	245-277	10	0.944	0.866	0.838	-0.057
	S	245-273	9	0.933	0.858	0.824	-0.051
FCA733 [b*]	K	194-238	12	0.811	0.864	0.836	0.027
	S	198-238	11	0.733	0.875	0.845	0.079
F53 [a*]	K	156-188	11	0.757	0.857	0.829	0.057
	S	164-192	7	0.864	0.852	0.811	-0.016
FCA747 [b*]	K	150-174	10	0.757	0.826	0.791	0.045
	S	150-170	9	0.720	0.827	0.785	0.061
FCA077 [a*]	K	151-165	8	0.622	0.825	0.788	0.135
	S	143-193	9	0.667	0.844	0.805	0.112
FCA310 [a*]	K	132-152	8	0.676	0.822	0.787	0.082
	S	132-208	15	0.600	0.888	0.862	0.191
FCA441 [a*]	K	158-178	8	0.838	0.801	0.762	-0.033
	S	158-196	13	0.767	0.838	0.808	0.028
FCA223 [a*]	K	224-254	8	0.556	0.797	0.761	0.173
	S	228-258	8	0.567	0.732	0.681	0.120
F124 [a*]	K	223-251	8	0.270	0.786	0.744	0.485
	S	225-259	11	0.345	0.767	0.734	0.388
FCA229 [a*]	K	176-182	7	0.351	0.738	0.683	0.363
	S	174-196	6	0.433	0.808	0.763	0.289
FCA726 [b*]	K	237-259	9	0.297	0.732	0.677	0.438
	S	237-257	8	0.652	0.846	0.807	0.127
FCA096 [a*]	K	221-233	4	0.568	0.652	0.575	0.060
	S	219-255	10	0.367	0.741	0.709	0.345
FCA586 [a*]	K	210-234	6	0.135	0.638	0.571	0.658
	S	210-246	8	0.300	0.727	0.688	0.409
FCA124 [a*]	K	124-144	4	0.703	0.622	0.561	-0.069
	S	124-156	7	0.367	0.732	0.682	0.309
F42 [a*]	K	228-244	5	0.378	0.628	0.552	0.249
	S	218-244	5	0.250	0.650	0.565	0.432
FCA045 [a*]	K	172-180	3	0.595	0.588	0.489	-0.030
	S	172-188	9	0.300	0.789	0.748	0.425
FCA132 [a*]	K	149, 163	2	0.405	0.520	0.394	0.119
	S	149-169	6	0.667	0.770	0.716	0.037
FCA391 [a*]	K	262-278	5	0.270	0.424	0.386	0.282
	S	256-284	7	0.533	0.712	0.667	0.128
FCA201 [a*]	K	158-168	3	0.342	0.330	0.281	-0.032
	S	158-198	8	0.357	0.731	0.678	0.319
FCA126 [a*]	K	162, 174	2	0.405	0.328	0.271	-0.111
	S	162, 174	2	0.500	0.384	0.305	-0.142
FCA596 [a*]	K	164-184	3	0.081	0.080	0.077	-0.012
	S	162-192	6	0.143	0.627	0.588	0.623
FCA290 [a]	K	236, 242	2	0.054	0.053	0.051	-0.006
	S	236, 242	2	0.100	0.210	0.185	0.345
FCA298 [a]	K	252, 244	2	0.027	0.027	0.026	-0.002
	S	252, 244	2	0.067	0.066	0.062	-0.008
FCA665 [a]	K	254	1	0.000	0.000	0.000	Na
	S	254	1	0.000	0.000	0.000	Na
FCA105 [a]	K	208	1	0.000	0.000	0.000	Na
	S	208	1	0.000	0.000	0.000	Na
FCA075 [a]	K	162	1	0.000	0.000	0.000	Na
	S	162	1	0.000	0.000	0.000	Na
FCA220 [a]	K	234	1	0.000	0.000	0.000	Na
	S	234	1	0.000	0.000	0.000	Na

Published primer see reference; [a] = Menotti-Raymond et al., 1999; [b] = Menotti-Raymond et al., 2005; * = marker suitable for genetic diversity in Korat and Siamese cats; Na = not identified

In the Korat cat, 12, 7 and 9 markers had *PIC* value ≥ 0.6, 0.59-0.30 and < 0.30, respectively (Figure 1). In Siamese cat, 19, 3 and 6 markers had *PIC* value ≥ 0.6, 0.59-0.30 and < 0.30, respectively (Figure 2). The highest *PIC* was found in microsatellite marker FCA008 in Korat cat and FCA310 in Siamese cat. The lowest *PIC* value except for 4 monomorphic markers (*PIC* = 0) was found in microsatellite marker FCA298 in both breeds depicting low polymorphism in this marker locus.

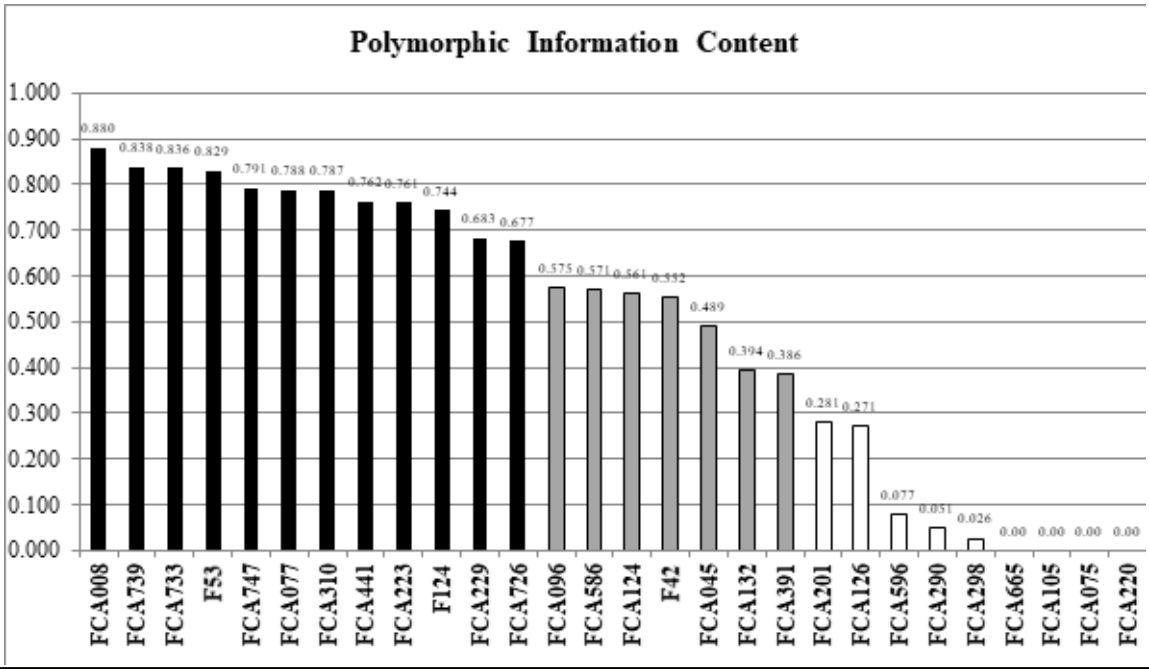

Figure 1 Polymorphic information content (*PIC*) of 28 microsatellite markers in 37 Korat cats. Black bar represented high polymorphism (*PIC* >o.6), gray bar represented moderate polymorphism (*PIC* 0.30- 0.59) and white bar represented low polymorphism (*PIC* < 0.3).

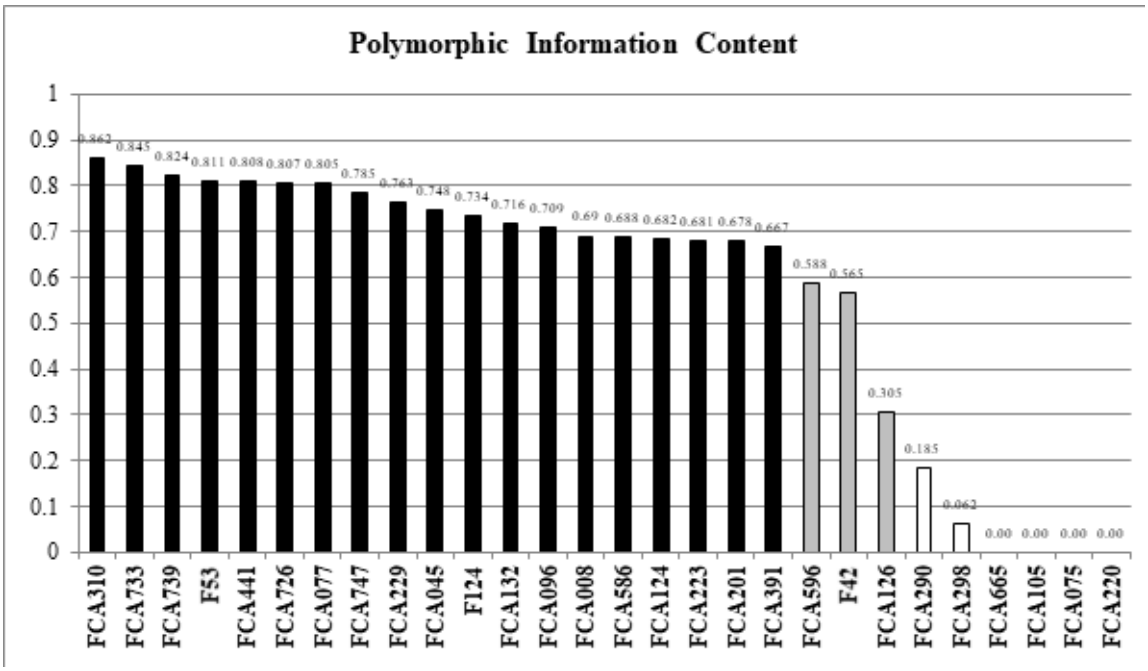

Figure 2 Polymorphic information content (*PIC*) of 28 microsatellite markers in 30 Siamese cats. Black bar represented high polymorphism (*PIC* >0.6), gray bar represented moderate polymorphism (*PIC* 0.30- 0.59) and white bar represented low polymorphism (*PIC* < 0.3).

The average parameters were performed by data from 28 microsatellite markers. The average *PIC*, *Ho*, *He* and *f* were 0.471, 0.413, 0.510 and 0.215 in Korat cats, 0.551, 0.417, 0.587 and 0.261 in Siamese cats and 0.511, 0.415, 0.549 and 0.238 in both breeds, respectively (Table 2). When compared *Ho* and *He* under the condition of Hardy-Weinberg Equilibrium (WHE). Both breeds show that an average observed heterozygosity level (0.415) was a few less than the expected heterozygosity (0.549), which meant a deviation from Hardy-Weinberg Equilibrium (Table 2). Overall, low observed heterozygosity and higher value of inbreeding coefficient had a correlation. It may be the few effects of breeding selection of breed by the breeders. This means that the genetic diversity of these 2 Thai native cats evaluated by a microsatellite markers method was in moderate level in Thailand.

Table 2 The average polymorphic information content (*PIC*), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and inbreeding coefficient (*f*) analyzed from 28 microsatellite markers in 37 Korat (K) and 30 Siamese cats (S).

Breed	PIC	*Ho*	*He*	*f*
Siamese cat	0.551	0.417	0.587	0.261
Korat cat	0.471	0.413	0.510	0.215
both breeds	0.511	0.415	0.549	0.238

Population structure of Korat and Siamese cats

The STRUCTURE software version 2.3 was used to investigate the population structure. The program identified populations that characterized by the allele frequencies from the genetic data and assigned individual to that population or genetic groups (K). The number of populations was set as K=2 or K=3 to assign 67 cats (number 1-37 were Korat cats and number 38-67 were Siamese cats) to the populations. The Korat cat samples came from 5 conservative catteries; 4 catteries from Nakhon Ratchasima province; cattery 1 (n=13) = no. 1-11, 28-29, cattery 2 (n=5) = no. 12-16, cattery 3 (n=4) = no. 17-20, cattery 4 (n=7) = no. 21-27 and cattery 5 from Bangkok (n=8) no. 30-37. For Siamese cats, samples came from 4 sources; Samut Songkhram province (n=6) no. 38-43, Nakhon Ratchasima province (n=2) no. 44-45, Bangkok (n=21) no. 47-67 and Nakhon Pathom province (n=1) no. 46. An individual cat was assigned to the given population, which was identified by color (Figure 3). The first split (K=2) separated Korat cat and Siamese cats. Every cat was assigned to the exact cat breed except cat number 2 that assigned both in Korat and Siamese cats. Siamese cats were divided into 2 subpopulations while Korat cats still group together in the same breed at K=3.

DISCUSSION

Microsatellite markers were a better indicator of more recent genetic diversity. Therefore, this study focused on microsatellite markers to evaluate genetic diversity in 2 Thai native cat breeds. The polymorphic information content reveals the features of the marker. There were 4 monomorphic microsatellite markers (FCA665, FCA105, FCA075 and FCA220), which were not suitable for studying the genetic diversity in Korat and Siamese cats.

The high *PIC* values > 0.6 were found in 12 markers of Korat cat and 19 markers of Siamese cat. The high to moderate informative markers were 19 markers in Korat cat and 22 markers in Siamese cats. In low *PIC* value markers, one marker (FCA201) significantly found high *PIC* value in Siamese cat but low *PIC* value in Korat cat. Two markers (FCA126, FCA596) had low polymorphism in Korat cats but were moderately polymorphic in Siamese cat and 2 markers (FCA290, FCA298) had low polymorphism in both cat breeds. This depicts that 22 selected markers except for FCA290 and FCA298 markers are efficient and polymorphic in these Thai cat breeds. Therefore, this selected set of markers was suitable for genetic diversity evaluation within each breed and useful for individual identification, parentage testing and forensic casework in future studies. In the previous study by Moreno et al., (2006), FCA008 locus had a high number of alleles in Jaguar (12 alleles), Korat cat (14 alleles) and Siamese cat (13 alleles) that correlate with our study. The allele sizes of 3 microsatellite markers FCA077 (140-154 bp), FCA008 (122-146 bp) and FCA096 (183-225 bp) of the domestic cat in South Africa (Wiseman et al., 2000) were overlapped with Korat cat and Siamese cat. That depict that these markers are highly conserved in Felidae.

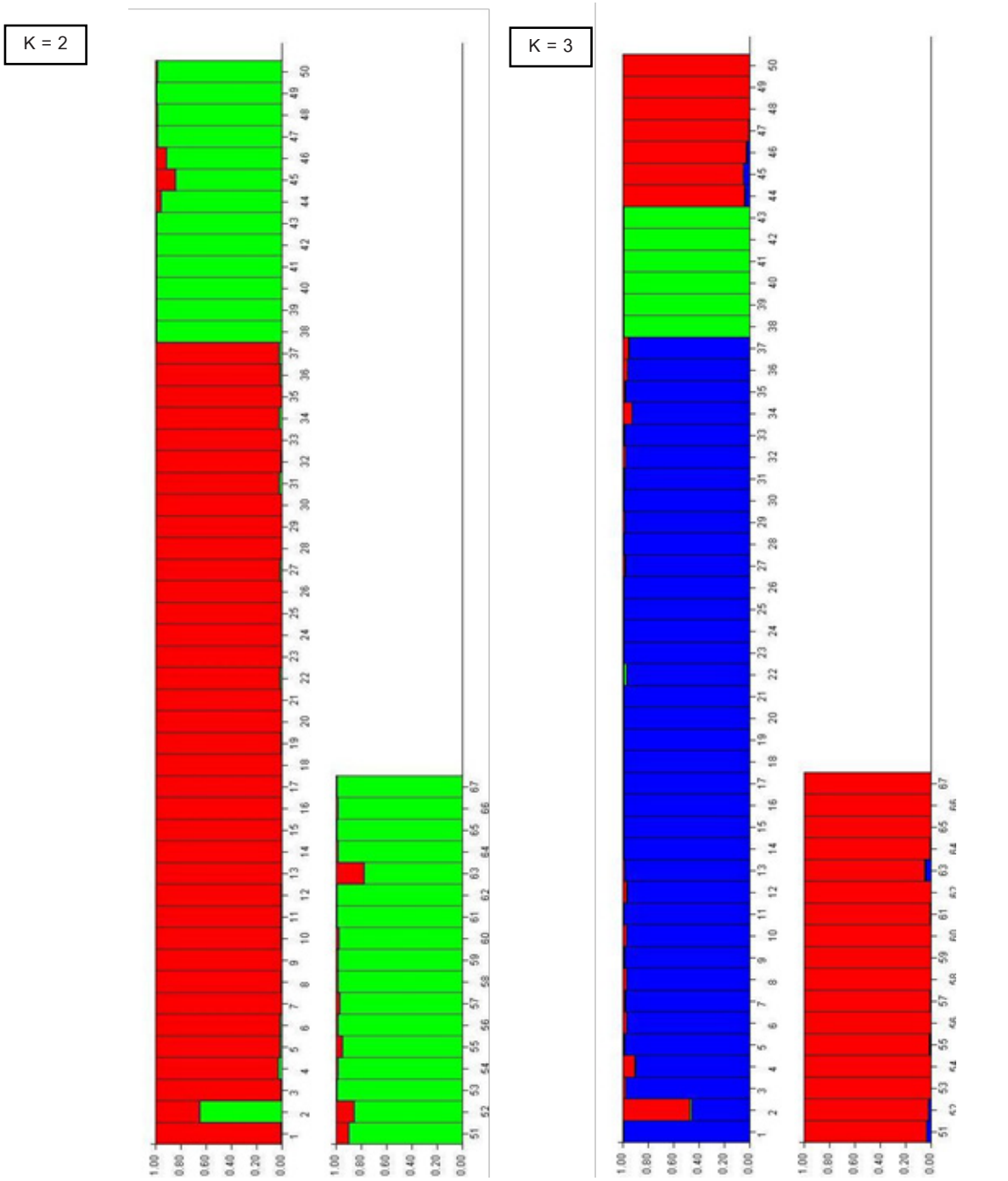

Figure 3 Histogram demonstrating the population structure of 2 cat breeds. Each column represents an individual cat. The y-axis represents the proportional estimate of genetic data to the given K (populations or genetic groups). Number 1-37 were Korat cats and number 38-67 were Siamese cats. An individual cat was assigned to the given population, which was identified by color. K=2 divided 67 cats into 2 populations; red was population 1 and green was population 2. K=3 assigned Korat cats (number 1-37) to the same population (blue color) and separated Siamese cats (number 38-67) into 2 subpopulations as shown in blue and red colors.

The genetic diversity was identified by *Ho*, *He* and *f* values. Lipinski et al. (2008) had studied genetic diversity in 39 Korat cat and 32 Siamese cats by using 38 microsatellite markers. The comparative all values of this study and the previously studied were shown in Table 3. The expected heterozygosity level in this study was 0.51 and 0.59 in Korat and Siamese cat that had the same values as the cats in the United State of America (USA) (0.52, 0.57) values (Lipinski et al., 2008). The observed heterozygosity level in this study was 0.41 and 0.42 in Korat and Siamese cat. The Ho values of Korat and Siamese cat in the previous report had higher *Ho* values (Lipinski et al., 2008, Menotti-Raymond et al., 2008). These data revealed the changed situation of genetic diversity of Korat and Siamese cats in the USA 10 years ago and this study. Genetic diversity of Korat and Siamese cat in Thailand was reduced compared to these cats in the USA for the past 10 years that was supported by the higher inbreeding coefficient 0.22 in Korat cat and 0.26 Siamese cats. However, the *f* value was nearly in range when compared with average *f* value of 22 natural breeds (0.02-0.23) (Table 3). These depict that the effect on the breeding selection of breed by the breeders was similar between Thai cat and foreign cat breeds. Thai cat breeders are the main factors with genetic variation effect in Thai native cats. Mating choice decided by the breeders such as mating between close relatives with the famous sire and queen may increase the proportion of homozygous alleles that decrease *Ho* and increase *f* value. Most of their gene pools were limited in a local area and most of them can only be found in the breeding kennel and ancient Thai cat conservation center. Therefore, the opportunity of the new gene transfer into a population is usually limited.

Table 3 Observed heterozygosity (*Ho*), expected heterozygosity (*He*) and inbreeding coefficient (*f*) between the previous report and this study.

Breed	N	*Ho*	*He*	*f*	Sources
Korat cat	39	0.56	0.57	0.02	Lipinski et al.,2008
	11	0.53	ND	ND	Menotti-Raymond et al., 2012
	37	0.41	0.51	0.22	This study
Siamese cat	32	0.47	0.52	0.10	Lipinski et al.,2008
	35	0.62	ND	ND	Menotti-Raymond et al., 2012
	30	0.42	0.59	0.26	This study
22 natural breeds	1176	0.34-0.69	0.38-0.73	0.02-0.23	Lipinski et al.,2008

ND = no data

The Governing Council of the Cat Fancy (GCCF) in the United Kingdom offers some comment if inbreeding coefficient is more than 0.25 represents a close mating risk of inherited disease in the cat population (https://www.gccfcats.org/). Our data indicated that the level of inbreeding is higher than we thought. The average *f* value was 0.22 in Korat cat and 0.26 in Siamese cats. In Thailand, there was no scientific evidence of inherited cat diseases in Korat and Siamese cats. However, the increase in *f* value, especially in a Siamese cat, should remind us how to prevent inbreeding depression in the future. This raises the conservation concern of Korat and Siamese cats, including other Thai cat breeds. The breeders were the important factor. Therefore, to prevent further inbreeding, it should recommend the Thai breeders for selective breeding and maintain the genetic integrity of these Thai native cats.

Based on the previous study (Lipinski et al., 2008), Bayesian clustering by STRUCTURE program discovered uniform modern cats based on the microsatellite markers. Genetic data were derived from over 1100 individuals, representing seventeen random bred populations from five continents and twenty-two breeds. The result showed Asian cats, including Korat and Siamese cat appeared to separate early and expand in relative isolation. In this study, STRUCTURE program was assigned individual cat to the right cat breeds. Korat and Siamese cats had shown uniform and genetically distinct of their breeds. This study served as a valuable genetic data and used as a model for the future investigation of the Thai native cat. Thai native cat breeders in Thailand did not have scientific data system for mating and breeding selection. Moreover, crossbreeding with free-ranging domestic cats or Thai feral cats is usually accidental finding especially in the urban area. If the owners ignore, it was one of the main threat to their purebred status. Breeding management and pedigree recording should be considered to prevent the incorporation of feral cat genes into the Thai purebred cat gene pool, prevent inbreeding in the future and maintain their genetic integrity.

CONCLUSION

This set of microsatellite markers is efficient and suitable for genetic study in the Korat and Siamese cats. The results revealed a moderate level of genetic diversity that correlates with inbreeding coefficient. The level of inbreeding is higher than we thought. Even though, there was no report of inherited cat diseases in these cats. However, it raises the awareness to conserve the pure Thai cat breed. The information on the inbreeding situation should be provided to the Thai cat breeder in order to collaborate together to prevent inbreeding depression in the future.

CONFLICT of INTEREST

There is no conflict of interest.

REFERENCES

Department of livestock, 2016. A Survey of dog and cat population [Online]. www.http://dcontrol.dld.go.th/dcontrol/index.php/rabies/747-dogpop2016.

Ellegren, H., 2004. Microsatellites: simple sequences with complex evolution. Nat. Rev. Genet. 5, 435-444.

Halliburton, R., 2004. Introduction to population genetics. Pearson Education, Inc., Upper Saddle River, NJ. pp. 650.

Imes, D.L., Geary, L.A., Grahn, R.A., Lyons, L.A., 2006. Albinism in the domestic cat (*Felis catus*) is associated with a tyrosinase (TYR) mutation. Anim. Genet. 37, 175–178.

Lipinski, M.J., Froenicke, L., Baysac, K.C., Billings, N.C., Leutenegger, C.M., Levy, A.M., Longeri, M., Niini, T., Ozpinar, H., Slater, M.R., Pedersen, C.N., Lyons, L.A., 2008. The ascent of cat breeds: genetic evaluations of breeds and worldwide random-bred populations. Genomics. 91, 12-21.

Litt, M., Luty, J.A., 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. 44, 388–396.

Mateescu, R.G., Zhang, Z., Tsai, K., Phavaphutanon, J., Burton-Wurster, N.I., Lust, G., Quaas, R., Murphy, K., Acland, G.M., Todhunter, R.J., 2005. Analysis of allele fidelity, polymorphic information content, and density of microsatellites in a genome-wide screening for hip dysplasia in a crossbreed pedigree. J. Hered. 96, 847-853.

Menotti-Raymond, M., David, V.A., Lyons, L.A., Schaffer, A.A., Tomlin, J.F., Hutton, M.K., O'Brienet, S.J., 1999. A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics. 57, 9-23.

Menotti-Raymond, M., David, V.A., Pflueger, S.M., Lindblad-Toh, K., Wade, C.M., O'Brien, S.J., Johnsonet, W.E., 2008. Patterns of molecular genetic variation among cat breeds. Genomics. 91, 1-11.

Menotti-Raymond, M., David, V.A., Schaffer, A.A., Tomlin, J.F., Eizirik, E., Phillip, C., Wells, D., Pontius, J.U., Hannah, S.S., O'Brien, S.J., 2009. An autosomal genetic linkage map of the domestic cat, *Felis silvestris catus*. Genomics. 93, 305-313.

Menotti-Raymond, M.A., David, V.A., Wacher, L. L., Butler, J.M., O'Brien, S.J., 2005. An STR forensic typing system for genetic individualization of domestic cat (*Felis catus*) samples. J. Forensic Sci. 50, 1061-1070.

Menotti-Raymond, M., David, V.A., Weir, B.S., O'Brien, S.J., 2012. A population genetic database of cat Breeds developed in coordination with a domestic cat STR multiplex. J. Forensic Sci. 57, 596-601.

Moreno, V.R., Grisolia, A.B., Campagnari, F., Milazzotto, M., Adania, C.H., Garcia, J.F., Souza, E.B., 2006. Genetic variability of *Herpailurus yagouaroundi*, *Puma concolor* and *Panthera onca* (Mammalia, Felidae) studied using *Felis catus* microsatellites. Gen. Mol. Biol. 29, 290-293.

Perez-Jimenez, M., Besnard, G, Dorado, G., Hernandez, P., 2013. Varietal tracing of virgin olive oils based on plastid DNA variation profiling. PLOS One 8:e70507.

Phumichai, C., Phumichai, T., Wongkaew, A., 2015. Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild Hevea rubber. Plant Mol. Biol. Report. 33, 1486-1498.

Pritchard, J.K., Falus, D., 2009. Documentation for structure software version 2.3. The University of Chicago Press, Chicago.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Sciences. 239, 487–491.

Sambrook, J., Russell, D.W., 2001. Molecular cloning: A laboratory manual 3rd ed. vol.1. Cold Spring Harbor Laboratory Press, New York. pp. 643.

The Governing Council of the Cat Fancy (GCCF) in United Kingdom, 2016. GCCF Outcrossing Policy [Online]. http://www.gccfcats.org/.

Weber, J.L., May, P.E., 1989. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44, 388–396.

Wiseman, R., O'Ryan, C., Harley, E.H., 2000. Microsatellite analysis reveals that domestic cat (*Felis catus*) and southern African wild cat (*F. lybica*) are genetically distinct. Anim. Conserv. 3, 221-228.

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