

9-1-2011

Evaluation of Microsatellite Polymorphism and Genetic Variability in Thai Ridgeback and Bangkaew Dogs

Janjira Phavaphutanon

Sudtisa Laopiem

Follow this and additional works at: <https://digital.car.chula.ac.th/tjvm>



Part of the [Veterinary Medicine Commons](#)

Recommended Citation

Phavaphutanon, Janjira and Laopiem, Sudtisa (2011) "Evaluation of Microsatellite Polymorphism and Genetic Variability in Thai Ridgeback and Bangkaew Dogs," *The Thai Journal of Veterinary Medicine*: Vol. 41: Iss. 3, Article 3.

DOI: <https://doi.org/10.56808/2985-1130.2310>

Available at: <https://digital.car.chula.ac.th/tjvm/vol41/iss3/3>

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Veterinary Medicine by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Evaluation of Microsatellite Polymorphism and Genetic Variability in Thai Ridgeback and Bangkaew Dogs

Janjira Phavaphutanon^{1*,2,3} Sudtisa Laopiem⁴

Abstract

The origin of dogs came from the limited number of founders leading to inbreeding or genetic disease within or among breeds. Thai Ridgeback (TR) and Bangkaew (BK) dogs were the only native dog breeds that we believed to originate in Thailand. The study of genetic variability in Thai dogs (39 Thai Ridgeback dogs, 82 Bangkaew dogs) was defined by using 12 microsatellite markers. These DNA markers were highly informative and distributed through the canine genome. The result from these markers revealed the higher number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) in TR and BK dogs. The average Ho, He and PIC were 0.72, 0.77, 0.73 in Bangkaew and 0.81, 0.78, 0.75 in Thai Ridgeback dogs. The average inbreeding coefficient was 0.072 and -0.019 in Bangkaew and Thai Ridgeback dogs, respectively. The low and negative inbreeding coefficient values revealed no inbreeding problem especially in Thai Ridgeback dog. The information generated in this study could be used for further genetic study in Thai dogs and to investigate the population structure in these dog breeds.

Keywords: Bangkaew dogs, dog, genetic variability, microsatellite, Thai Ridgeback dogs

¹Department of Companion Animals Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

²Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

³Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand

⁴Kamphaeng Saen Veterinary Diagnosis Center, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom 73140, Thailand

*Corresponding author E-mail: foetjrp@ku.ac.th

บทคัดย่อ

การประเมินความหลากหลายของเครื่องหมายดีเอ็นเอไมโครแซทเทลไลต์และความหลากหลายทางพันธุกรรมในสุนัขไทยหลังอานและสุนัขบางแก้ว

จันทร์จิรา ภาวภูตานนท์^{1*,2,3} สุทธิษา เหล่าเปี่ยม⁴

ต้นกำเนิดของสุนัขพบว่ามาจากสุนัขต้นกำเนิดจำนวนจำกัด ทำให้เกิดปัญหาการผสมเลือดชิดและโรคทางพันธุกรรมภายในสุนัขสายพันธุ์เดียวกันหรือต่างสายพันธุ์ สุนัขไทยหลังอานและสุนัขบางแก้วเป็นสุนัขพื้นเมืองที่คาดว่าจะมีต้นกำเนิดในประเทศไทย จากการศึกษาความหลากหลายทางพันธุกรรมในสุนัขไทย (สุนัขไทยหลังอาน 39 ตัว สุนัขบางแก้ว 82 ตัว) โดยใช้เครื่องหมายดีเอ็นเอไมโครแซทเทลไลต์จำนวน 12 เครื่องหมาย เครื่องหมายดีเอ็นเอชนิดนี้พบว่ามีหลากหลายและกระจายอยู่ทั่วทั้งจีโนมในสุนัข ผลจากการศึกษาเครื่องหมายดีเอ็นเอเหล่านี้พบจำนวนอัลลีล ค่า observed heterozygosity (HO) ค่า expected heterozygosity (He) และค่า polymorphic information content (PIC) สูงทั้งในสุนัขไทยหลังอานและบางแก้ว ค่าเฉลี่ยของ Ho, He และ PIC เท่ากับ 0.72, 0.77, 0.73 ในสุนัขบางแก้ว และ 0.81, 0.78, 0.75 ในสุนัขไทยหลังอาน ค่าเฉลี่ยของอัตราเลือดชิดเท่ากับ 0.072 และ -0.019 ในสุนัขบางแก้วและสุนัขไทยหลังอานตามลำดับ ค่าอัตราเลือดชิดที่ต่ำและติดลบบ่งบอกว่าไม่น่ามีปัญหาการผสมเลือดชิดในสุนัขเหล่านี้ โดยเฉพาะในสุนัขไทยหลังอาน ข้อมูลที่ได้จากการศึกษาในครั้งนี้สามารถนำไปใช้ศึกษาลักษณะทางพันธุกรรมในสุนัขไทยเพื่อสำรวจโครงสร้างทางประชากรในสุนัขเหล่านี้ต่อไป

คำสำคัญ: สุนัขบางแก้ว สุนัข ความหลากหลายทางพันธุกรรม ไมโครแซทเทลไลต์ สุนัขไทยหลังอาน

¹ภาควิชาเวชศาสตร์คลินิกสัตว์เลี้ยง คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน นครปฐม 73140

²ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

³ศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตร สำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยี สำนักงานคณะกรรมการการอุดมศึกษา กรุงเทพฯ 10900

⁴หน่วยงานชั้นสูตโรครัสต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน นครปฐม 73140

*ผู้รับผิดชอบบทความ E-mail: fvetjrp@ku.ac.th

Introduction

DNA is the genetic material of organisms, hence DNA variations reflect the genetic difference between individuals. Mitochondrial DNA data indicate the origin of domestic dogs date back to 15,000 yr B.P. from at least 5 female wolf lines. Among these, the clade from East Asian dog showed highly genetic variation and had evidence that the haplotypes in Europe and Southwest Asia derive from a subset of the East Asian types (Savolainen et al., 2002). However, the mitochondrial DNA evolution is too slow to identify the relationships among modern dog breeds because these dogs were developed for fewer than 400 years.

A wide variety of techniques can be used to measure DNA variation; direct sequencing of DNA or PCR technique to test the difference of DNA fragment lengths. Nowadays, the advancement and discovery of DNA-based genetic markers give a new hope for investigators to identify genetic variabilities in many populations. These markers have the ability to detect

genetic variations at the DNA level without interfering the expression of phenotype. Genetic variability can be detected from polymorphic of the markers in the population such as allelic diversity, observed heterozygosity, expected heterozygosity and polymorphic information content. Therefore, there has been an explosion in the use of marker-based methods in genetic studies.

The common repeats of microsatellite in the canine genome are (CA)_n, (GATA)_n or (CAG)_n (Ostrander et al., 1992). The most frequent repeats are (CA)_n or (GT)_n which are distributed approximately every 43 kb. Repeat numbers of tri- or tetranucleotide are found every 320 kb and the most polymorphic tetra-nucleotide repeat is (GAAA)_n (Ruvinsky and Sampson, 2001). Although tetranucleotide repeat is less frequent than dinucleotide repeat, it proves to have more polymorphism and gives less stutter bands. In dogs, microsatellite loci have heterozygosity values ranging from 36-55% within breed (Holmes et al., 1993; Fredholm and Wintero, 1995; Zajc et al., 1997 and Zajc and Sampson, 1999). Microsatellite alleles are differentiated by their sizes (number of repeats) which

can be detected by gel electrophoresis. They show abundance, uniform distribution and a high degree of polymorphism in genomes. Therefore, they are useful tools for mapping, paternity testing, individual identification, forensic application and population study in human beings and animals.

There were reports of microsatellite markers succeeding in assign dogs from many breeds to their breed of origin (Koskinen and Bredbacka, 2000; Parker et al., 2004). Currently, the World Canine Organization (Federation Cynologique Internationale, FCI) classified 347 modern dog breeds including Thai Ridgeback dog into 10 groups according to their function and area of origin (Sundqvist et al., 2006). These dog breeds differ widely in size, behavior, characteristic and physiology (Wayne and Vila, 2001). The extreme phenotypic variation between breeds of dogs is possibly originated by human selection or their multiple origins with a limited gene pool. Mating between close relatives may increase the proportion of homozygous recessive alleles, the major cause of heritable genetic disease. To maintain the desired characteristics and decrease the genetic abnormality, researchers and breeders are now showing increasing interest in conserving genetic variability within dog breeds.

Bangkaew and Thai Ridgeback dogs are the only pure-bred dogs that are believed to be indigenous to Thailand. Thai Ridgeback is an ancient breed of dog and one of only 3 breeds (Thai Ridgeback, Rhodesian Ridgeback and Phu Quoc Ridgeback) that has a ridge of hair that runs along its back in the opposite direction to the rest of the coat. The origin of the Thai Ridgeback dog is undocumented, but the breed was developed in eastern Thailand. Bangkaew dog is an old breed. The breed traces its ancestry back to a cross between a native black & white female dog and a now-extinct wild dog. It is a medium size, double-coated dog. The coats form the ruff around the neck and shoulders forming a lion-like and a pluming tail, which is more noticeable on male than on female. The color is usually white with shades of red, gray, brown, and black in a wide variety of patterns. These dogs are indigenous to Thailand and are believed that their ancestors descended from wild wolves. The dog populations developed and carried characteristic genetic signatures appropriate for living in Thailand, so most of their gene pools are limited in a local area. Moreover, breeding management limits to the specific group of breeder who lead and control the characteristic of the dogs. To maintain breed characteristic in the line breeding, close relative, cousin or popular sire are factors of selective breeding. However, the cause of increase in tendency of inbreeding and inheriting genetic disease in these two dog breeds still remain unknown. No data have ever been reported for their genetics that can be used for selection and breeding plan to improve genetic variability and prevent inbreeding in the population.

The aim of this study was to investigate genetic variation in Thai Ridgeback and Bangkaew dogs. Inbreeding coefficient for each marker position

in each breed was also analyzed to investigate the degree of inbreeding among these dog breeds.

Materials and Methods

Sample collection and DNA extraction: The sample comprised 121 individuals representing the dog breeds in Thailand: Bangkaews dogs (BK; n=82) and Thai Ridgeback dogs (TR; n=39). Genomic DNA of 82 Bangkaew and 39 Thai Ridgeback dogs were extracted from whole blood by the standard phenol-chloroform (Sambrook and Russell, 2001). The quality and concentration of DNA was examined by UV-spectrophotometer and the concentration was adjusted to 50 ng/μl.

Marker selection, PCR amplification and fragment analysis: Twelve microsatellite markers were chosen from different chromosomes to perform the analysis (FH2004, FH2097, FH2132, FH2608, FH2161, FH2016, FH2158, FH2626, FH2010, FH2138, FH2422 and FH2145). The primer sequences were available at http://research.nhgri.nih.gov/dog_genome/guyon2003/guyon_data/mss2.html. All these microsatellite markers were amplified with a thermal cycle program: 5-min denaturation at 94°C followed by 30 cycles of 1-min denaturation at 94°C, 1-min annealing at 60°C, and 1-min extension at 72°C, and final 15-min extension at 72°C. For fragment analysis, 2 μl of PCR product were mixed with 8 μl of formamide dye solution, denatured for 5 min at 95°C and loaded into 6% polyacrylamide 7 M urea sequencing gel. The band patterns were visualized by silver staining.

Statistical analysis: The polymorphisms per locus were analyzed as number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC) and inbreeding coefficient of each locus were performed using the software PowerMarker V3.25 (Liu and Muse, 2005).

Results

Analysis of genotypes from 12 microsatellite markers in Thai Ridgeback and Bangkaew dogs yielded several findings. The details of markers such as location on chromosome, repeat numbers and major allele frequency (MAF) are given in Table 1. Major allele frequency ranged from 0.20-0.46. The highest value was found in Thai Ridgeback dogs (FH2010) and the lowest was in Bangkaew dogs (FH2016).

The number of alleles from Thai Ridgeback and Bangkaew dogs were compared with alleles derived from published papers of several dog breeds (Fig 1) (Koskinen and Bredbacka, 2000; Ichikawa et al., 2001; Clark et al., 2004; Klukowska et al., 2003; Irion et al., 2005; Puja et al., 2005; Lupke and Distl, 2005; Verardi et al., 2006; Kang et al., 2009).

The number and distribution of alleles varied among markers and between dog breeds, ranging from 3-27 alleles per marker. Bangkaew and Thai Ridgeback dogs gave the average number of

alleles at 8.83 and 8.0. The total numbers of alleles observed within each breed were 106 in Bangkaew and 96 in Thai Ridgeback dogs. Most of the markers in Thai Ridgeback and Bangkaew dogs were in the group of high number of alleles (≥ 6 alleles) except FH2010 (3 alleles) and FH2145 (4 alleles) in Thai Ridgeback dogs. The dog from Asia such as Poongsan, Jindo (Kank et al., 2009), Bali street dogs (Irion et al., 2005), Kintamani and Bali dogs (Puja et al., 2005) showed high number of alleles in several markers especially in marker FH2004 and FH2132 (Fig 1). The number of alleles per breed mirrored the level of heterozygosity in dogs (Irion et al., 2003)

Table 1 Marker location on chromosome and major allele frequency (MAF) from 12 microsatellite markers in Bangkaew (BK) and Thai Ridgeback (TR) dogs.

Markers	chromosome	Repeat	Major allele frequency	
			BK	TR
FH2004	CFA11	(GAAA)13	0.38	0.45
FH2097	CFA04	(GAAA)16	0.36	0.30
FH2132	CFA02	(GAAA)35	0.43	0.20
FH2608	CFA02	Tetra-repeat	0.38	0.42
FH2161	CFA21	(GAAA)21	0.34	0.28
FH2016	CFA01	(GAAA)25	0.20	0.33
FH2158	CFA20	(GAAA)44	0.27	0.34
FH2626	CFA22	Tetra-repeat	0.38	0.23
FH2010	CFA24	(CAAA)10	0.41	0.46
FH2138	CFA08	(GAAA)28	0.34	0.26
FH2422	CFA10	Tetra-repeat	0.44	0.34
FH2145	CFA03	(GAAA)8	0.43	0.30
Mean			0.36	0.33

The data of many microsatellite markers from different articles were presented in dog breed from various populations (Fig 2) (Koskinen and Bredbacka, 2000; Lupke and Distl, 2005; Verardi et al., 2006; Kang et al., 2009; Kanthaswamy et al., 2009). The number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) in several breed were demonstrated. Unfortunately, no information from Thai Ridgeback and Bangkaew dogs has ever been reported. This study represented the variability of microsatellite markers within Thai Ridgeback and Bangkaew dog breeds and between several dog breeds from many articles. The average Ho, He and PIC were 0.72, 0.77, 0.73 in Bangkaew dogs and 0.81, 0.78, 0.75 in Thai Ridgeback dogs. Thai Ridgeback and Bangkaew dogs displayed highly informative values of these markers compared to other dog breeds. Dog breed from Asia (Poongsan and Jindo from Korea; Bali street dogs, Kintamani and Bali dogs from Indonesia) also showed high value of genetic variability (Fig 2). The observed heterozygosity in these dog breeds ranged from 0.13 (Rottweiler) -0.92 (Toy Poodle) in FH2004, 0.54 (Pembroke Welsh Corgi) -0.95 (Thai Ridgeback) in FH2132, 0.78 (Bangkaew) -0.84 (Thai Ridgeback) in FH2097, 0.78 (Thai Ridgeback) -0.82 (Bangkaew) in FH2608, 0.52

(Bangkaew) -0.75 (Poongsan) in FH2161, 0.82 (Hanoverian Hound) -0.91 (Thai Ridgeback, Bangkaew) in FH2016, 0.70 (Bangkaew) -0.76 (Thai Ridgeback) in FH2158, 0.66 (Bangkaew) -0.91 (Thai Ridgeback) in FH2626, 0.64 (Bangkaew) -0.74 (Thai Ridgeback) in FH2138, 0.75 (Bangkaew) -0.89 (Thai Ridgeback) in FH2422, 0.25 (Shih Tzu, Standard Poodle) -1.00 (Yorkshire Terrier) in FH2010 and 0.76 (Thai Ridgeback) -0.80 (Bangkaew) in FH2145.

The range and average of gene diversity (expected heterozygosity) of 12 markers were 0.59-0.88 and 0.78 in Thai Ridgeback and 0.69-0.85 and 0.77 in Bangkaew dogs. Out of these markers, most breeds had gene diversity > 0.5 except Rottweiler (0.13), Pembroke Welsh Corgi (0.15) and Bedlington Terrier (0.35) in FH2004, Miniature Poodle (0.4) and Standard Poodle (0.4) in FH2010. Polymorphic information content was highest in FH2132 and lowest in FH2161. However, most of the markers were informative (PIC values ≥ 0.5) except FH2161 in German Shepherd (0.44) (Fig 2).

There were 11 markers that Thai Ridgeback and Bangkaew dogs had some alleles in the same allelic sizes, but in different allelic frequency; 5 alleles in FH2004 (232, 236, 240, 244 and 248 bp), 6 alleles in FH2097 (276, 280, 284, 288, 292 and 296 bp), 6 alleles in FH2608 (244, 248, 252, 258, 262 and 356 bp), 4 alleles in FH2161 (248, 252, 256 and 260 bp), 5 alleles in FH2016 (288, 296, 304, 308 and 317 bp), 3 alleles in FH2158 (302, 314 and 318 bp), 4 alleles in FH2626 (214, 226, 230 and 232 bp), 2 alleles in FH2010 (230 and 238 bp), 4 alleles in FH2138 (266, 270, 344 and 406 bp), 3 alleles in FH2422 (184, 192 and 204 bp) and 3 alleles in FH2145 (276, 280 and 292 bp). Only marker FH2132 had the unique alleles both in Thai Ridgeback and Bangkaew dogs. Allele frequencies for each marker were presented in Fig 3.

In general, most dog breeds had similar ranges of allelic sizes. The widest range of alleles was in FH2138 (238 bp) and the smallest range was in FH2097 and FH2145 (24 bp) (Table 2). There was small differences in allelic calling (1 bp) between this study in FH2004 (Klukowska, et al., 2003) and FH2016, FH2138 (Francisco et al., 1996). The allele 228, 229 bp and 244, 245 bp in FH2004, 317, 318 bp in FH2016 and 270, 271 in FH2138 should be the same allele. This situation can occur in different laboratories that use a variety of analytical equipment. However, the difference should not more than one base pair.

Inbreeding coefficient that measures the reduction of heterozygotes ranged from a high value of 0.322 in FH2161 to a low value of -0.316 in FH2010. The average inbreeding coefficient in Bangkaew dogs was 0.072 and in Thai Ridgeback dogs was -0.019. In Thai Ridgeback dogs, more than half of the markers had negative inbreeding coefficient. The negative values indicated an excess of heterozygotes than expected value under Hardy Weinberg Equilibrium (HWE).

Table 2 Allelic sizes of each marker in this study and studies from previous publications.

Locus	Breed	Allelic size (bp)	References
FH2004	Thai Ridgeback	232, 236, 240, 244, 248, 304	Present study
	Bangkaew	228, 232, 236, 240, 244, 248, <u>296</u> , <u>300</u> , <u>312</u>	
	Wolf	<u>102-196</u>	Verardi et al., 2006
	Red fox	209-229	Klukowska et al., 2003
	Arctic fox	209-245	
	Beagle	224, 236, 240, 244, 248, <u>316</u> , <u>320</u> , <u>324</u>	Ichikawa et al., 2001
	Labrador Retriever	236, 240, 244, 304	
FH2097	Thai Ridgeback	276, 280, 284, 288, 292, 296	Present study
	Bangkaew	<u>272</u> , 276, 280, <u>282</u> , 284, 288, <u>290</u> , 292, 296	
	nd	288	Francisco et al., 1996
FH2132	Thai Ridgeback	<u>240</u> , <u>248</u> , 270, 278, 282, 286, 326, 334, <u>351</u> , <u>360</u> , <u>368</u> , <u>380</u>	Present study
	Bangkaew	264, 280, 284, 288, 306, 310, 318, 342, <u>358</u> , <u>362</u> , <u>372</u> , <u>392</u>	
	Hanoverian Hound	256-344	Lupke and Distl, 2005
	nd	272	Francisco et al., 1996
FH2608	Thai Ridgeback	244, 248, 252, 258, 262, <u>266</u> , <u>278</u> , 356, <u>364</u> , <u>375</u> , <u>384</u>	Present study
	Bangkaew	<u>238</u> , 244, <u>246</u> , 248, 252, 258, 262, 356, <u>360</u> , <u>368</u>	
FH2161	Thai Ridgeback	<u>244</u> , 248, 252, 256, 260, <u>264</u> , <u>268</u>	Present study
	Bangkaew	<u>236</u> , <u>238</u> , <u>242</u> , <u>246</u> , 248, 252, 256, 260	
	nd	<u>250</u>	Francisco et al., 1996
FH2016	Thai Ridgeback	<u>275</u> , 288, 296, 300, 304, 308, 312, 317	Present study
	Bangkaew	<u>280</u> , 284, 288, 292, 296, 298, 304, 308, 310, 317	
	Hanoverian Hound	284-312	Lupke and Distl, 2005
	nd	318	Francisco et al., 1996
FH2158	Thai Ridgeback	<u>267</u> , <u>272</u> , <u>288</u> , <u>292</u> , 302, 314, 318, <u>336</u> , <u>368</u> , <u>376</u>	Present study
	Bangkaew	<u>298</u> , 302, <u>306</u> , <u>310</u> , 314, 318, <u>334</u> , 338, <u>341</u> , <u>344</u> , <u>352</u>	
	nd	274	Francisco et al., 1996
FH2626	Thai Ridgeback	214, <u>218</u> , <u>224</u> , 226, 230, 232, <u>236</u> , <u>240</u> , <u>244</u> , <u>248</u>	Present study
	Bangkaew	214, <u>222</u> , 226, <u>228</u> , 230, 232, <u>234</u>	
FH2010	Thai Ridgeback	230, 234, 238	Present study
	Bangkaew	<u>211</u> , 224, 228, 230, 238, <u>242</u>	
	nd	224, 228, 232, 236	Eichmann et al., 2004
	Red fox	216-232	Klukowska et al., 2003
	Arctic fox	216-236	
	Beagle	226, 230, 234, 238	Ichikawa et al., 2001
	Labrador Retriever	226, 230, 234, 238	
	Beagle	226, 230, 234, 238	Ichikawa et al., 2001
	Labrador Retriever	226, 230, 234, 238	
	German Shepherd	220, 224, 232	Padar et al., 2001
	nd	228	Francisco et al., 1996
FH2138	Thai Ridgeback	266, 270, 274, <u>306</u> , <u>332</u> , 344, <u>378</u> , <u>386</u> , <u>390</u> , <u>398</u> , 406	Present study
	Bangkaew	<u>258</u> , <u>262</u> , 266, 270, 344, <u>400</u> , 406, <u>417</u> , <u>428</u> , <u>444</u> , <u>448</u> , <u>474</u> ,	
	nd	271	Francisco et al., 1996
FH2422	Thai Ridgeback	<u>149</u> , <u>161</u> , <u>171</u> , 184, 192, 204, <u>210</u> , <u>216</u>	Present study
	Bangkaew	<u>178</u> , 184, <u>188</u> , 192, 204	
FH2145	Thai Ridgeback	276, 280, <u>286</u> , 292	Present study
	Bangkaew	<u>268</u> , 276, 280, <u>284</u> , <u>288</u> , 292	
	nd	280	Francisco et al., 1996

nd: no data of dog breeds, underline: alleles found only in one breed

Discussion

The aim of this study was to identify genetic variability in Thai Ridgeback and Bangkaew dogs by using the polymorphic of microsatellite markers. The results of heterozygosity values were high in both Thai breeds. When compared with other previous studies that use the same microsatellite markers, mean observed heterozygosity values in Thai Ridgeback (0.81) and Bangkaew dog (0.72) were

similar to 3 dog breeds from South Korea; Poongsan (0.71), Jindo (0.86), Beagle (0.78) (Kang et al., 2009); 5 Spanish dog breeds (0.70-0.77) (Morera et al., 1999), but higher than Golden Retriever (0.61), German Shepherd (0.62), Wirehaired Dachshund (0.67), Pembroke Welsh Corgi (0.55), Bedlington Terrier (0.55) (Koskinen and Bredbacka, 2000); German Shepherd (0.65) and Greyhound (0.66) (Kang et al., 2009).

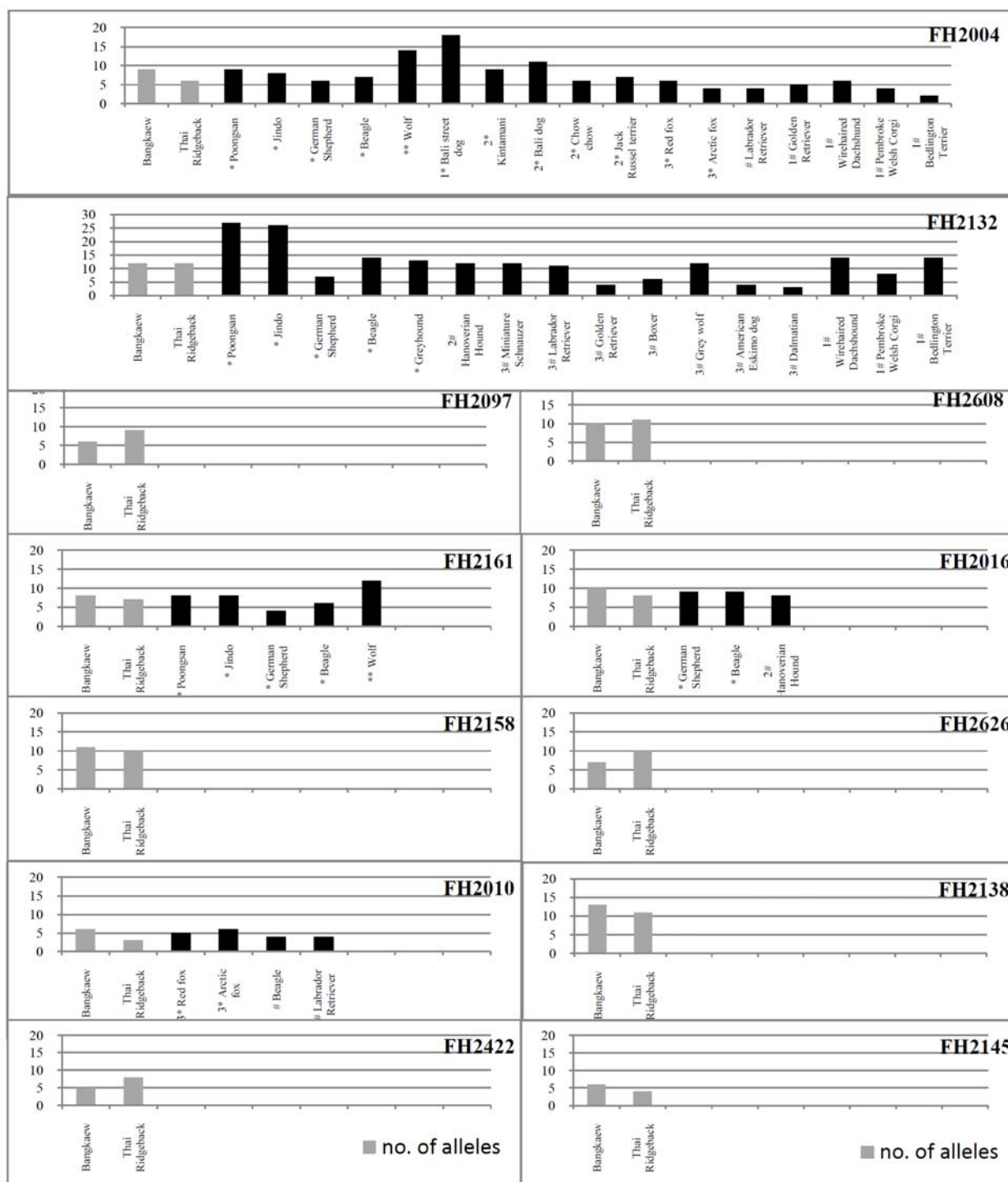


Figure 1 Number of alleles in Thai Ridgeback and Bangkaew dogs compared to other dog breeds from others studies.

* Kang et al., 2009,** Verardi et al., 2006, 1* Irion et al., 2005, 2* Puja et al., 2005, 3* Klukowska et al., 2003, #Ichikawa et al., 2001, 1# Koskinen and Bredbacka, 2000, 2# Lupke and Distl, 2005, 3# Clark et al., 2004

Gene diversity (expected heterozygosity) within breeds had high values in Thai Ridgeback (0.78) and Bangkaew dogs (0.77), but lower than Poongsan (0.84) and Jindo (0.85) (Kang et al., 2009). However, the values were close to Turkey dogs; Kangal (0.74) and Turkish Greyhound (0.71) (Altunok et al., 2005). However, the values were higher than in

East Asia village dogs (0.55-0.68) (Boyko et al., 2009) and wolf (0.52) (Verardi et al., 2006). The present study also demonstrated that every marker showed their unique alleles which differed among dog breeds. However, it requires more samples from many dog breeds to test with these markers before the final conclusion.

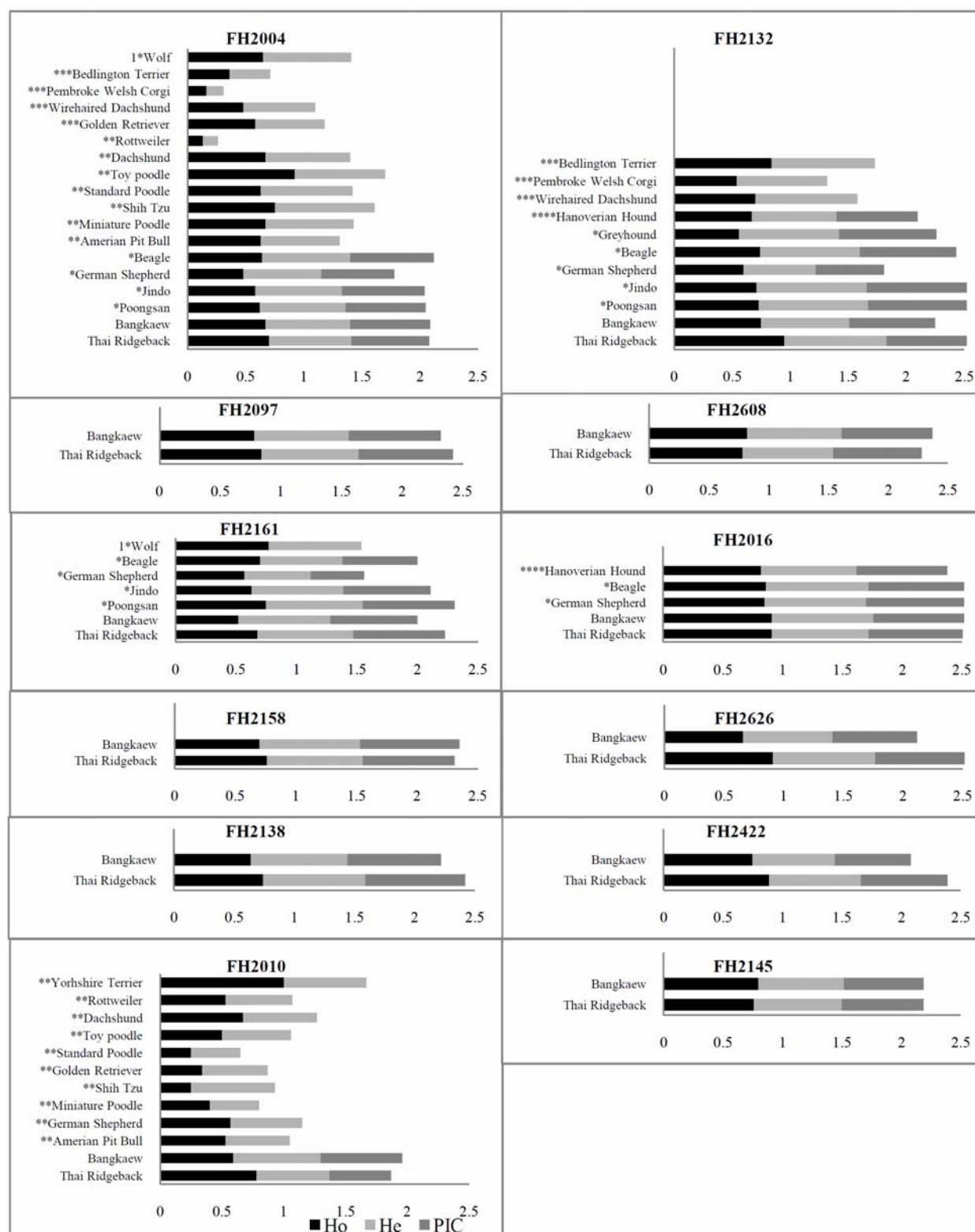


Figure 2 Results of observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content values (PIC) obtained for each microsatellite marker in Thai Ridgeback and Bangkaew dogs from this study compared to data from others studies.

* Kang et al., 2009, ** Kanthaswamy et al., 2009, 1* Verardi et al., 2006, *** Koskinen and Bredbacka, 2000, ****Lupke and Disl, 2005

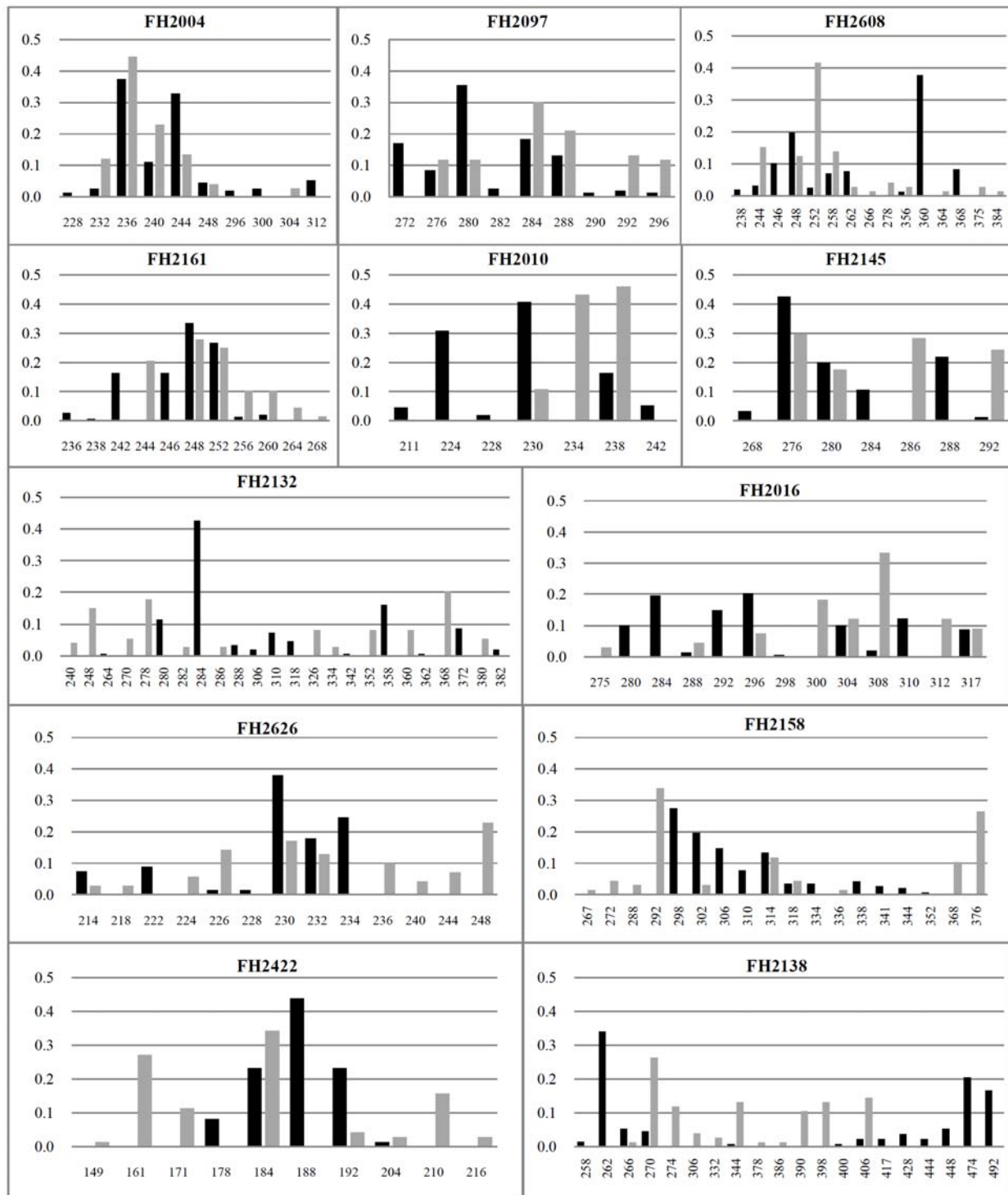


Figure 3 Allele frequencies and distribution of 12 microsatellite markers measured in Bangkaew and Thai Ridgeback dogs.

Inbreeding coefficient in Bangkaew (0.072) and Thai Ridgeback dogs (-0.019) was the same as in Lancashire Heeler (0.04-0.1) (Maki, 2010), but lower than in 5 pointing dog breeds (0.33-0.37) (Parra et al., 2008) and Nova Scotia Duck Tolling Retriever (0.24-0.32) (Maki, 2010). The highly polymorphic of the markers in this study indicated that Thai Ridgeback and Bangkaew dogs appeared to have high genetic variability within breeds. The low and negative inbreeding coefficient values revealed no inbreeding problem of them especially in Thai Ridgeback dogs.

High inbreeding coefficient happens when 2 closely related animals are mated or breed origin comes from a small number of founders. The possible explanation for this result is that these Thai dog breeds may originate from founder of wide genetic diversity, which they were genetic flow among populations. Moreover, breeding selection is no limit to only some popular sires or dams, but they have opportunity to share their genetic material with other population. Therefore, artificial selection and close breeding are not intense in these dogs. Thus, inbreeding should have only a minor impact on the genetic variability of

these breeds. However, this study is the beginning of genetic study in Thai dogs that helps to understand the genetic background within breeds.

Acknowledgements

The authors would like to thank Creative Thai Dog Club and others Thai breeders for providing the blood samples in this study.

References

- Altunok, V., Koban, E., Chikhi, L., Schaffer, A., Pedersen, N.C., Nizamlioglu, M. and Togan, I. 2005. Genetic evidence for the distinctness of Kangal dogs. *Bull Vet Inst Pulawy* 49: 249-254.
- Boyko, A.R., Boyko, R.H., Boyko, C.M., Parker, H.G., Castellhano, M., Corey, L., Degenhardt, J.D., Auton, A., Hedimbi, M., Kityo, R., Ostrander, E.A., Schoenbeck, J., Todhunter, R.J., Jones, P. and Bustamante, C.D. 2009. Complex population structure in African village dogs and its implications for inferring dog domestication history. *PNAS* 106(33): 13903-13908.
- Clark, L.A., Famula, T.R. and Murphy, K.E. 2004. Evaluation of a rapid single multiplex microsatellite-based assay for use in forensic genetic investigations in dogs. *Am J Vet Res*. 65(10): 1446-1450.
- Francisco, L.V., Langston, A.A., Mellers, C.S., Neal, C.L. and Ostrander, E.A. 1996. A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. *Mamm Genome* 7: 359-362.
- Fredholm, M., and Wintero, A.K. 1995. Variation of short tandem repeats within and between species belonging to Canidae family. *Mamm Genome*. 6: 11-18.
- Fred Hutchinson Cancer Research Center. 2002. "Dog Genome Project. Microsatellite Markers." [Online]. Available: <http://www.fhcrc.org/science/dog-genome/dog.html>.
- Holmes, N.G., Mellers, C.S., Humphreys, S.J., Binns, M.M., Holliman, A., Curtis, R. and Sampson, J. 1993. Isolation and characterization of microsatellites from canine genome. *Anim Genet*. 24: 289-292.
- Ichikawa, Y., Takagi, K., Tsumagari, S., Ishihama, K., Morita, M., Kanemaki, M., Takeishi, M. and Takahashi, H. 2001. Canine parentage testing based on microsatellite polymorphisms. *J Vet Med Sci*. 63(11): 1209-1213.
- Irion, D.N., Schaffer, A.L., Grant, S., Wilton, A.N. and Pedersen, N.C. 2005. Genetic variation analysis of the Bali street dog using microsatellites. *BMC Genet*. 6(6): 1-13.
- Irion, D.N., Schaffer, A.L., Famula, T.R., Eggleston, M.L., Hughes, S.S. and Pedersen, N.C. 2003. Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. *J Hered*. 94(1): 81-87.
- Kang, B-T., kim, K-S., Min, M-S., Chae, Y-J., Kang, J-W., Yoon, J., Choi, J., Seong, J-K., Park, H-C., An, J., Lee, M-H., Park, H-M. and Lee, H. 2009. Microsatellite loci analysis for the genetic variability and the parentage test of five dog breeds in South Korea. *Genes Genet Syst*. 84: 245-251.
- Kanthaswamy, S., Tom, B.K., Mattila, A-M., Johnston, E., Dayton, M., Kinaga, J., Erickson, B. J-A., Halverson, J., Fantin, D., DeNise, S., Kou, A., Malladi, V., Satkoski, J., Budowle, B., Smith, D.G. and Koskinen, M.T. 2009. Canine population data generated from a multiplex STR kit for use in forensic casework. *J Forensic Sci*. 54(4): 829-840.
- Klukowska, J., Strabel, T., Mackowski, M. and Switonski, W. 2003. Microsatellite polymorphism and genetic distances between the dog, red fox and arctic fox. *J Anim Breed Genet*. 120: 88-94.
- Koskinen, M.T. and Bredbacka, P. 2000. Assessment of the population structure of five Finnish dog breeds with microsatellites. *Anim Genet*. 31: 310-317.
- Liu, K. and Muse, S.V. 2005. Power marker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21(9): 2128-2129.
- Lupke, L. and Distl, O. 2005. Microsatellite marker analysis of the genetic variability in Havoverian Hounds. *J Anim Breed Genet*. 122: 131-139.
- Maki, K. 2010. Population structure and genetic diversity of worldwide Nova Scotia Duck Tolling Retriever and Lancashire Heeler dog populations. *J Anim Breed Genet*. 127: 318-326.
- Morera, L., Barba, C.J., Garrido, J.J., Barbancho, M. and Andres, F. de. 1999. Genetic variation detected by microsatellite in five Spanish dog breeds. *J Hered*. 90(6): 654-656.
- Ostrander, E.A., Jong, P.M., Rine, J. and Duyk, G. 1992. Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. *Proc Natl Acad Sci*. 89: 3419-3423.
- Padar, Z., Angyal, M., Egyed, B., Furedi, S., Woller, J., Zoldag, L. and Fekete, S. 2001. Canine microsatellite polymorphisms as the resolution of an illegal animal death case in a Hungarian Zoological Gardens. *Int J Legal Med*. 115: 79-81.
- Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A. and Kruglyak, L. 2004. Genetic structure of the purebred domestic dog. *Sci*. 304: 1160-1164.
- Parra, D., Mendez, S., Canon, J. and Dunner, S. 2008. Genetic differentiation in pointing dog breeds inferred from microsatellites and mitochondrial DNA sequence. *Anim Genet*. 39: 1-7.
- Puja, I.K., Irion, D.N., Schaffer, A.L. and Pedersen, N.C. 2005. The Kintamani dog: Genetic profile of an emerging breed from Bali, Indonesia. *J Hered*. 96(7): 854-859.
- Ruvinsky, A. and Sampson, J. 2001. *The Genetics of the Dog*. CABI publishing, Wallingford, UK. 564 pp.
- Sambrook, J. and Russell, D.W. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd ed. (vol.1). Cold Spring Harbor Laboratory Press, Cold

- Spring Harbor, New York. 643 pp.
- Savolainen, P., Zhang, Y., Luo, J., Lundeberg, J. and Leitner, T. 2002. Genetic evidence for an East Asian origin of domestic dogs. *Sci.* 298: 1610-1613.
- Sundqvist, A.K., Björnerfeldt, S., Leonard, J.A., Hailer, F., Hedhammar, A., Ellegren, H. and Vila, C. 2006. Unequal contribution of sexes in the origin of dog breeds. *Genet.* 172: 1121-1128.
- Verardi, A., Lucchini, V. and Randi, E. 2006. Detection introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. *Mol Ecol.* 15: 2845-2855.
- Wayne, R.K. and Vila, C. 2001. Phylogeny and origin of the domestic dog. In: *The Genetics of the Dog*. 1st ed. by A. Ruvinsky and J. Sampson (eds). CABI Publishing, New York: 1-13.
- Wright, B.D. 2002. Clinical pain management techniques for cats. *Clin Tech Small Anim Pract.* 17(4): 151-157.
- Zajc, I., Mellersh, C.S. and Sampson, J. 1997. Variability of canine microsatellites within and between different dog breeds. *Mamm Genome.* 8: 182-185.
- Zajc, I. and Sampson, J. 1999. Utility of canine microsatellite in revealing the relationships of pure bred dogs. *J Hered.* 90(1): 104-107.