Biochemical Genetics
https://doi.org/10.1007/s10528-024-10858-7

ORIGINAL ARTICLE

Small but Mighty: Genetic Diversity of the Thai Ridgeback Dog Population

Chadaphon Thatukan[1,2,3] · Chananya Patta[1,2,3] · Worapong Singchat[1,4] · Wattanawan Jaito[1,2,3] · Nichakorn Kumnan[1,2,3] · Piangjai Chalermwong[1,2,3] · Thitipong Panthum[1,4] · Wongsathit Wongloet[1,4] · Pish Wattanadilokchatkun[1] · Thanyapat Thong[1] · Syed Farhan Ahmad[1,4] · Narongrit Muangmai[1,5] · Kyudong Han[1,6,7,8] · Akihiko Koga[1] · Prateep Duengkae[1,4] · Ratthanin Patcharakulvorawat[3] · Kornsorn Srikulnath[1,2,4,9,10]

Received: 19 December 2023 / Accepted: 3 June 2024

Abstract

Originating in Thailand, the Thai Ridgeback dog is known for its unique fur ridge that grows in the opposite direction along its back. Selective breeding and a limited populations in Thailand have led to significant close inbreeding among related individuals. The current Thai Ridgeback population is assumed to have experienced a loss of genetic diversity and bottleneck events. Furthermore, studies on the genetic diversity and structure of Thai Ridgeback dogs are limited. Therefore, the aim of this study was to assess the genetic diversity in Thai Ridgeback dogs. Microsatellite genotyping and mitochondrial DNA D-loop sequences were used to assess genetic diversity in 105 Thai Ridgeback dogs from various farms throughout Thailand. Significant genetic diversity and minimal inbreeding were observed in the current Thai Ridgeback population. Signs of bottlenecks were not observed because the exchange of genetic material among Thai Ridgeback owners effectively preserved the genetic diversity. Moreover, the genetic parameters in this study supported owner-to-owner exchanges animals for mating programs. To sustain the genetic diversity of Thai Ridgeback dogs, the use of genetic parameters to manage genetic closeness while preserving breed characteristics is essential. These data are crucial for ensuring demographic stability, which is pivotal for long-term conservation and effective population management.

Keywords Bottleneck · Dog · Gene flow · Inbreeding · Microsatellite

Extended author information available on the last page of the article

Published online: 12 June 2024

Introduction

Domestic dogs are considered the most phenotypically diverse vertebrate species (Bigi et al. 2015). The socioeconomic significance of dogs is enhanced by their use for therapy, sports, herding, hunting, research, and companionship and specialized roles in the police, customs, military, rescue, and security sectors; thus, the socioeconomic significance of dogs has impacted their domestication (Hart and Yamamoto 2016; Vonholdt and Driscoll 2016). Most pure-breed dogs are morphologically distinct and differ in their behavior, physical properties, and specific inherited diseases (Teng et al. 2016). Worldwide, more than 400 dog breeds have been registered by various organizations, including the World Canine Organization (FCI, Federation Cynologique Internationale), American Kennel Club (2023a, 2023b), and British Kennel Club (http://www.fci.be; https://www.akc.org; www.thekennelclub.org.uk). Domestic dog breeding, which is characterized by strong selection for specific traits, is often conducted through mating between closely related lines, backcrossing, and inbreeding. Increased homozygosity and reduced genetic diversity are known outcomes of this practice, which affects entire breeds and the variation within breeds. However, recent research has suggested that genetic diversity within breeds is rapidly decreasing, which poses challenges for the long-term maintenance of separate breeds (Bartnikowska and Kania-Gierdziewicz 2023). The abundance of deleterious recessive alleles may also increase the risk of genetic disorders (Axelsson et al. 2021). Despite efforts to exchange genetic material, breeders cannot maintain the same level of heterogeneity in newly established subpopulations compared with that in the initial population. Consequently, heterozygosity decreases and inbreeding increases in subsequent generations. The maintenance of genetic diversity in domesticated dogs with socioeconomic and cultural value is considered essential. Accordingly, the development and implementation of strategies to safeguard the genetic diversity of such species have been advocated, such as in Aichi Target 13 (http://www.cbd.int/sp/targets). Increasing focus is being directed toward the genetic diversity of domestic animal populations, particularly dogs.

The Thai Ridgeback dog (*Canis lupus familiaris*), also known as the Thai Lang An dog, has been recognized as an ancient breed through archaeological records from Thailand. It has existed as a Thai domestic breed for several hundred years, and although its origin remains undocumented, its development has been traced to eastern Thailand. The Thai Ridgeback has been officially recognized by both the FCI and AKC and is classified under Group 5: Spitz and Primitive Types, Section 7: Primitive Type-Hunting Dogs (http://www.fci.be; https://www.akc.org). It received provisional recognition from the FCI in 1993 and definitive recognition in 2003 (http://www.fci.be; Fédération Cynologique Internationale 2004, 2023). This breed is distinguished by medium size, short smooth coat, and distinctive ridge of fur on its back, which is formed by hair that grows in the direction opposite to the rest of its coat. In addition to its striking appearance, the Thai Ridgeback is recognized for its muscular and athletic build and loyalty and intelligence (http://www.fci.be; https://www.akc.org; The Kennel Club 2022). The popularity

of the Thai Ridgeback as a dog breed is attributed to its distinctive characteristics. The Thai Ridgeback is primarily found in Thailand, where its small number of individuals and limited gene pool have led to the emergence of various defects that are likely genetically determined. The loss of genetic diversity in dog breeds, which is often associated with breeding in closed populations and the extensive use of a small number of popular sires, may result in the reduction genetic diversity within a population. In Thailand, only total dog populations are currently recorded without specifying breeds, including Thai Ridgeback dogs, which have been studied in very limited numbers. A decade ago, only a few studies had been conducted on the genetic diversity and structure of Thai Ridgeback dogs (Phavaphutanon and Laopiem 2011; Zhang et al. 2020). Effective conservation decisions and management strategies for the Thai Ridgeback depend on understanding its genetic diversity. Comprehensive knowledge of breed characteristics is necessary for the effective management of farm dog resources. In the present study, genetic diversity was assessed in a population of 105 Thai Ridgeback dogs from several farms throughout Thailand using microsatellite genotyping and mitochondrial DNA D-loop sequences.

Materials and Methods

Specimen Collection and DNA Extraction

Whole blood specimens were collected from the lateral saphenous vein using a 24-gauge needle (Nipro Corporation, Tokyo, Japan), with ethylenediaminetetraacetic acid as an anticoagulant (Greiner Bio-One, Kremsmünster, Austria), and then stored at − 20 °C. Alternatively, buccal cell specimens were collected from the cheek area using a sterile swab (THAI GAUZE Co., Ltd., Bangkok, Thailand) and stored at 4 °C in a dry paper Ziplock bag (Seethong 555 Co., Ltd., Samut Sakhon, Thailand). The type of specimens permitted (blood or buccal cell) depends on the owner's permission. Specimens were collected from a total of 105 Thai Ridgeback dogs (32 males and 73 females) from 11 localities in Thailand, including Pathum Thani (PTE), Suphan Buri (SPB), Surat Thani (SNI), Nakhon Si Thammarat (NST), Chiang Mai (CNX) Chum Phon (CPN), Chachoengsao (CCO), Amnat Charoen (ACR), Chonburi (CBI), Trat (TRT), and Rayong (RYG). Samples were collected from only 11 farms in response to our outreach to numerous farms across Thailand for Thai Ridgeback dog breeding. Permission was granted by the farm owners, and all Thai Ridgeback dogs were released immediately after sample collection. Samples from three Thai Bangkaews, two Shih Tzus, three Chihuahuas, one Jack Russell, and two French Bulldogs were also collected as outgroups. Detailed information on the sampled individuals is provided in Tables S1 and S2. The DNA extraction and DNA quality and quantity assessment were performed using the same methods as in previous studies (Supikamolseni et al. 2015) (Supplementary material). Ethical approval for the experimental procedures outlined in this study was granted by the Kasetsart

University Animal Experiment Committee (Approval No: ACKU65-SCI-030) and ARRIVE guidelines (https://arriveguidelines.org).

Microsatellite Genotyping and Data Analysis

Fifteen microsatellite primer sets were sourced from previous studies (Francisco et al. 1996; Altet et al. 2001; Richman et al. 2001; Radko and Slota 2009) (Table S3). A fluorescent dye (carboxyfluorescein, 6-FAM; or hexachloro-fluorescein, HEX) (Macrogen Inc., Seoul, Korea) was affixed to the 5' end of the forward primer within each primer set. PCR amplification was performed in a 15 µL of reaction volume that consisted of 1 × standard reaction buffer (Apsalagen Co., Ltd., Bangkok, Thailand), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primers, 0.5 U of *Taq* polymerase (Apsalagen), and 25 ng of genomic DNA. The amplification process included initial denaturation at 98 °C for 5 min, followed by 35 cycles of 98 °C for 30 s, 30 s at the annealing temperature specified in Table S3, and 72 °C for 30 s. Amplification was concluded with a final extension step at 72 °C for 5 min. PCR products were detected by electrophoresis on a 1% agarose gel. Fluorescent DNA fragment length analysis was then performed using an ABI 3730XL Automatic Sequencer (Applied Biosystems, Foster City, CA, USA) at the DNA sequencing service of Macrogen Inc. Allele size was determined using a Peak Scanner version 1.0 (Applied Biosystems). The genotypic data resulting from this study were deposited in the Dryad Digital Repository Dataset (https://datadryad.org/stash/share/WCImm18VKNgl8wvFZxtTmtCSudx). We used the same methods as previous studies for PCR amplification to analyze genetic diversity and population structure of the Thai Ridgeback populations (Jangtarwan et al. 2020; Ariyaraphong et al. 2023) (Supplementary Material).

Construction of Individual Probability Tests

The marker set's effectiveness for individual identification of Thai Ridgeback dogs was evaluated. Probabilities for all populations were calculated using GenAlEx version 6.5 (Peakall and Smouse 2012). The following formulae were applied:

Matching probability (MP) values indicate the likelihood of two individuals having the same genotypic profile by chance (Allendorf et al. 2012).

$$\mathrm{MP} = \prod p_{\mathrm{i}}^2 \times \prod 2p_{\mathrm{i}}p_{\mathrm{j}},$$

where $\prod$ indicates chain multiplication across each locus, p_{i} is the frequency of the allele homozygous loci, and pi and pj are the frequencies of alleles at heterozygous loci.

Probability of identity (PI) assesses the likelihood of random drawing of two individuals with identical genotypes from the population (Waits et al. 2001).

$$\mathrm{PI} = 2\left(\sum pi^2\right)^2 - \sum pi^4,$$

where p_i is the frequency of the i^{th} allele at a locus.

Probability of identity between siblings (PI_{sibs}) indicates the probability that two siblings share the same multilocus genotype (Waits et al. 2001).

$$\mathrm{PI}_{sibs} = 0.25 + (0.5\Sigma pi^2) + [0.5(\Sigma pi^2)^2] - (0.25\Sigma pi^4),$$

where p_i is the frequency of the i^{th} allele at a locus.

Probability of exclusion (PE) indicates the probability that a parent can be excluded as the parent based on genetic testing results when the genotype of one parent is known (Jamieson 1965).

$$\mathrm{PE} = 1 - 4\Sigma pi^2 + 2(\Sigma pi^2)^2 + 4\Sigma pi^3 - 3\Sigma pi^4,$$

where p_i is the frequency of the i^{th} allele at a locus.

Mitochondrial DNA D-Loop Sequencing and Data Analysis

The mtDNA D-loop sequences of DNA fragments were amplified using the primers H15422/L16106F (5′-CTCTTGCTCCACCATCAGC-3′) and H15422/L16106R (5′-AAACTATATGTCCTGAAACC-3′) (Murgia et al. 2006). PCR amplification was conducted by using 15 µL of 1X standard reaction buffer (Apsalagen), 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primers, 0.5 U *Taq* polymerase (Apsalagen), and 25 ng of genomic DNA. The PCR conditions were as follows: initial denaturation at 94 °C for 2 min, then 35 cycles of 94 °C for 15 s, 56 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products underwent purification with the MEGAquick-spin plus Fragment DNA Purification Kit (iNtRON Biotechnology, Gyeonggi-do, Korea). Nucleotide sequences of the DNA fragments were determined by using the DNA sequencing service provided by First Base Laboratories Sdn Bhd (Seri Kembangan, Selangor, Malaysia). BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to query the nucleotide sequences within the National Center for Biotechnology Information database to verify the identity of the amplified DNA fragments. All sequences were deposited in the DNA Data Bank of Japan (DDBJ) (https://www.ddbj.nig.ac.jp/, accessed on September 14, 2023) (accession numbers: LC779393–LC779497) (Table S1). The 105 partial mtDNA D-loop sequences were subjected to multiple alignments using the default parameters of the Geneious Prime software version 2023.2.1 (Biomatters Ltd., Auckland, New Zealand; http://www.geneious.com). All nonalignable and gap-containing sites were meticulously removed and trimmed from the dataset. We used the same methods as previous studies to analyze genetic diversity of the

Table 1 Genetic diversity in 11 populations of the Thai Ridgeback (*Canis lupus familiaris*) estimated using 15 microsatellite loci

Population[a]		*N*	N_a	*AR*	N_{ea}	*I*	H_o	H_e	*M* ratio	*PIC*	*F*
PTE	Mean	12	4.60	4.60	2.85	1.17	0.61	0.60	0.29	0.56	− 0.02
	S.E		0.38	0.38	0.27	0.09	0.05	0.04	0.02	0.04	0.06
SPB	Mean	4	2.80	2.80	2.29	0.82	0.52	0.48	0.40	0.42	− 0.06
	S.E		0.30	0.30	0.26	0.12	0.10	0.06	0.06	0.06	0.14
SNI	Mean	6	3.07	3.07	2.17	0.85	0.57	0.49	0.35	0.44	− 0.13
	S.E		0.23	0.23	0.18	0.08	0.07	0.04	0.04	0.04	0.07
NST	Mean	15	4.73	4.73	3.14	1.24	0.66	0.65	0.31	0.59	− 0.01
	S.E		0.41	0.41	0.32	0.08	0.04	0.03	0.03	0.03	0.05
CNX	Mean	5	3.87	3.87	2.94	1.15	0.56	0.62	0.30	0.57	0.10
	S.E		0.31	0.31	0.27	0.09	0.07	0.03	0.02	0.04	0.11
CPN	Mean	4	3.27	3.27	2.59	0.97	0.55	0.55	0.40	0.49	0.01
	S.E		0.30	0.30	0.29	0.10	0.07	0.05	0.07	0.05	0.09
CCO	Mean	5	3.67	3.67	2.73	1.08	0.56	0.59	0.30	0.54	0.03
	S.E		0.27	0.27	0.23	0.09	0.07	0.04	0.02	0.04	0.09
ACR	Mean	24	5.73	5.73	3.33	1.35	0.62	0.67	0.31	0.62	0.08
	S.E		0.44	0.44	0.31	0.08	0.04	0.02	0.03	0.03	0.05
CBI	Mean	7	4.40	4.40	3.12	1.26	0.52	0.65	0.34	0.61	0.20
	S.E		0.24	0.24	0.23	0.07	0.07	0.03	0.02	0.03	0.09
TRT	Mean	5	3.60	3.60	2.86	1.09	0.65	0.61	0.30	0.55	− 0.05
	S.E		0.29	0.29	0.27	0.08	0.07	0.03	0.03	0.04	0.11
RYG	Mean	18	5.67	5.67	3.39	1.33	0.59	0.65	0.35	0.61	0.10
	S.E		0.40	0.40	0.38	0.09	0.06	0.04	0.03	0.04	0.06
All Population	Mean	105	4.13	4.13	2.86	1.12	0.58	0.60	0.29	0.55	0.02
	S.E		0.30	0.30	0.12	0.05	0.01	0.02	0.02	0.02	0.03

Detailed information of 105 individuals analyzed in this study is presented in Supplementary Table S1

N Sample size N_a number of alleles, *AR* allelic richness, N_{ea} number of effective alleles, *I* Shannon's information index, H_o observed heterozygosity, H_e expected heterozygosity, *PIC* polymorphic information content, *F* fixation index

[a]*PTE* Pathum Thani, *SPB* Suphan Buri, *SNI* Surat Thani, *NST* Nakhon Si Thammarat, *CNX* Chiang Mai, *CPN* Chum Phon, *CCO* Chachoengsao, *ACR* Amnat Charoen, *CBI* Chonburi, *TRT* Trat, *RYG* Rayong

Thai Ridgeback populations (Ariyaraphong et al. 2023; Budi et al. 2023) (Supplementary Material).

Results

Genetic Variability of Thai Ridgeback Populations Based on Microsatellite Data

The 11 populations of Thai Ridgeback had a total of 104 alleles, with a mean number of 4.13 ± 0.30 alleles per locus (Table 1). Most allelic frequencies in the

Table 2 Observed (H_o) and expected (H_e) heterozygosity in three populations of the Thai Ridgeback dog (*Canis lupus familiaris*) estimated by the Welch's *t* test using 15 microsatellite loci

Population[a]	H_o	H_e	df	*t* test	*p* value
PTE	0.61 ± 0.05	0.60 ± 0.04	0.01	0.14	0.89
SPB	0.52 ± 0.10	0.47 ± 0.06	0.04	0.36	0.73
SNI	0.57 ± 0.07	0.49 ± 0.04	0.08	0.90	0.39
NST	0.66 ± 0.04	0.65 ± 0.02	0.01	0.18	0.86
CNX	0.56 ± 0.07	0.62 ± 0.03	− 0.06	− 0.74	0.49
CPN	0.55 ± 0.06	0.55 ± 0.05	0.00	0.05	0.96
CCO	0.56 ± 0.06	0.59 ± 0.04	− 0.03	− 0.35	0.74
ACR	0.62 ± 0.04	0.67 ± 0.02	− 0.05	− 1.09	0.29
CBI	0.52 ± 0.07	0.65 ± 0.04	− 0.13	− 1.71	0.13
TRT	0.65 ± 0.07	0.61 ± 0.03	0.04	0.51	0.63
RYG	0.59 ± 0.06	0.65 ± 0.04	− 0.07	− 0.98	0.34

[a]*PTE* Pathum Thani, *SPB* Suphan Buri, *SNI* Surat Thani, *NST* Nakhon Si Thammarat, *CNX* Chiang Mai, *CPN* Chum Phon, *CCO* Chachoengsao, *ACR* Amnat Charoen, *CBI* Chonburi, *TRT* Trat, *RYG* Rayong

population were not significantly different from expected under Hardy–Weinberg equilibrium and linkage disequilibrium. Null alleles were detected at the INU005 locus; however, the markers were treated in the same manner as the other alleles. Positive fixation index (*F*) values were recorded in 6 of 11 populations of Thai Ridgeback dogs: CNX, CPN, CCO, ACR, CBI, and RYG. The polymorphic information content (*PIC*) across all populations ranged from 0.42 to 0.62, and the

Table 3 Inbreeding coefficients, relatedness values, effective population size, and ratio of effective population size to census population size (N_e/N) in 11 populations of the Thai Ridgeback (*Canis lupus familiaris*)

Population[a]	*N*	F_{IS}	Relatedness (*r*)	Estimated N_e	95% CIs for N_e	N_e/N
PTE	12	− 0.09 ± 0.04	− 0.04 ± 0.06	23.20	10.60–11.30	1.93
SPB	4	− 0.06 ± 0.03	− 0.15 ± 0.09	Infinite	30.40–30.40	Infinite
SNI	6	− 0.08 ± 0.05	− 0.08 ± 0.08	15.30	2.50–2.50	2.55
NST	15	− 0.10 ± 0.04	− 0.04 ± 0.07	24.60	13.90–16.10	1.64
CNX	5	− 0.07 ± 0.07	− 0.13 ± 0.08	132.00	5.40–5.40	26.40
CPN	4	− 0.05 ± 0.10	− 0.16 ± 0.10	32.30	2.00–2.00	8.08
CCO	5	− 0.05 ± 0.05	− 0.13 ± 0.07	Infinite	8.50–8.50	Infinite
ACR	24	− 0.09 ± 0.05	− 0.02 ± 0.06	35.20	23.00–31.30	1.47
CBI	7	− 0.04 ± 0.06	− 0.09 ± 0.07	Infinite	16.10–16.10	Infinite
TRT	5	− 0.11 ± 0.03	− 0.12 ± 0.08	10.50	2.30–2.30	2.10
RYG	18	− 0.07 ± 0.07	− 0.03 ± 0.05	70.00	32.80–43.20	3.89

N Sample size, F_{IS} inbreeding coefficient, N_e effective population size, N_e/N ratio of effective population size to census population size

[a]*PTE* Pathum Thani, *SPB* Suphan Buri, *SNI* Surat Thani, *NST* Nakhon Si Thammarat, *CNX* Chiang Mai, *CPN* Chum Phon, *CCO* Chachoengsao, *ACR* Amnat Charoen, *CBI* Chonburi, *TRT* Trat, *RYG* Rayong

Shannon's information index (*I*) values ranged from 0.82 to 1.35. The observed heterozygosity (H_o) values ranged from 0.52 to 0.66 (mean ± standard error (SE): 0.58 ± 0.01) and the expected heterozygosity (H_e) values ranged from 0.47 to 0.67 (0.60 ± 0.02) (Table 1). Welch's *t* test revealed that H_o was not significantly different from H_e (Table 2). Statistical differences were observed in the pairwise H_o values between populations for the seven pairs (SPB x ACR, SNI x NST, SNI x CNX, SNI x ACR, SNI x CBI, SNI x TRT, and SNI x RYG). The allelic richness (*AR*) value of all populations was 4.16 ± 0.30. The standard genetic diversity indices are summarized in Table 1.

The mean relatedness values (*r*) for all 105 sampled individuals were − 0.09 ± 0.07. The lowest value was found at CPN (− 0.16), and the highest was at ACR (− 0.02) (Table 3). The distribution of *r* values for Thai Ridgeback dogs varied among the populations (Fig. S1A, Table S5). The inbreeding coefficient (F_{IS}) in the eleven populations varied between − 0.11 (TRT) and − 0.04 (CBI), with an average of − 0.07 ± 0.05 (Table 3). However, the distribution of F_{IS} across all populations did not show statistically significant differences from one another (Fig. S1B, Table S5). Number of effective alleles (N_{ea}) of the Thai Ridgeback dogs varied among the different populations (Table 3). Significant differences ($p < 0.05$) were observed in the estimates of Wright's *F*-statistics for subpopulations within the total population (F_{ST}) between populations after 110 permutations (Table S5). Nei's genetic distances and genetic distances between population (R_{ST}) showed that the CNX population was closer to the other populations than the CCO population (Tables S5 and S6). The analysis of molecular variance (AMOVA) revealed 86% genetic variation within individuals, 9% among individuals, and 5% among populations (Table S7).

Recent gene flow estimates from BayesAss ranged from 0.68 ± 0.02 to 0.83 ± 0.04 within populations and from 0.01 ± 0.01 to 0.17 ± 0.03 between populations (Fig. 1A, Table S8). Microsatellite genotyping datasets were subsequently used in independent runs for the MIGRATE-N analysis to estimate the historic gene flow (Fig. 1B, Table S9). A diverse range of mutation-scaled immigration rates (*M*) from 4.33 to 965.67 was observed in the MIGRATE-N analysis. The highest *M* value was observed from ARC to CPN, indicating that the migration rate from population ARC to population CPN relative to the mutation rate is high compared to other population pairs. Mutation-scaled population size (Θ) values ranging from 0.00 to 0.10 were observed across populations with the highest value observed in CBI, indicating that the effective population size of CBI is relatively large compared to the mutation rate. NST exhibited lower Θ values. Deficient gene flow is generally indicated by the calculated effective number of migrants per generation or the gene flow rate (N_m) in most populations. A diverse range of N_m values was observed for gene flow among the 11 populations, ranging from 0.00 to 22.35. The highest N_m value was observed from SPB to CBI, indicating relatively high gene flow between these populations (Table S10). However, based on the OptM function in TreeMix, the optimum number of major gene flow events was determined to be three: (1) SPB and CCO, (2) SPB and inter-TRT-CNX, and (3) CPN and RYG (Fig. 2A, Fig. S2). After accounting for the three major introgression events, the residuals indicated the potential remaining admixtures within

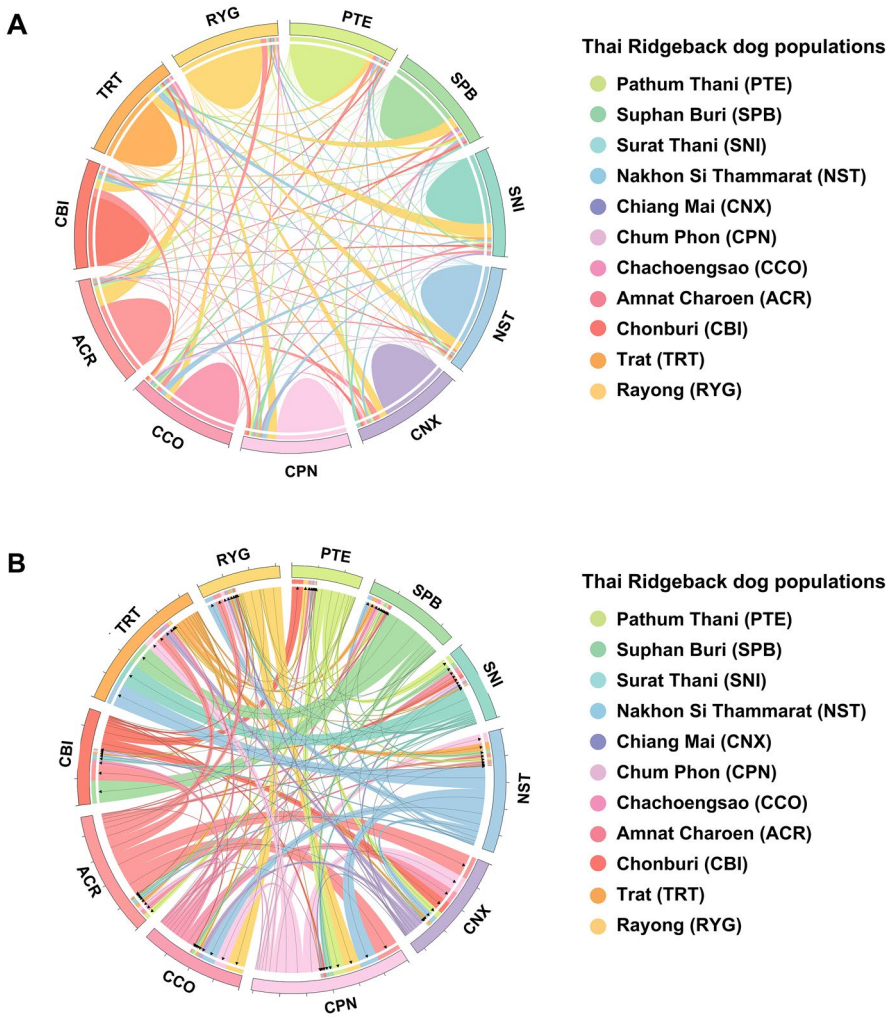

Fig. 1 Source-sink migration dynamics revealed by Circos version 0.69–8. **A** Current migration directionality estimated using BayesAss version 3.0.5. **B** Historical migration represented using MIGRATE-N version 4.4.3. The width of the migration curves indicates the relative magnitude of migration. Each population is represented by a color

the 11 populations (Fig. 2B). The Wilcoxon signed-rank test was performed to determine whether bottlenecks existed in the 11 populations; SMM and TPM values ranged from 0.06 to 0.98 and from 0.01 to 0.66, respectively, which showed a normal L-shaped mode shift and shifted mode (Table S11). The *M* ratio for all the populations was 0.290, as estimated by Garza and Williamson (2008) (Table 1).

Based on discriminant analysis of principal components (DAPC), no substantial discrete clusters were observed, which was consistent with the principal coordinate analysis (PCoA) results and indicated dispersion among the 11 Thai Ridgeback

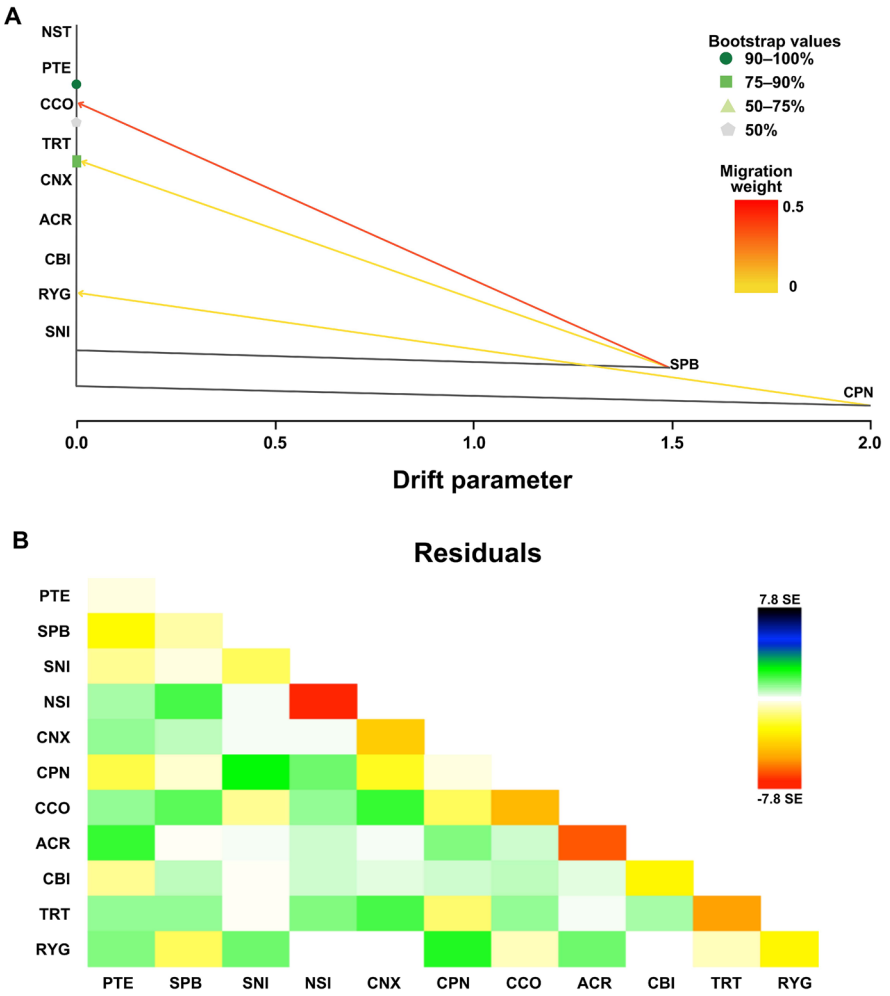

Fig. 2 TreeMix was employed to construct maximum likelihood trees for the 11 Thai Ridgeback populations. **A** Maximum likelihood tree and **B** residual fit evaluation for one migration event. The scale bar indicates ten times the average standard error of the values in the covariance matrix

populations without distinct separation (Figs. S3 and S4). Differentiation among these populations was established by utilizing the first, second, and third principal components, which contributed 10.61%, 7.87%, and 5.95% of the total variation, respectively. STRUCTURE analysis revealed the highest posterior probability with one peak ($K=2$) based on Evanno's ΔK, while the mean ln P (K) showed a different peak ($K=10$) (Fig. 3, Fig. S5). Thai Ridgeback exhibited a variety of gene pool patterns that generally differed from those of other dog breeds.

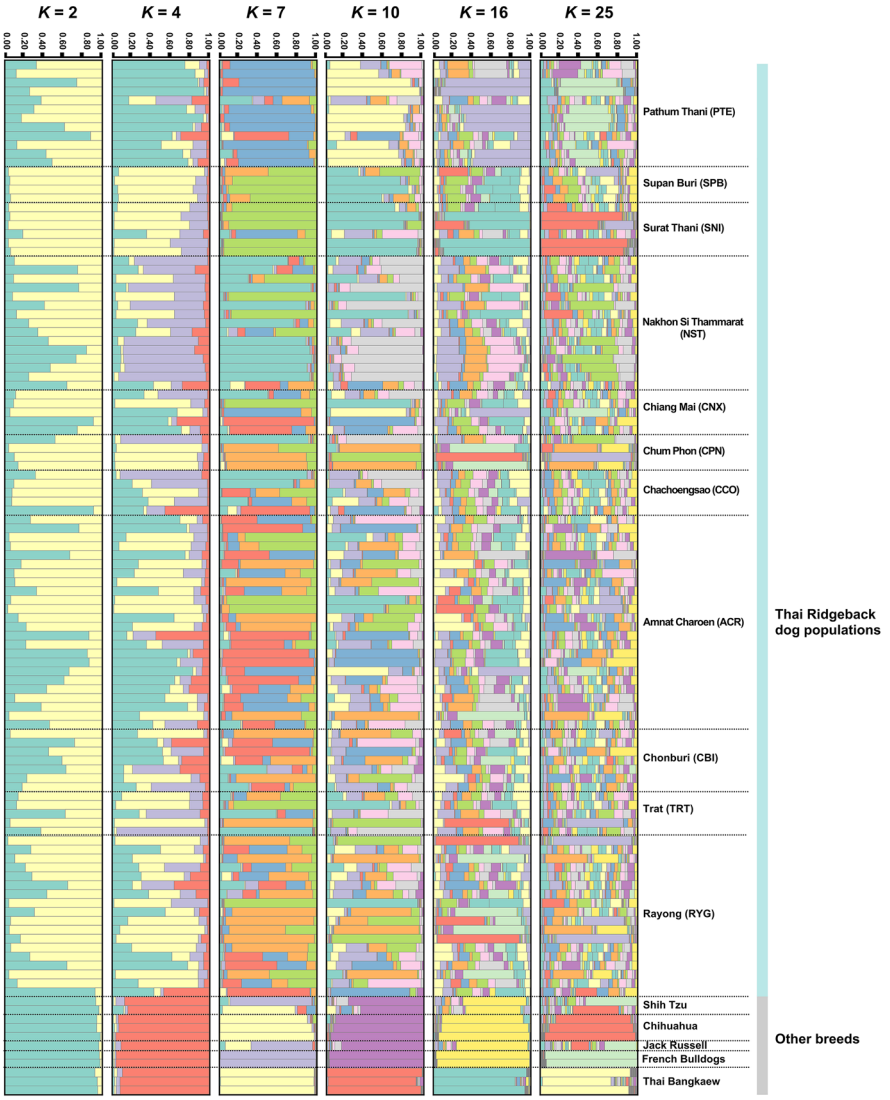

Fig. 3 Genetic structures of 11 Thai Ridgeback populations revealed by the Bayesian structural analysis. Each vertical bar on the *x*-axis represents an individual, and the *y*-axis represents the proportion of membership in each genetic cluster. All individuals from 11 populations are superimposed on the plot. Black vertical lines indicate the boundaries. Each color is assigned to represent a distinct ancestry component, signifying unique genetic variation. Genetic distinctiveness is indicated by solid colors within populations, while shared colors denote similarity

Probability of Individual Identification

MP was calculated for each locus in the 105 Thai Ridgeback dogs. The results revealed the lowest value in locus FH2016 (3.6×10^{-2}) and the highest value in

locus CPH9 (3.1×10^{-1}). The PI and PI_{sibs} for each locus were low, ranging from 3.6×10^{-2} to 3.10^{-1} and 3.3×10^{-1} to 5.8×10^{-1}, respectively. When considering the combinations of the 15 microsatellite loci, the PI value was 4.2×10^{-6} and PI_{sibs} value was 4.5×10^{-14}. When only one parent was known, the lowest value was noted in CPH9 (12.68%) and the highest value in FH2016 (55.38%). When the 15 microsatellite loci were combined, the PE value was 99.63% (Fig. S6, Table S12).

Genetic Variability of Thai Ridgeback Populations Based on Mitochondrial DNA D-Loop Sequences

The aligned mtDNA D-loop sequence was 701 bp. A total of 18 haplotypes were found across all populations of Thai Ridgeback. The overall haplotype and nucleotide diversities were 0.91 ± 0.01 and 0.01 ± 0.00, respectively (Table 4). Theta (Per Site) from *S* values ranged from 0.00 to 0.01. Average number of nucleotide differences (*k*) was 6.00 (Table 4). We constructed a complex haplotype network using numerous polymorphic sites and haplotypes. The most common haplotype in the Thai Ridgeback population was HAP30. Fourteen haplotypes (HAP03, HAP06, HAP12, HAP14, HAP15, HAP21, HAP22, HAP24, HAP25, HAP26, HAP29, HAP30, HAP31, and HAP34) were shared among the populations or other breed (Fig. S7). The phylogenetic tree indicated that seven Thai Ridgeback samples from SPB, ACR, CBI, and RYG were associated with breeds such as Shiba, Dachshund, Cavalier, and Jack Russell. Additionally, three ACR samples were linked to Shih Tzu and Mongolian native breeds, whereas two samples from NST and CNX were related to the Chow Chow breed (Fig. S8). All Thai Ridgeback sequences in this study fell within haplogroups A and B based on the haplogroup classification in dogs (Zhang et al. 2020).

To assess genetic differentiation among the eleven populations, we computed several indices including Wright's F-statistics for subpopulations within the total population (F_{ST}), the genetic differentiation coefficient (G_{ST}), correlation of random haplotypes within populations (Φ_{ST}), the average number of nucleotide substitutions per site between populations (D_{xy}), and the net nucleotide substitutions per site between populations (D_a). The F_{ST} values varied between -0.17 and 0.72, whereas the G_{ST} values ranged from − 0.03 to 0.41. Additionally, the Φ_{ST} values spanned from 0.01 to 0.62, D_{xy} values ranged from 0.00 to 0.02, and D_a values ranged from 0.00 to 0.01 for the mtD-loop sequences (Table S13). Well-defined posterior probability distributions for each parameter were observed in the MIGRATE-N version 4.4.3 analysis (Table S14). The mutation-scaled immigration rate (*M*) varied from 0.30 to 387.70, with the highest *M* value of observed between NST and CNX. Mutation-scaled population size (Θ) values ranged from 0.08 to 0.10, with the highest value observed in SNI, CPN, and RYG (Table S14). A diverse range of N_m values was observed, from 0.01 to 8.57, and the highest was observed from CCO to CPN (Table S15). Non-significant for Tajima's D (ranging from − 1.23 to 0.50), Fu and Li's *F** (− 1.60 to 0.92), and Fu and Li's *D* (− 1.43 to 1.37) (Table S16).

Table 4 The genetic diversity of mitochondrial DNA D-loop sequences in 11 populations of the Thai Ridgeback (*Canis lupus familiaris*)

Population[a]	*N*	Number of haplotypes (H)	Theta (Per Site) from *S*	Average number of nucleotide differences (*k*)	Haplotype diversity (*h*)	Nucleotide diversity (π)
PTE	12	7	0.01	3.46	0.86 ± 0.08	0.01 ± 0.00
SPB	4	2	0.01	7.50	0.50 ± 0.26	0.01 ± 0.01
SNI	6	2	0.00	1.33	0.33 ± 0.21	0.00 ± 0.00
NST	15	6	0.01	6.13	0.81 ± 0.08	0.01 ± 0.00
CNX	5	5	0.01	3.60	1.00 ± 0.13	0.01 ± 0.00
CPN	4	3	0.01	9.33	0.83 ± 0.22	0.01 ± 0.00
CCO	5	3	0.01	3.60	0.80 ± 0.16	0.01 ± 0.00
ACR	24	9	0.01	4.84	0.89 ± 0.03	0.01 ± 0.00
CBI	7	6	0.01	8.62	0.95 ± 0.10	0.01 ± 0.00
TRT	5	4	0.01	8.20	0.90 ± 0.16	0.01 ± 0.00
RYG	18	9	0.01	6.88	0.89 ± 0.05	0.01 ± 0.00
All populations	105	18	0.01	6.00	0.91 ± 0.01	0.01 ± 0.00

[a]*PTE* Pathum Thani, *SPB* Suphan Buri, *SNI* Surat Thani, *NST* Nakhon Si Thammarat, *CNX* Chiang Mai, *CPN* Chum Phon, *CCO* Chachoengsao, *ACR* Amnat Charoen, *CBI* Chonburi, *TRT* Trat, *RYG* Rayong

Discussion

According to the Kennel Association of Thailand, most Thai Ridgeback dogs are owned by individuals unaffiliated with the association and are intended solely as pets, not for breeding. From 2020 to 2023, only 5% of the Thai Ridgeback dogs registered from 2020 to 2023 are used for breeding or commercial trading. The conservation of the original Thai Ridgeback as an indigenous breed may face serious challenges owing to its limited population and lack of recorded breeding, which could lead to genetic problems, such as inbreeding and bottlenecks. Breeding programs aimed at specific physical traits in dogs, such as Thai Ridgeback, often lead to inherited health issues, which pose a significant challenge. Genetic monitoring with markers is used to assess genetic diversity and inbreeding levels when pedigree knowledge is lacking or pedigree errors occur, which introduce a bias of approximately 1–10% (Ostrander et al. 2005; Leroy 2011). This information can be strategically used to design conservation and breeding programs to curtail inbreeding and preserve genetic diversity.

Thai Ridgeback Population Presents Exceptional Genetic Diversity and is Free from Inbreeding and Marked by High Heterozygosity

Constrained by limited genetic diversity and selective breeding from a small reproductive pool, many dog breeds face increased inherited defects due to close relative mating. When selected by humans for desirable phenotypes, their health and welfare are often compromised. High inbreeding rates and population bottlenecks (Leroy 2011) can result from breeding strategies that focus on only a few animals, leading to a significant loss of genetic diversity. The worldwide dispersion of dog hobby breeding has created a new form of the bottleneck effect. In this study, higher genetic diversity (average $H_e = 0.60$) in the Thai Ridgeback breed was observed, which is consistent with the findings of Phavaphutanon and Laopiem (2011) (0.39–0.76) but inconsistent with the lower diversity observed in long-established breeds. According to Foulley and Ollivier (2006), *AR* is more sensitive to bottlenecks than heterozygosity. The current Thai Ridgeback breed likely has not experienced bottlenecks. Our demographic analysis revealed a historical reduction in all the examined populations; however, no recent bottlenecks were detected. This was supported by haplotype network analysis using mtDNA D-loop sequences and demographic data of microsatellite genotypes. The comparison of H_e and H_o shows no significant difference, indicating low potential for inbreeding and outbreeding events. This is consistent with the remarkably low values of F_{IS} and r. Many Thai Ridgeback dogs effectively transmit genetic components within the population, thereby maintaining a high N_e/N ratio, which helps to prevent long-term inbreeding depression and ensures the long-term performance of the population. High h values were observed in all populations in the maternal lineage analysis using mtDNA D-loop sequences. These findings may be attributed to the highly variable gene pool of the Thai Ridgeback breed. Alternatively, breeds with higher inbreeding values may show further increases in h values, whereas breeds with historically high initial heterozygosity

may maintain higher current values of H_e. Notably, although 2/3 of the specimens were from females, the unequal sex ratio factor in the analysis did not affect the sex ratio within the population (Dubois et al. 2018). Moreover, reliable genetic diversity estimates were demonstrated with small samples by Pruett and Winker (2008). Thus, specimen division by province may not significantly affect results in this study.

Successful Genetic Variability in Thai Ridgeback Populations Through Genetic Material Exchange Among Owners

Dog breeders often struggle to maintain the same genetic diversity in new subpopulations as in the original population, despite efforts to exchange genetic material. As generations pass, heterozygosity decreases and inbreeding increases. However, low positive or negative F values were observed in all populations in this study, indicating the presence of very small potential subpopulations within each population. Evidence of introgression into the Thai Ridgeback breed was found in the Treemix and Bayesian structural analyses. This may have occurred before the restoration process when the subpopulation levels were low. This results in the potential for remaining admixture or a shared gene pool within the current Thai Ridgeback populations. The Bayesian structural, PCoA, and DAPC analyses strongly suggested that no clear genetic structuring could be identified. This aligns with the F_{ST} results, which indicated minimal genetic differentiation in some but not all population pairs. The absence of significant drift or isolated populations may result from extensive gene flow between populations, which is facilitated by the artificial migration of populations within the Thai Ridgeback community. N_m values exceeding 1.0 indicate that gene flow predominates over genetic drift (Hannachi et al. 1998). Extensive gene flow and subsequent genetic mixing between populations have led to a reduction in genetic variation across populations. The AMOVA results also highlighted that a substantial portion of the genetic variation resides within populations rather than between them. Significant introgression was observed in the SPB population, which is consistent with the historical gene flow, as indicated by the MIGRATE-N results. However, recent gene flow has also been detected in TRT and RYG populations in eastern Thailand. This aligns with the renowned breeding stock of Thai Ridgeback dogs, which are well regarded in eastern Thailand. The exchange of genetic material among Thai Ridgeback owners is highly effective in mitigating the loss of genetic diversity.

Despite the small population size, the genetic characterization revealed significant genetic diversity in Thai Ridgeback breeds—a crucial aspect to be preserved. Implementing effective breeding management schemes in these dog breeds is advisable to prevent excessive inbreeding, thereby avoiding significant inbreeding depression and the loss of genetic variation. In small and declining populations, the focus should be on mating strategies that minimize relatedness and promoting crossbreeding and outcrossing to maintain genetic diversity and health. The utilization of microsatellite genetic markers for individual identification of Thai Ridgeback dogs was based on standard genetic parameters, such as *PIC*, PI, and PI_{sibs}. In our study, the mean *PIC* values were greater than 0.5, which is considered informative

and similar to those of many other investigated dog breeds (Goleman et al. 2019; Radko and Podbielska 2021). The discrimination power of a microsatellite genotyping panel indicates its usefulness for individual identification. A higher power of discrimination increases the chance of using the technique to identify parental dogs and individual exchanges in mating between owners, and it can also help prevent inbreeding. The power of discrimination with 16 microsatellite loci in Shiba was very high at > 99.99% (Radko and Podbielska 2021). In our study of 15 loci in Thai Ridgeback, the prevalence was > 99.99%. This shows their strong potential for individual identification and assessment of genetic diversity. Previous PI values estimated for 15 microsatellite loci in dogs amounted to 10^{-8} (Eichmann et al. 2005), whereas the PI was 10^{-14} in this study. This should be sufficient to distinguish individual Thai Ridgeback dogs. The use of 17 or 18 microsatellite loci resulted in PE values of 0.99998% and 0.99996%, respectively, whereas a previous panel of 10 microsatellite loci obtained a PE of 0.994 (Dodd et al. 2001; Radko et al. 2018). The microsatellite platform used in this study for the Thai Ridgeback breed achieved a PE > 0.99635. The use of genetic parameters in this study provided a baseline for identifying and assessing at-risk Thai Ridgeback dogs, underscoring the importance of preserving genetic diversity and conducting individual investigations. Breeders and clubs should consider these findings valuable.

Conclusions

Thai Ridgeback dogs present distinct genetic traits adapted to Thailand's local environment. The genetic profile of Thai Ridgeback dogs revealed robust genetic diversity and a low level of inbreeding. Genetic admixture was detected among the 11 populations in Thailand, although a distinct genetic pattern or gene pool was not observed. This may be because the owners exchange genetic material, which supports long-term genetic diversity. However, genetic diversity assessments using genetic markers may be biased by sampling errors resulting from the small number of genotyped individuals. A larger number of Thai Ridgeback dogs is needed for analysis with genotyping data, particularly in populations with a small size. To maintain genetic diversity, owners of Thai Ridgeback dogs should use genetic parameters to identify and manage genetic closeness while preserving breed characteristics. Management of breeding programs requires avoiding pairs of dogs with high relatedness while maintaining unique traits through line breeding of Thai Ridgeback dogs. These crucial data are utilized to inform genetic profiles, guiding strategies for long-term conservation and effective management.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10528-024-10858-7.

Acknowledgements We would like to thank the breeders and owners of the dogs for providing samples including Kok Muang Thai Farm (Pathum Thani, Thailand), BB Thai Ridgeback Farm (Suphanburi, Thailand), Thai Lang An Baan Khun Thee Farm (Surat Thani, Thailand), Thai Lang An Baan Suk San Farm (Nakhon Si Thammarat, Thailand), Thai Lang An San Pa Tong Farm (Chiang Mai, Thailand),

Nora-singha Farm (Chachoengsao, Thailand), Thai Lang An Baan Kan Ta Farm (Chum Phon, Thailand), Kok Sunak So Thai Lang An Amnat Charoen Farm (Amnat Charoen, Thailand), Pin Pin Thai Lang An Farm (Chonburi, Thailand), Thai Lang An Baan Makham Khu (Trat, Thailand), and Thai Lang An Damrong Thai Farm (Rayong, Thailand). We thank the Higher Education for Industry Consortium (Hi-FI) under Experiential learning program, Office of The Permanent Secretary (OPS), Ministry of Higher Education, Science, Research, and Innovation for financial support. We thank the Center for Agricultural Biotechnology (CAB) at Kasetsart University, Kamphaeng Saen Campus and the NSTDA Supercomputer Center (ThaiSC) for their support with server analysis services. We also thank the Faculty of Science of Kasetsart University for providing the research facilities.

Author Contributions Conceptualization: CT, SFA, and KS. Data curation: CT, CP, WJ, NK, PC, WW, PW, and KS. Formal analysis: CT, CP, WJ, NK, PC, WW, and KS. Funding acquisition: KS. Investigation: CT, CP, WJ, NK, PC, and WW. Methodology: CT, WS, TP, and KS. Project administration: KS. Visualization: CT, CP, WW, TP, and KS. Writing—original draft: CT, WS, RP PW, and KS. Writing—review and editing: CT, CP, WS, WJ, NK, PC, TP, WW, PW, TT, SFA, NM, KH, AK, PD, RP, and KS. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded in part by Higher Education for Industry Consortium (Hi–FI) under Experiential learning program, Office of The Permanent Secretary (OPS), Ministry of Higher Education, Science, Research, and Innovation grants (6514400892, 6514400906, 6514400931, 6514400914, 6514400949) awarded to CT, CP, WJ, NK, and PC. Funds from Kasetsart University Research and Development Institute funds (FF(KU)25.64 and FF(KU)51.67) were awarded to WS, SFA, and KS. A Thailand Science Research and Innovation (TSRI) grant through the Kasetsart University Reinventing University Program 2021 (3/2564) was awarded to TP and KS. The High-Quality Research Graduate Development Cooperation Project between Kasetsart University and National Science and Technology Development Agency (NSTDA) awarded funds to TP and KS, and International SciKU Branding (ISB), Faculty of Science, Kasetsart University, awarded funds to WS, SFA, and KS. No funding source was involved in the study design, collection, analysis, and interpretation of the data, writing of the report, or decision to submit the article for publication.

Data Availability The full dataset and metadata from this study are available from the Dryad Digital Repository (https://datadryad.org/stash/share/WCImm18VKNgl8wvFZxtTmtCSudx-pGWnhqY6lu1z0SM) (accessed 06 December 2023). All sequences were deposited in the DNA Data Bank of Japan (DDBJ) (https://www.ddbj.nig.ac.jp/; accession numbers: LC779393–LC779497) (accessed 14 September 2023).

Declarations

Conflict of interest The authors declare no competing financial interests or personal relationships that influenced this study.

Ethical Approval All experimental procedures were approved by the Animal Experiment Committee of Kasetsart University, Thailand (Approval No: ACKU65-SCI-030) and conducted in accordance with the Regulations on Animal Experiments at Kasetsart University and ARRIVE guidelines (https://arriveguidelines.org).

References

Allendorf FW, Luikart GH, Aitken SN (2012) Conservation and the genetics of populations, 2nd edn. Hoboken, USA

Altet L, Francino O, Sánchez A (2001) Microsatellite polymorphism in closely related dogs. J Hered 92:276–279. https://doi.org/10.1093/jhered/92.3.276

American Kennel Club (2023a) Becoming Recognized by the AKC. http://www.fci.be/en/Presentation-of-our-organisation-4.html. Accessed 19 Sept 2023

American Kennel Club (2023b) Thai Ridgeback dog. https://www.akc.org/dog-breeds/thai-ridgeback/. Accessed 19 Sept 2023

Ariyaraphong N, Wongloet W, Wattanadilokchatkun P, Panthum T, Singchat W, Thong T, Lisachov A, Ahmad SF, Muangmai N, Han K, Duengkae P, Temsiripong Y, Srikulnath K (2023) Should the identification guidelines for Siamese crocodiles be revised? Differing post-occipital scute scale numbers show phenotypic variation does not result from hybridization with saltwater crocodiles. Biology 12:535. https://doi.org/10.3390/biology12040535

Axelsson E, Ljungvall I, Bhoumik P, Conn LB, Muren E, Ohlsson Å, Olsen LH, Engdahl K, Hagman R, Hanson J, Kryvokhyzha D, Pettersson M, Grenet O, Moggs J, Rio-Espinola AD, Epe C, Taillon B, Tawari N, Mane S, Hawkins T, Hedhammar Å, Gruet P, Häggström J, Lindblad-Toh K (2021) The genetic consequences of dog breed formation—Accumulation of deleterious genetic variation and fixation of mutations associated with myxomatous mitral valve disease in cavalier King Charles spaniels. PLoS Genet 17:e1009726

Bartnikowska A, Kania-Gierdziewicz J (2023) Effect of inbreeding on the occurrence of genetic defects in Chinese crested dogs. Med Weter 79:291–301. https://doi.org/10.21521/mw.6765

Bigi D, Marelli SP, Randi E, Polli M (2015) Genetic characterization of four native Italian shepherd dog breeds and analysis of their relationship to cosmopolitan dog breeds using microsatellite markers. Animal 9:1921–1928. https://doi.org/10.1017/S1751731115001561

Budi T, Singchat W, Tanglertpaibul N, Wongloet W, Chaiyes A, Ariyaraphong N, Thienpreecha W, Wannakan W, Mungmee A, Thong T, Wattanadilokchatkun P, Panthum T, Ahmad SF, Lisachov A, Muangmai N, Chuenka R, Prapattong P, Nunome M, Chamchumroon W, Han K, Pornpipatsiri S, Supnithi T, Peng M, Han J, Matsuda Y, Duengkae P, Noinafai P, Srikulnath K (2023) Thai local chicken breeds, Chee Fah and Fah Luang, originated from Chinese black-boned chicken with introgression of red Junglefowl and domestic chicken breeds. Sustainability 15:6878. https://doi.org/10.3390/su15086878

Dodd J, Morris B, Oliveira D, Bernoco D (2001) DNA testing for parentage verification and individual identification in seven breeds of dogs. Rev Bras De Reprod Anim 25:35–40

Dubois Q, Lebigre C, Schtickzelle N, Turlure C (2018) Sex, size and timing: sampling design for reliable population genetics analyses using microsatellite data. Methods Ecol Evol 9:1036–1048. https://doi.org/10.1111/2041-210X.12948

Eichmann C, Berger B, Steinlechner M, Parson W (2005) Estimating the probability of identity in a random dog population using 15 highly polymorphic canine STR markers. Forensic Sci Int 151:37–44. https://doi.org/10.1016/j.forsciint.2004.07.002

Fédération Cynologique Internationale (2004) Thai Ridgeback dog. http://www.fci.be/Nomenclature/Standards/338g05-en.pdf. Accessed 19 Sept 2023

Fédération Cynologique Internationale (2023) Presentation of our organization. http://www.fci.be/en/Presentation-of-our-organisation-4.html. Accessed 19 Sept 2023

Foulley JL, Ollivier L (2006) Estimating allelic richness and its diversity. Livest Sci 101:150–158. https://doi.org/10.1016/j.livprodsci.2005.10.021

Francisco LV, Langston AA, Mellersh CS, Neal CL, Ostrander EA (1996) A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. Mamm Genome 7:359–362. https://doi.org/10.1007/s003359900104

Garza JC, Williamson EG (2008) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318. https://doi.org/10.1046/j.1365-294X.2001.01190.x

Goleman M, Balicki I, Radko A, Jakubczak A, Fornal A (2019) Genetic diversity of the polish hunting dog population based on pedigree analyses and molecular studies. Livest Sci 229:114–117. https://doi.org/10.1016/j.livsci.2019.09.017

Hannachi AS, Boussaid M, Marrakchi M (1998) Genetic variability organisation and gene flow in natural populations of *Medicago polymorpha* L. prospected in Tunisia. Genet Sel Evol 30:121–135. https://doi.org/10.1186/1297-9686-30-S1-S121

Hart LA, Yamamoto M (2016) Dogs as helping partners and companions for humans, 2nd edn. Cambridge University, Cambridge, pp 247–270

Jamieson A (1965) The genetics of transferrins in cattle. Hered 20:419–441. https://doi.org/10.1038/hdy.1965.54

Jangtarwan K, Kamsongkram P, Subpayakom N, Sillapaprayoon S, Muangmai N, Kongphoemph A, Wongsodchuen A, Intapan S, Chamchumroon W, Safoowong M, Peyachoknagul S, Duengkae P, Srikulnath K (2020) Predictive genetic plan for a captive population of the Chinese goral

(*Naemorhedus griseus*) and prescriptive action for *ex situ* and *in situ* conservation management in Thailand. PLoS ONE 15:e0234064. https://doi.org/10.1371/journal.pone.0234064
Leroy G (2011) Genetic diversity inbreeding and breeding practices in dogs: results from pedigree analyses. Vet J 189:177–182. https://doi.org/10.1016/j.tvjl.2011.06.016
Murgia C, Pritchard JK, Kim SY, Fassati A, Weiss RA (2006) Clonal origin and evolution of a transmissible cancer. Cell 126:477–487. https://doi.org/10.1016/j.cell.2006.05.051
Ostrander EA, Lindblad-Toh K, Lander ES (2005) Sequencing the genome of the domestic dog Canis familiaris. National Human Genome Research Institute. http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/CanineSEQedited.pdf. Accessed 9 Sept 2023
Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460
Phavaphutanon J, Laopiem S (2011) Evaluation of microsatellite polymorphism and genetic variability in Thai Ridgeback and Bangkaew dogs. Thai J Vet Med 41(3):273–282. https://doi.org/10.56808/2985-1130.2310
Pruett C, Winker K (2008) The effects of sample size on population genetic diversity estimates in song sparrows *Melospiza melodia*. J Avian Biol 39:252–256. https://doi.org/10.1111/j.0908-8857.2008.04094.x
Radko A, Podbielska A (2021) Microsatellite DNA analysis of genetic diversity and parentage testing in the popular dog breeds in Poland. Genes 12:485. https://doi.org/10.3390/genes12040485
Radko A, Słota E (2009) Application of 19 microsatellite DNA markers for parentage control in Borzoi dogs. Pol J Vet Sci 12:113–117
Radko A, Rubiś D, Szumiec A (2018) Analysis of microsatellite DNA polymorphism in the tatra shepherd dog. J Appl Poult Res 46:254–256. https://doi.org/10.1080/09712119.2017.1292912
Richman M, Mellersh CS, André C, Galibert F, Ostrander EA (2001) Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. J Biochem Biophys Methods 47:137–149. https://doi.org/10.1016/s0165-022x(00)00160-3
Supikamolseni A, Ngaoburanawit N, Sumontha M, Chanhome L, Suntrarachun S, Peyachoknagul S, Srikulnath K (2015) Molecular barcoding of venomous snakes and species-specific multiplex PCR assay to identify snake groups for which antivenom is available in Thailand. Genet Mol Res 14:13981–13997
Teng KT, McGreevy PD, Toribio J-ALML, Dhand NK (2016) Trends in popularity of some morphological traits of purebred dogs in Australia. Canine Genet Epidemiol 3:2. https://doi.org/10.1186/s40575-016-0032-2
The Kennel Club (2022) Annual review 2022. https://kc-media-production.azureedge.net/Media/4103/the-kennel-club-annual-report.pdf. Accessed 19 Sept 2023
Vonholdt BM, Driscoll CA (2016) Origins of the dog: genetic insights into dog domestication. Cambridge University, Cambridge, pp 22–41
Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10:249–256. https://doi.org/10.1046/j.1365-294X.2001.01185.x
Zhang L, Liu Y, Ke QT, Ardalan A, Boonyaprakob U, Savolainen P (2020) Complete range of the universal mtDNA gene pool and high genetic diversity in the Thai dog population. Genes 11:253. https://doi.org/10.3390/genes11030253

Authors and Affiliations

Chadaphon Thatukan[1,2,3] · Chananya Patta[1,2,3] · Worapong Singchat[1,4] · Wattanawan Jaito[1,2,3] · Nichakorn Kumnan[1,2,3] · Piangjai Chalermwong[1,2,3] · Thitipong Panthum[1,4] · Wongsathit Wongloet[1,4] · Pish Wattanadilokchatkun[1] · Thanyapat Thong[1] · Syed Farhan Ahmad[1,4] · Narongrit Muangmai[1,5] · Kyudong Han[1,6,7,8] · Akihiko Koga[1] · Prateep Duengkae[1,4] · Ratthanin Patcharakulvorawat[3] · Kornsorn Srikulnath[1,2,4,9,10]

✉ Kornsorn Srikulnath
kornsorn.s@ku.ac.th

1 Animal Genomics and Bioresources Research Unit (AGB Research Unit), Faculty of Science, Kasetsart University, 50 Ngamwongwan, Chatuchak, Bangkok 10900, Thailand

2 Sciences for Industry, Faculty of Science, Kasetsart University, 50 Ngamwongwan, Chatuchak, Bangkok 10900, Thailand

3 Mind Pets Animal Hospital, 169/10, Khlong Song Ton Nun, Lat Krabang, Bangkok 10520, Thailand

4 Special Research Unit for Wildlife Genomics (SRUWG), Department of Forest Biology, Faculty of Forestry, Kasetsart University, 50 Ngamwongwan, Chatuchak, Bangkok 10900, Thailand

5 Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand

6 Department of Microbiology, Dankook University, Cheonan 31116, Korea

7 Bio-Medical Engineering Core Facility Research Center, Dankook University, Cheonan 31116, Korea

8 Smart Animal Bio Institute, Dankook University, Cheonan 31116, Republic of Korea

9 Department of Genetics, Faculty of Science, Kasetsart University, 50 Ngamwongwan, Chatuchak, Bangkok 10900, Thailand

10 Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University, Kasetsart University, Bangkok 10900, Thailand