FULL PAPER

Wildlife Science

Population structure of the raccoon dog (*Nyctereutes procyonoides*) using microsatellite loci analysis in South Korea: Implications for disease management

Yoon Jee HONG[1], Kyung Seok KIM[2], Mi-Sook MIN[1]* and Hang LEE[1]*

[1]Conservation Genome Resource Bank for Korean Wildlife (CGRB), Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
[2]Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA 50011, U.S.A.

ABSTRACT. The prevention and control of infectious diseases transmitted by wildlife are gaining importance. To establish effective management strategies, it is essential to understand the population structure of animals. Raccoon dogs (*Nyctereutes procyonoides*) in South Korea play a key role in the maintenance of food web stability and possess genetic compositions that are unique compared to those in other areas. However, wild raccoon dogs play another role as the main host of various infectious diseases. To establish long-term strategies for disease management, we investigated the genetic structure and possible geographic barriers that influence the raccoon dog population in South Korea by analyzing 16 microsatellite loci. The present study showed that mountains were the major factors responsible for genetic structuring, along with distance. We proposed potential management units (MUs) for raccoon dogs based on the genetic structuring and gene-flow barrier data obtained in this study. Four MUs were suggested for the Korean raccoon dog population (Northern, Central, Southwestern, and Southeastern). The Korean raccoon dog population structure determined in this study and the proposed MUs will be helpful to establish pragmatic strategies for managing Korean raccoon dog population and for preventing the transmission of infectious diseases.

KEY WORDS: management unit (MU), microsatellites, *Nyctereutes procyonoides*, population structure, raccoon dog

The raccoon dog is a true omnivore [11] that maintains the food web stability in ecosystems [1] and also functions as an effective scavenger [21]. Raccoon dogs play an important role as one of the medium-sized predators or scavengers because of the reduction or extinction of their competitors and other major terrestrial predators, assisting in maintaining the ecological balance in the Korean Peninsula [8]. A phylogeographic study using complete mtDNA cytochrome *b* sequences suggested that Korean raccoon dogs are a unique population that have adapted to the particular environment of Korean Peninsula in northeast Asia and are different from the other continental and Japanese raccoon dog populations [14]. This study showed that the Korean raccoon dog population should be considered as a valuable biological resource requiring proper management and conservation strategies.

Wild raccoon dogs (*Nyctereutes procyonoides*) play another role as the main host of various infectious diseases, such as rabies, canine distemper, and parasites, which can be transmitted among domestic dogs and wild Canidae species [11, 35, 37]. Its high adaptability to various environments and the reduction of its competitors and predators might assist in increasing its population size in South Korea [9]. The increasing Korean raccoon dog population has raised public concerns over their potential role as disease and parasite vectors, particularly for contagious zoonotic diseases [8, 22]. Some pathogens and parasites are transmitted between dogs and wild canids, and from rodents to foxes, raccoon dogs, dogs, and even humans [11]. Potential issues regarding the prevention and control of wildlife-related infectious diseases are becoming increasingly important. Hence, as part of the effort to establish proper disease risk management strategies, it is essential to understand the population structure and connectivity among the animal populations of epidemiological interest [3, 31]. Lack of information on the movement of individuals of the vector and/ or host population makes it difficult to control and prevent disease outbreaks and transmission. In South Korea, rabies has been mostly reported in wild and domestic animals, and in humans in the northern part of Seoul/Gyeonggi and Gangwon Provinces of South Korea; and preventive measures against rabies are focused only in these areas [35]. However, limited scientific data are

*Correspondence to: Min, M.-S.: minbio@yahoo.co.kr, Lee, H.: hanglee@snu.ac.kr
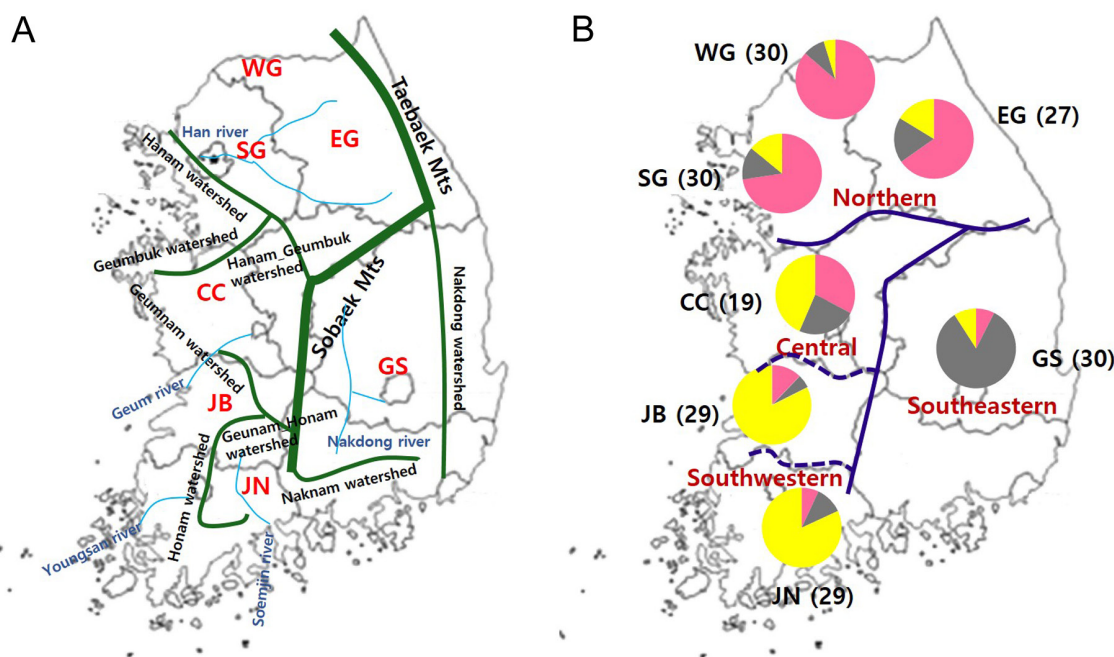
Fig. 1. Study areas including geographical features (A) and sampling information (B) of raccoon dogs. Thick and thin solid lines in green indicate representative mountains and watersheds, respectively. Solid lines in blue mean rivers. Pie charts show proportions of the STRUCTURE clusters of each subdivided group in Fig. 3. Solid (strong) and dotted (weak) lines indicate the genetic barriers generated by BARRIER program using Monmonier's algorithm. SG: Seoul/Gyeonggi, WG: Western Gangwon, EG: Eastern Gangwon, CC: Chungcheong, JB: Jeonbuk, JN: Jeonnam, GS: Gyeongsang.

available on the dispersal or movement pattern of raccoon dogs, which is the major wild rabies host in South Korea with respect to the geographical features such as rivers and mountain ranges in South Korea. This information might be critical for establishing appropriate strategies to prevent and manage infectious diseases transmitted by raccoon dogs. The genetic structure of wild animal population is formed by limited dispersal owing to artificial or natural barriers. Therefore, investigation on the genetic structure of populations will be of immense help to provide information for the proper management of wild animal populations and their diseases.

Several studies have been conducted on wild animal population structure in South Korea by using microsatellites: wild boar (*Sus scrofa*) [2], water deer (*Hydropotes inermis*) [16], Siberian roe deer (*Capreolus pygargus*) [17], and striped field mouse (*Apodemus agrarius*) [10]. In case of wild boars, the authors did not show a clear population structure but a gradual geographic gradient from north to south and the high mountain range (Baekdudaegan: Taebaek, and Sobaek mountains, Fig. 1) was suggested as the geographic barrier. Generally, raccoon dogs prefer lower altitude (below 300 m) [21] and low temperature (below 0°C) that limit their migration [12]. Hong *et al.* [8] also reported the possibility that high altitude mountains (Sobaek mountains, Fig. 1) can act as a geographical barrier for raccoon dogs. In this study, we mainly investigated whether mountain ranges affected raccoon dog population in South Korea. In addition, impacts of the geographical distance on the population differentiation were tested.

Microsatellite markers are widely used to identify individuals and to study the phylogeographic relationships, genetic variation, population structuring, and genetic differentiation in various wildlife species [8, 17]. Information obtained by analyzing microsatellite loci is used for determining the genetic structure and recent gene flow among natural populations and serves as the basis for establishing management and conservation strategies for a population, e.g., for defining conservation or management units (MUs) [23]. To help establish long-term strategies for effective population and disease management of raccoon dogs in South Korea, we examined the genetic structure of seven regional populations, by focusing on their genetic diversity and the potential barriers of gene flow among the populations. The findings of the study would provide a valuable resource that might be applied to other mammalian species and related infectious disease for developing better management strategies.

MATERIALS AND METHODS

DNA samples and genotyping

We analyzed the tissue samples of 194 raccoon dogs collected from seven provinces of South Korea (SG: Seoul/Gyeonggi, WG: Western Gangwon, EG: Eastern Gangwon, CC: Chungcheong, JB: Jeonbuk, JN: Jeonnam, and GS: Gyeongsang; Fig. 1). All the experimental materials were collected legally and provided by the Conservation Genome Resource Bank for Korean Wildlife (CGRB) for this study. All procedures followed when working with animal samples were in accordance with the guidelines of Seoul

Table 1. Genetic diversity estimates for raccoon dogs

Location	N	No. of alleles	Allelic diversity	Allelic richness	H_E	H_O	HWE *P*-value	Number and loci list with null allele
SG	30	104	5.8	4.457	0.711	0.622	0.001	2 (*Nyct2, Nyct6*)
WG	30	95	5.9	4.157	0.689	0.693	0.017	none
EG	27	95	6.4	4.248	0.720	0.556	0.002	2 (*Nyct6, Nyct9*)
CC	19	103	6.5	4.875	0.708	0.681	0.000	3 (*Nyct2, Nyct3, Nyct5*)
JB	29	86	5.4	3.940	0.663	0.609	0.051	3 (*Nyct2,Nyct4, Nyct9*)
JN	29	88	5.5	4.001	0.658	0.651	0.357	none
GS	30	93	5.9	4.220	0.683	0.646	0.000	4 (*Nyct2, Nyct4, Nyct6, Nyct10*)

N: Number of individual, No. of alleles: Number of alleles, Allelic diversity: Mean number of alleles, H_O: Observed heterozygosity, H_E: Expected heterozygosity, HWE *P*-value: The probability of Hardy-Weinberg equilibrium ($P<0.05$: significant departure from Hardy-Weinberg equilibrium).

National University Institutional Animal Care and Use Committee (SNU-IACUC). Details of sampling locations and sample sizes are shown in Table 1. In the case of Gangwon individuals, the western area was very close to Seoul/Gyeonggi; therefore, these individuals were analyzed separately from the eastern individuals of Gangwon. Sixteen microsatellite loci were used to determine the genotypes. Four microsatellite loci (*ZuBeCa 15, ZuBeCa 17, ZuBeCa 18,* and *ZuBeCa 26*) were originally developed for the dog, *Canis lupus* [29, 30]. The other 12 microsatellite loci (*Nyct1* to *Nyct12*) were developed for the Korean raccoon dog, *Nyctereutes procyonoides koreensis* [8]. The genomic DNA of all dogs was extracted using a DNeasy Tissue kit (QIAGEN, Valencia, CA, U.S.A.) and amplified using PCR under the following touchdown conditions: initial denaturation for 3 min at 94°C; followed by 20 cycles at 94°C for 1 min, 60–50°C (decreased by 0.5°C per cycle) for 1 min, 72°C for 1 min; again followed by 20 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR reaction mixture comprised 1.5 mM MgCl$_2$, 200 μM of each dNTP, 0.5 U i-Star *Taq* DNA polymerase (iNtRON Inc., Seongnam, Gyeonggi, Korea), 0.3 μM each of the fluorescent labeled forward primer and non-fluorescent reverse primers, and 10–50 *n*g template DNA. Alleles were genotyped using an ABI Prism 3730 XL DNA Analyzer (Applied Biosystems, Calsbad, CA, U.S.A.) and a GeneScan-500 ROX size standard (Applied Biosystems), and subsequently analyzed with GeneMarker v1.9 software (SoftGenetics, State College, PA, U.S.A.).

Data analysis

Genetic diversity measurements, including the mean number of alleles, and observed (H_O) and expected (H_E) heterozygosity under Hardy-Weinberg assumptions, were obtained using GenAlEx v6.1 [24]. Allelic richness, an important measure of genetic diversity, was determined using the program FSTAT 2.9.3.2 [5]. Deviations from Hardy-Weinberg equilibrium for each population were estimated using the exact probability test with Fisher's method [6] implemented in GENEPOP v3.3 [27]. Null alleles for each locus and population were checked using MICROCHECKER 2.2.3 [32]. To analyze the genetic relationships among populations, pairwise F_{ST} [33] and Nei's genetic distance were calculated using FSTAT 2.9.3.2 [5] and GenAlEx v6.1 [24], respectively. We applied the Bonferroni correction to significance levels to account for multiple tests [28]. GenAlEx v6.1 [24] was further used to conduct principal coordinate analysis (PCoA) for the construction of a scatter diagram to visualize geometric relationships among populations. We investigated the genetic structure using a hierarchical method based on Bayesian clustering function of STRUCTURE 2.2 [26]. Data were represented by *K* separate clusters and the log posterior probability for a given *K*, ln Pr (*X/K*), was generated for each of the 30 STRUCTURE runs at *K* values of 1–7 for the locations of the raccoon dog samples. The initial burn-in period was 100,000 replications, followed by another 200,000 replications. Two models, the admixture and the correlated allele frequencies, were chosen for the analysis. The Δ*K* was also calculated using the method described by Evanno *et al*. [4]. Isolation-by-distance (IBD) [34] was obtained by regression of genetic distance ($F_{ST}/(1-F_{ST})$) on geographic distance [Ln(dis):Km] between pairs of populations. Mantel test was carried out with 999 permutations for correlations. We applied Monmonier's algorithm to detect the genetic barriers among populations using BARRIER 2.2 [20], and geographical coordinates were used for each population to obtain Delaunay triangulation connecting locations. *F*-statistics (F_{RT}, F_{SR}, F_{ST}, F_{IS}, and F_{IT}) were estimated via an analysis of molecular variance (AMOVA) among populations and regions, using GenAlEx v6.1 [24]. Finally, to detect the evidence of a recent genetic bottleneck, we performed Wilcoxon sign rank test to determine heterozygosity excess [19] and a mode-shift in allelic frequency distribution [18] using BOTTLENECK version 1.2.02 [25]. In the Wilcoxon sign rank test, we chose a two-phase mutation model (TPM) employing 10% multiple-step mutations and 90% single-step mutations with 1,000 simulations. AGARst version 3.3 [7] was used to calculate the Garza and Williamson's *M* ratio for the number of alleles to the range of allele size, to detect reductions in both recent and historical population sizes.

RESULTS

Genetic characteristics and diversity

In total, 144 alleles were detected in 194 raccoon dog samples from seven populations using 16 microsatellite markers, ranging from 88 (Jeonnam) to 104 alleles (Seoul/Gyeonggi) as shown in Fig. 1 and Table 1. Sixteen private alleles were found to be unique to single populations. Most of these alleles had a frequency of <5%; however, only one allele had a frequency of >15% in the Gyeongsang population. Mean values of expected and observed heterozygosity were 0.690 (0.658–0.720) and 0.637 (0.556–0.693),

Table 2. Pairwise F_{ST} below the diagonal and gene flow (Nm, above the diagonal) between raccoon dog populations

F_{ST} \ Nm	1	2	3	4	5	6	7
1. SG (30)		21.987	17.863	8.355	8.005	5.992	7.261
2. WG (30)	0.006[a]		14.288	6.045	5.454	4.856	6.183
3. EG (27)	0.009[a]	0.015[a]		8.723	8.446	6.464	7.056
4. CC (19)	0.027[a]	0.047[a]	0.025[a]		10.969	6.158	6.166
5. JB (29)	0.038[a]	0.065[a]	0.036[a]	0.016[ns]		10.225	4.902
6. JN (29)	0.056[a]	0.072[a]	0.051[a]	0.049[a]	0.029[a]		5.467
7. GS (30)	0.048[a]	0.059[a]	0.049[a]	0.050[a]	0.076[a]	0.066[a]	

a) Significant after Bonferroni correction ($P<0.003$); ns, not significant; Indirect indicator of gene flow (Nm) was calculated among geographic populations using the equation, $Nm = 1/4\{(1-F_{ST})/F_{ST}\}$.
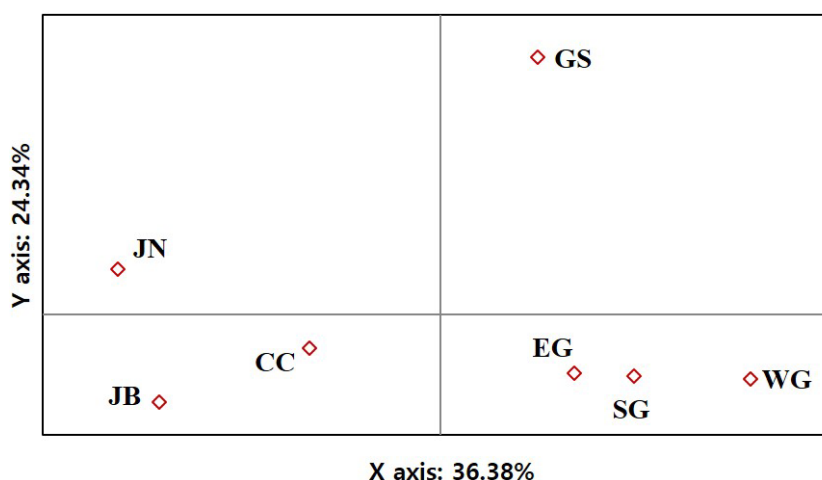

Fig. 2. Scatter diagram generated from the principal coordinate analysis of geographical locations of raccoon dogs.

respectively. The mean number of alleles across all the loci ranged from 5.4 (Jeonbuk population) to 6.5 (Chungcheong population). High allelic richness was observed in Chungcheong region, and null alleles were detected at more than one loci for each population, except two populations (Western Gangwon and Jeonnam populations, Table 1). Most populations revealed low frequencies (approximately 0.10) of null alleles. However, some loci in Chungcheong and Gyeongsang populations showed null allele frequencies more than 0.10. All the loci were included for further analyses because the loci with null alleles were present only in a subset of populations and some missing data for each locus and population might have influenced the detection of null alleles. Most populations, except Jeonnam, showed heterozygosity that deviated significantly from the expected heterozygosity under Hardy-Weinberg equilibrium (Table 1). The Wahlund effect or the presence of null alleles accounted for significant deviations from Hardy–Weinberg equilibrium.

Genetic differentiation and gene flow

Pairwise F_{ST}, a measure of genetic differentiation, and gene flow (Nm) between populations are shown in Table 2. Values of F_{ST} and Nm ranged from 0.006/21.987 (Seoul/Gyeonggi-Western Gangwon) to 0.076/4.856 (Jeonbuk-Gyeongsang). Negligible genetic differentiation was observed among Korean raccoon dog populations, with mean F_{ST} being 0.042 (Nm=8.613). Notably, the level of differentiation among Seoul/Gyeonggi, Western Gangwon, and Eastern Gangwon populations was relatively low (F_{ST}=0.006–0.015/Nm=14.288–21.987).

PCoA showed that Korean populations had three genetic clusters, i.e., populations from (1) Seoul/Gyeonggi, Western Gangwon, and Eastern Gangwon; (2) Chungcheong, Jeonbuk, and Jeonnam; and (3) Gyeongsang (Fig. 2).

Genetic structure

STRUCTURE analysis revealed that ΔK value was the highest when K was set to 3, implying that raccoon dogs of the seven areas comprised three distinct populations (Fig. 3). Except for the Chungcheong population, other Korean subpopulations were mainly divided based on their geographical location, i.e., (1) northern region (Seoul/Gyeonggi, Western Gangwon and Eastern Gangwon), (2) southwestern region (Jeonbuk and Jeonnam), and (3) southeastern region (Gyeongsang). The Chungcheong region,
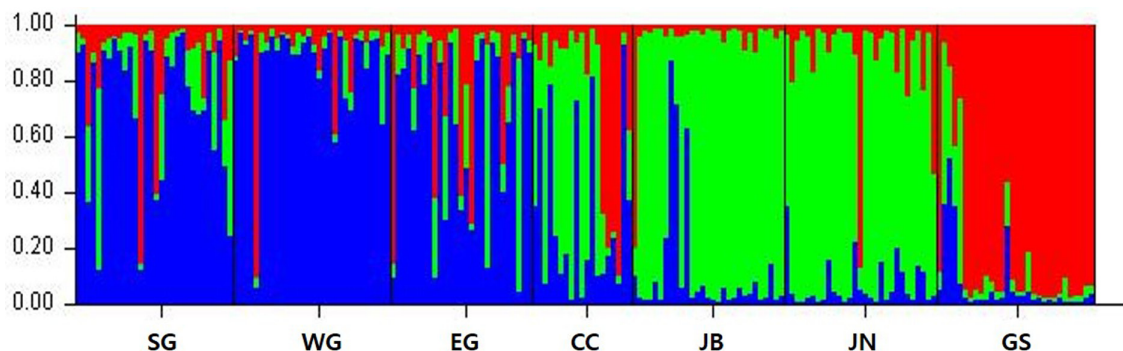
Fig. 3. Bar plots of raccoon dog population identified by STRUCTURE analysis.
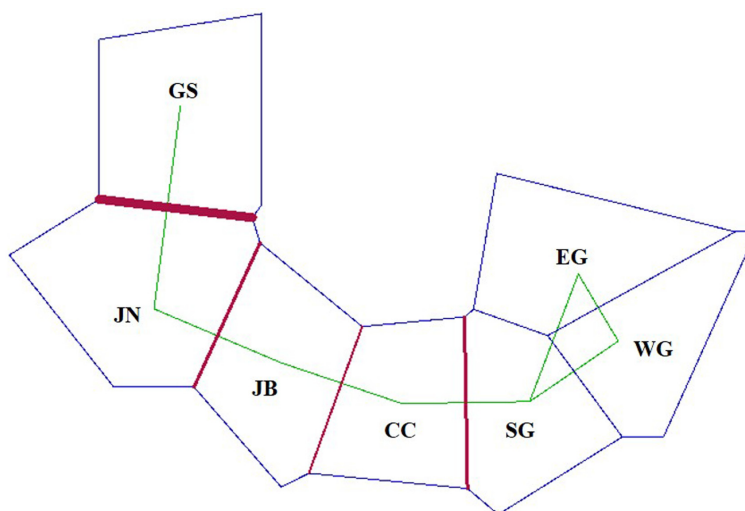

Fig. 4. Geographical barriers of limited gene flow determined by BARRIER using Monmonier's algorithm. The bold lines showing genetic boundaries are proportional to the strength of the barriers. The thicker lines, the stronger barriers.

which is located in the center of South Korea and is grouped within the southwestern region (Jeonbuk and Jeonnam), shared its genetic structure with all the three sub-structuring areas. Although Jeolla (Jeonbuk and Jeonnam) and Gyeongsang regions occupied the neighboring areas, highest genetic differentiation was detected between the two regions (mean F_{ST}=0.071; Table 2).

A strong barrier existed between the southeastern region (Gyeongsang) and other regions, including the northern (Seoul/Gyeonggi, Western Gangwon, and Eastern Gangwon), central, and southwestern (Chungcheong, Jeonbuk, and Jeonnam) regions in South Korea (Figs. 1 and 4). Weak barriers were detected between the central and southwestern regions and between the two southwestern regions. However, no barrier was detected among the northern regions (Fig. 4). Regression analysis for the genetic isolation by geographic distance (IBD) revealed significant correlation (Fig. 5). Results of genetic clustering (obtained from STRUCTURE analysis) and AMOVA among populations and regions suggested three regions ([1] northern, [2] central and southwestern, and [3] southeastern regions). Hierarchical AMOVA indicated that genetic differentiation was higher among regions (F_{RT}) than among populations within regions (F_{SR}) (Table 3).

Bottleneck analysis did not detect any bottleneck event under the TPM model at P=0.05, and the distribution of allele frequencies was a normal L-shaped distribution in all the populations (Table 4). Garza and Williamson's M value ranged from 0.763 to 0.827, implying that all the raccoon dog populations maintained a stable size over 100 generations (Table 4).

DISCUSSION

We investigated the genetic diversity and structure of the raccoon dog population in South Korea. The raccoon dog population in South Korea appeared to have three subpopulations, namely, northern (Seoul/Gyeonggi, western Gangwon and eastern Gangwon), southwestern (Chungcheong, Jeonbuk and Jeonnam) and southeastern (Gyeongsang) subpopulations. The Chungcheong subpopulation showed more active genetic exchange with adjacent areas owing to the geographical location of Chungcheong, which is the center of South Korea. A previous genetic study involving Korean raccoon dogs and 12 microsatellite markers

Table 3. Analysis of molecular variance (AMOVA) of raccoon dog populations

Source of variation	df	SS	MS	Est. var.	%	*F*-statistics	Value	*P*-value
Among regions	2	73.071	36.535	0.183	3	F_{RT}	0.030	0.001
Among populations	4	56.546	14.137	0.141	2	F_{SR}	0.024	0.001
Among individuals	187	1,209.976	6.470	0.753	12	F_{ST}	0.054	0.001
Within individuals	194	963.000	4.964	4.964	82	F_{IS}	0.132	0.001
Total	387	2,302.593		6.042	100	F_{IT}	0.178	0.001

Three Korean regions_1. SG, EG, and WG (Northern) 2. CC, JB, and JN (Central and South-western) 3. GS (South-eastern).
df, degree of freedom; SS, sum of squares; MS, mean squares.

Table 4. Analysis to detect a recent population bottleneck or past population reduction event within populations

Locality	Wilcoxon Test (TPM) H excess probability[a]	Mode-Shift	Garza & Williamson's M-ratio[b]
SG	0.46994	normal L-shaped distribution	0.799
WG	0.48997	normal L-shaped distribution	0.795
EG	0.39098	normal L-shaped distribution	0.792
CC	0.16125	normal L-shaped distribution	0.803
JB	0.12611	normal L-shaped distribution	0.810
JN	0.31609	normal L-shaped distribution	0.827
GS	0.29829	normal L-shaped distribution	0.763

a) One-tail probability for an excess of observed heterozygosity relative to the expected equilibrium heterozygosity, computed from the observed number of alleles under mutation-drift equilibrium; TPM: two-phase model of mutation. b) M-ratio=Mean ratio of the number of allele size.
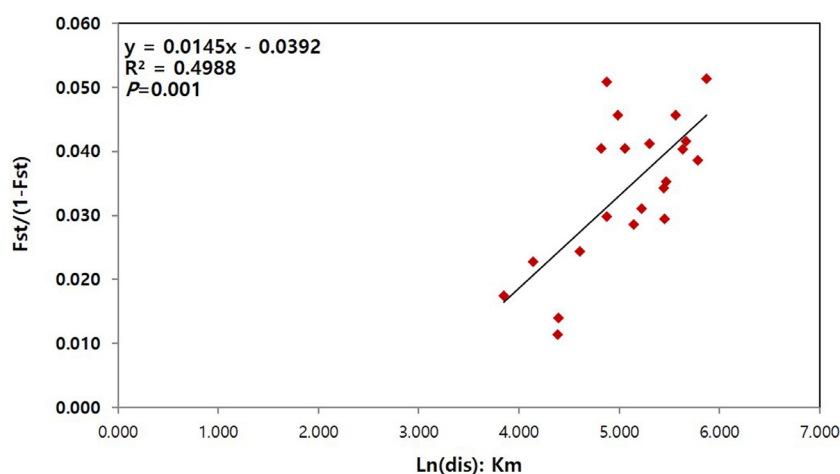

Fig. 5. Regression analysis for genetic isolation by geographic distance (IBD) of raccoon dog populations. Mantel test was carried out with 999 permutations for correlations.

also demonstrated that the Chungcheong raccoon dog subpopulation formed an intermediate group and that the southeastern subpopulation (Gyeongsang) was differentiated from other subpopulations. This study asserted that gene flow was limited by the Sobaek mountains [8], wherein the high altitude of the Sobaek mountains between the southeastern and southwestern subpopulations led to a high differentiation between these subpopulations. The results of the present study also supported the hypothesis that mountains are the major factors responsible for genetic structuring of this species. Melis *et al.* [21] observed that raccoon dogs prefer open landscapes, agricultural lands, lakeshores, and regions with elevations lower than 300 m, but can occasionally live at up to 800 m. Two watersheds, Hanam and Geumbuk, at elevations more than 400–600 m, exist between the northern and central subpopulations [15]. Although their altitudes were not high for limiting the migration of raccoon dog, double blocking effects by the two watersheds might play a role. Moreover, high Hanam Geumbuk watershed (492–1,058 m) which is a joint watershed of the two, must act as an effective barrier. The Geunam watershed (average 624 m) located between the central and southwestern subpopulations could function as partial barrier to the migration of raccoon dogs. On the other hand, the Honam watershed (average 627 m) present between Jeonbuk and Jeonam does not seem to be a barrier for them [15]. Moreover, unlike home range of raccoon dogs in the rural areas of Japan (<2.78 km²) raccoon dogs in rural areas of South Korea has smaller home

range (<0.8 km^2) [13], implying that geographical distance is another important cause of genetic differentiation among the raccoon dog subpopulations. A significant correlation between the regression of genetic distance (F_{ST}) and geographical distance also supported this assumption (Fig. 5). Furthermore, river might not function as a geographical barrier to raccoon dog populations. We tested whether river influenced the migration of raccoon dogs using samples in SG, and the locations of the samples from other provinces were not adequate for testing the river's impact on raccoon dog population. The F_{ST} (0.016) and STRUCTURE (K=1) analyses revealed that the Han river, which runs between the north and south of Seoul (Fig. 1), does not seem to act as a barrier (data not shown).

We propose four tentative MUs in the South Korean raccoon dog population based on genetic analyses, namely, northern, central, southwestern, and southeastern subpopulations. Although the central (Chungcheong) subpopulation was grouped with the southwestern (Jeonbuk and Jeonnam) subpopulation, we suggest that the Chungcheong subpopulation is considered as another potential MU. Because the Chungcheong subpopulation had a diverse genetic composition resulting from the active interaction with raccoon dogs of other regions, it might function as a critical zone to regulate the spread of diseases by the animals. A previous study showed that an MU can be smaller than a "subpopulation" by reflecting spatial differences [36], and the Chungcheong region might support this. For instance, the caribou in Eastern Canada is grouped into six genetic groups; however, eight MUs were eventually distinguished, which is consistent with the ecological criteria [36]. Moreover, potential threats, including disease outbreaks, need to be considered to establish MUs [36]. Such tentative MUs might assist in establishing a national strategy for preventing and controlling infectious diseases and zoonoses, such as rabies, which is mainly transmitted by wild raccoon dog population. In South Korea, rabies has been reported in wild and domestic animals, and in humans mostly in the northern part of Seoul/Gyeonggi and Gangwon Provinces; therefore, preventive measures against rabies have been focused mainly in these areas. However, our results indicated that they were grouped as a single population with active genetic exchanges within the subpopulation of the entire Seoul/Gyeonggi and Gangwon Provinces. Therefore, it would be desirable to expand the present bait vaccination program to target all the regions in the northern subpopulation. Then, the geographical barrier between the tentative northern and central MUs might be used as the first line to prevent disease dissemination to central MU, which might further function as the second buffer zone for the southern MUs. Therefore, the status of infectious diseases in the central and northern raccoon dog subpopulations needs to be closely monitored with great concern.

Our study suggested potential MUs for managing the raccoon dog population in South Korea based on the observed population structure. However, the proposed MUs should be confirmed by additional studies using more samples from a wider range of areas that are not covered in the present study. In South Korea, we deduced that high mountain ranges, watersheds, and geographical distances are responsible for the observed population structure, such that they limit gene flow and migration and the dispersal of individuals among subpopulations. Therefore, further studies should be performed with fine-scaled sample collection from potential subpopulations on both sides of tentative gene-flow barriers such as mountains, rivers, or highways. For example, genetic samples of raccoon dog subpopulations in the eastern and western regions of Baekdudaegan, the most prominent mountain range that runs north–south along the eastern side of the Korean peninsula, in Gangwon Province can be analyzed to determine whether this mountain range acts as a gene-flow barrier. Because we analyzed only the samples of raccoon dogs from the western region of Baekdudaegan in Gangwon Province, the function of Baekdudaegan as a gene-flow barrier could not be assessed in our study. In addition, the role of rivers and artificial landscape structures such as express highways, railroads, and industrial or agricultural regions in generating the population structure should be further investigated.

The demilitarized zone (DMZ) between South and North Korea is one such example. Information on the movement pattern of wild animals across the DMZ is important to establish holistic management plans for wild animal populations in the Korean Peninsula. Particularly, appearance of rabies in the northern part of South Korea only casts suspicion on the origin of rabies; it might originate from wild animal populations in the DMZ or North Korea. Although wild animal populations in the DMZ and North Korea are thought to have been separated from those in South Korea by heavy fences for the last over 60 years, some small- and medium-sized mammals are suspected to have crossed these fences. However, this possibility has not been scientifically evaluated to date. Therefore, studies on fine-scale population structure and genetic diversity analyses might identify cryptic barriers of dispersal, cryptic or isolated subpopulations, or subpopulations with genetic deterioration.

In conclusion, our study determined the population genetic structure, gene-flow patterns, and genetic diversity of raccoon dog population in South Korea by genetic analysis of microsatellite markers. We proposed tentative MUs based on the population structure and geographical information for proper management and conservation of raccoon dog population and to develop strategies for preventing and controlling diseases transmitted by raccoon dogs.

REFERENCES

1. Ågren, G. I., Stenberg, J. A. and Björkman, C. 2012. Omnivores as plant bodyguards—A model of the importance of plant. *Basic Appl. Ecol.* **13**: 441–448. [CrossRef]
2. Choi, S. K., Lee, J. E., Kim, Y. J., Min, M. S., Voloshina, I., Myslenkov, A., Oh, J. G., Kim, T. H., Markov, N., Seryodkin, I., Ishiguro, N., Yu, L., Zhang, Y. P., Lee, H. and Kim, K. S. 2014. Genetic structure of wild boar (*Sus scrofa*) populations from East Asia based on microsatellite loci

analyses. *BMC Genet.* **15**: 85. [Medline] [CrossRef]

3. Drygala, F., Korablev, N., Ansorge, H., Fickel, J., Isomursu, M., Elmeros, M., Kowalczyk, R., Baltrunaite, L., Balciauskas, L., Saarma, U., Schulze, C., Borkenhagen, P. and Frantz, A. C. 2016. Homogenous population genetic structure of the non-native raccoon dog (*Nyctereutes procyonoides*) in Europe as a result of rapid population expansion. *Plos One* **11**: 4 e0153098. [CrossRef]

4. Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**: 2611–2620. [Medline] [CrossRef]

5. Goudet, J. 1995. FSTAT, Version 1.2, a computer program to calculate F-statistics. *J. Hered.* **86**: 485–486. [CrossRef]

6. Guo, S. W. and Thompson, E. A. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**: 361–372. [Medline] [CrossRef]

7. Harley, E. H. 2003. AGARst. A program for calculating allele frequencies, Gst and Rst from microsatellite data plus a number of other population genetic estimates and outputting files formatted for various other population genetic programs. Version 3.3.: Department of Chemical Pathology, University of Cape Town, Cape Town.

8. Hong, Y., Kim, K. S., Lee, H. and Min, M. S. 2013. Population genetic study of the raccoon dog (*Nyctereutes procyonoides*) in South Korea using newly developed 12 microsatellite markers. *Genes Genet. Syst.* **88**: 69–76. [Medline] [CrossRef]

9. Jo, Y. S. 2015. Mammals of Korea: Conservation and management. Ph.D. Dissertation. Texas Tech University, Lubbock.

10. Jo, Y. S., Kim, H. N., Baccus, J. T. and Jung, J. 2016. Genetic differentiation of the Korean striped field mouse, *Apodemus agrarius* (Muridae, Rodentia), based on microsatellite polymorphism. *Mammalia* **81**: 297–307.

11. Kauhala, K. and Kowalczyk, R. 2011. Invasion of the raccoon dog *Nyctereutes procyonoides* in Europe: History of colonization, features behind its success, and threats to native fauna. *Curr. Zool.* **57**: 584–598. [CrossRef]

12. Kauhala, K. and Saeki, M. 2016. Nyctereutes procyonoides*.* The IUCN Red List of Threatened Species.T14925A85658776.

13. Kim, B. J., Choi, T. Y., Park, J. H., Kim, Y. J. and Lee, H. 2008. A brief report of the short-term home range study of a pair of raccoon dogs (*Nyctereutes procyonoides koreensis*) in a rural area of Gurye, Chonnam province, South Korea using radiotracking method. *Kor. J. Env. Eco.* **22**: 230–240.

14. Kim, S. I., Park, S. K., Lee, H., Oshida, T., Kimura, J., Kim, Y. J., Nguyen, S. T., Sashika, M. and Min, M. S. 2013. Phylogeography of Korean raccoon dogs: implications of peripheral isolation of a forest mammal in East Asia. *J. Zool. (Lond.)* **290**: 225–235. [CrossRef]

15. Kim, Y. P., Lim, E. S. and Kim, Y. J. 2004. Focusing on mountain range analysis. pp. 145–154. *In*: Restructuring Mountain Systems in Korean Peninsula, Korea Research Institute for Human Settlements (KRIHS), Anyang.

16. Lee, Y. S., Choi, S. K., An, J., Park, H. C., Kim, S. I., Min, M. S., Kim, K. S. and Lee, H. 2011. Isolation and characterization of 12 microsatellite loci from Korean water deer (*Hydropotes inermis argyropus*) for population structure analysis in South Korea. *Genes Genomics* **33**: 535–540. [CrossRef]

17. Lee, Y. S., Markov, N., Voloshina, I., Argunov, A., Bayarlkhagva, D., Oh, J. G., Park, Y. S., Min, M. S., Lee, H. and Kim, K. S. 2015. Genetic diversity and genetic structure of the Siberian roe deer (*Capreolus pygargus*) populations from Asia. *BMC Genet.* **16**: 100. [Medline] [CrossRef]

18. Luikart, G., Allendorf, F. W., Cornuet, J. M. and Sherwin, W. B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Hered.* **89**: 238–247. [Medline] [CrossRef]

19. Luikart, G. and Cornuet, J. M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol.* **12**: 228–237. [CrossRef]

20. Manni, F., Guérard, E. and Heyer, E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Hum. Biol.* **76**: 173–190. [Medline] [CrossRef]

21. Melis, C., Nordgård, H., Herfindal, I., Kauhala, K., Åhlen, P. A., Strann, K. B. and Andersen, R. 2007. Raccoon dogs in Norway−potential expansion rate, distribution area and management implications. NTNU Vitenskapsmuseet. *Rapp. Zool. Ser.* **3**: 1–49.

22. Oh, S. Y., Kim, S. A., Kim, J. Y., Yoo, H. S., Lee, K. K. and Shin, N. S. 2012. Detection of antibodies against the rabies virus in Korean raccoon dogs (*Nyctereutes procyonoides koreensis*). *J. Zoo Wildl. Med.* **43**: 174–176. [Medline] [CrossRef]

23. Palsbøll, P. J., Bérubé, M. and Allendorf, F. W. 2007. Identification of management units using population genetic data. *Trends Ecol. Evol. (Amst.)* **22**: 11–16. [Medline] [CrossRef]

24. Peakall, R. and Smouse, P. E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288–295. [CrossRef]

25. Piry, S., Luikart, G. and Cornuet, J. M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.* **90**: 502–503. [CrossRef]

26. Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959. [Medline]

27. Raymond, M. and Rousset, F. 1995. GENEPOP version 4.0: population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249. [CrossRef]

28. Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225. [Medline] [CrossRef]

29. Schelling, C., Schläpfer, J., Vennos, C., Stahlberger-Saitbekova, N. and Dolf, G. 2000. Seven novel cosmid-derived canine microsatellites. *Anim. Genet.* **31**: 77. [Medline] [CrossRef]

30. Schläpfer, J., Dolf, G., Saitbekova, N. and Schelling, C. 1999. Twelve novel cosmid-derived canine microsatellites. *Anim. Genet.* **30**: 387–388. [Medline] [CrossRef]

31. Singer, A., Kauhala, K., Holmala, K. and Smith, G. C. 2009. Rabies in northeastern Europe-the threat from invasive raccoon dogs. *J. Wildl. Dis.* **45**: 1121–1137. [Medline] [CrossRef]

32. Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**: 535–538. [CrossRef]

33. Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370. [Medline]

34. Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* **15**: 323–354. [Medline] [CrossRef]

35. Yang, D. K., Park, Y. N., Hong, G. S., Kang, H. K., Oh, Y. I., Cho, S. D. and Song, J. Y. 2011. Molecular characterization of Korean rabies virus isolates. *J. Vet. Sci.* **12**: 57–63. [Medline] [CrossRef]

36. Yannic, G., St-Laurent, M. H., Ortego, J., Taillon, J., Beauchemin, A., Bernatchez, L., Dussault, C. and Côté, S. D. 2016. Integrating ecological and genetic structure to define management units for caribou in Eastern Canada. *Conserv. Genet.* **17**: 437–453. [CrossRef]

37. Zhao, J., Shi, N., Sun, Y., Martella, V., Nikolin, V., Zhu, C., Zhang, H., Hu, B., Bai, X. and Yan, X. 2015. Pathogenesis of canine distemper virus in experimentally infected raccoon dogs, foxes, and minks. *Antiviral Res.* **122**: 1–11. [Medline] [CrossRef]