수의학석사 학위논문

Ecological Genetic Study on Yellow-throated Martens (*Martes flavigula*) in Jirisan National Park Inferred from Microsatellite Loci Analysis : Individual Identification, Relatedness and Abundance

지리산에 서식하는 담비의 microsatellite 좌위 분석을 이용한 생태유전학적 연구 : 개체식별, 혈연관계 그리고 개체수 측정

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Abstract

Ecological Genetic Study on Yellow-throated Martens (*Martes flavigula*) in Jirisan National Park Inferred from Microsatellite Loci Analysis : Individual Identification, Relatedness and Abundance

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Understanding genetic relatedness and abundance estimate is necessary for developing efficient conservation strategies of social animal such as yellow-throated martens. Although social structure based on relatedness influences life style such as behavior, foraging, dispersal and mating system, social structure of yellow-throated

marten has not been revealed due to confusion with elusive species and limitation of traditional ecological surveying method. By using ten cross-species microsatellite markers, this study investigated genetic diversity and assessed the relationship among yellow-throated martens and abundance estimate from non-invasive samples. Total of 223 hair samples were collected from specially designed hair trap in Jirisan National Park and 21 genetic profiles were found from 50 genotypes. Genetic background samples of 12 *Martes flavigula* were collected across a wide geographic area in the Korean peninsula. Averages of expected heterozygosities of 12 genetic background samples and 21 individuals from hairs were 0.45 and 0.44, respectively. These values of genetic diversity were at moderate level compared to other species of the same genus. According to our results, mean relatedness of 21 individuals was −0.05 ± SD 0.42 (SE = 0.03) and −0.05 ± SD 0.27 (SE = 0.02) using Queller and Goodnight method and Lynch and Ritland method, respectively. The results show that kinship does not play a significant role in shaping social structure and dispersal can be predicted due to low degree of relatedness among martens. Abundance estimate from mark-recapture method for yellow-throated marten in intensive study area was 32 ± 6.8397 (95% CI; 25 - 54). However, abundance estimate for entire region of Jirisan National Park was difficult to estimate due to our limited sampling efforts. It was concluded that ten polymorphic microsatellite markers enabled individual identification, genetic relatedness and abundance estimate for yellow-throated martens.

keywords : *Martes flavigula*, yellow-throated marten, genetic relatedness, abundance, microsatellite, jirisan national park

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Table of Contents

List of Tables

List of Figures

Introduction

1.1 General Background

Yellow-throated marten (*Martes flavigula*) is distributed throughout western Asia, the Far East Russia and eastern Asia in a wide variety of habitats (Roberts, 1977; Sathyakumar, 1999) (Figure 1). It has been designated as an endangered species (category II) by the Korean Ministry of Environment since 1997 and listed as Least Concern on IUCN Red List (appendix III).

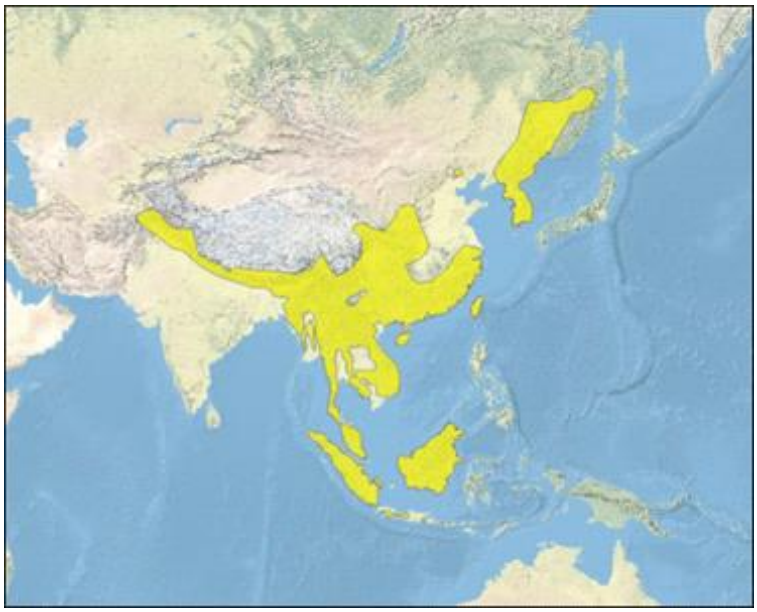

Figure 1. World-wide distribution of *Martes flavigula* (IUCN).

This species has wide habitat range (20-40km^2) and diverse diets such as rodents, birds, amphibians, insects, fruits and ungulates, and martens also play the role as seed disperser (Sathyakumar, 1999; Harrison et al., 2004; Zhou et al., 2008; Woo, 2014). Moreover, unlike other *Martes* species, yellow-throated martens have beneficial social structure such as foraging in a group, which ranges from one to seven individuals depending on the seasons (Sathyakumar, 1999; Grassman et al., 2005; Parr and Duckworth, 2007; Woo, 2014). Group foraging of *M. flavigula* serves as the most significant predator to control ungulate populations in South Korea and may impact populations of other various prey species (Woo, 2014). For these reasons, yellow-throated martens are regarded as one of the top-level predators and suggested to be the keystone species and umbrella species in the Korean peninsula (Woo, 2014).

Although several ecological studies of yellow-throated marten were conducted (Roberts, 1977; Sathyakumar, 1999; Harrison et al., 2004; Grassman et al., 2005; Parr and Duckworth, 2007; Zhou et al., 2008, Woo 2014), to further design efficient conservation strategies, fundamental information of the species, such as demography and social structure of *M. flavigula* is essential. Woo (2014) stated that yellow-throated martens share overlapping habitat range with each other and a few of these are of kin relationship. However, due to little sexual dimorphism and similar morphological characteristics, individual identification, population estimation and determination of social structure by traditional ecological method are limited for *M.*

flavigula. Hence, genetic study using non-invasively collected samples can be an alternative to the traditional ecological method.

1.2 Application of Genetics for Ecological Study

The recent application of molecular genetics to the investigation of individual identification, population estimation and relatedness between individuals in a population, has provided new information about the ecology of many species, including behavioral parameters, dispersal and social structure (Gompper and Wayne, 1996; Taylor et al., 1997; Frantz et al., 2004). Application of genetic analyses in the ecological study of endangered species should thus enhance the understanding of their ecology (Taylor et al., 1997). For example, in the genus *Martes*, Mowat and Paetkau (2002) reported population estimation of *Martes americana* using non-invasive samples; Mullins et al. (2010) studied sex, individual identification and evaluated census of free-ranging *Martes martes* populations by genotyping scat and plucked hair, and Ruiz-González et al. (2013) assessed species and individual identification of sympatric mustelid *Martes martes* and *Martes foina*.

Highly polymorphic genetic markers can be utilized in ecological studies of wild populations using non-invasive samples (Gompper and Wayne, 1996; Taylor et al., 1997; Frantz et al., 2004; Park et al., 2011). Non-invasive sampling methods from wild animals in the field

have become trending tools for wildlife management, particularly in elusive, endangered species because DNA can be extracted from samples such as feces, hair follicles, feathers and saliva without the need to capture the target animal (Taberlet and Bouvet, 1992; Morin and Woodruff, 1996; Saito et al., 2008; Park et al., 2011). Collecting plucked hairs are less harmful to free-living wild animals and researchers can collect genetic samples without directly handling them. Thus hair samples have been successfully used to study the genetics of wildlife animals including mustelid species (Johnson et al., 2001; Frantz et al., 2003, 2004; Scheppers et al., 2007; Mullins et al., 2010; Johnson et al., 2013; Sheehy, 2013).

1.2.1 Social Structure

Social structure is formulated by social network of animals between interactions of individuals or populations (Hinde, 1976; Biondo et al., 2014). The benefits of living in social structure for many vertebrate species are reduced risk of predation, increased access to resources and increased reproductive success (Gompper, 1996). Negative aspects of group living are increased potential for parasite and disease transmission (Krause and Ruxton, 2002).

Social structure based on relatedness can have strong influence on mating pattern, foraging behavior, dispersal, competition and distribution of individuals (Gompper, 1996; Gompper et al., 1998;

Banks et al., 2002; Blundell et al., 2004; Garroway et al., 2013). Previous studies of social structure revealed dispersal by DNA analysis. Gompper et al. (1998) examined the influence of dispersal on genetic relationships of *Nasua narica*. This study revealed that female white-nosed coatis are highly philopatric and adult males do not disperse from the home range of their natal band.

1.2.2 Population Estimation

Estimating the population of top-level predator is important to its conservation, prey and wildlife management, however, options for evaluation of population are few and difficult due to the dynamics of population, endangered and cryptic species (Mowat and Strobeck, 2000; Frantz et al., 2004). Moreover, one of the most common ecological method using camera trap is limited due to similar intraspecific morphological characteristics. However, the recent advancement of genetic techniques and analysis programs has made it possible to census population size (Miller et al., 2005). Using non-invasive samples, many previous studies of population estimation have been reported (Mowat and Strobeck, 2000; Frantz et al., 2004; Hájková et al., 2009). Moreover, Mondol et al. (2009) estimated population size of *Panthera tigris* using combination of camera trap and DNA survey in India. This study revealed that genetic study for

population estimation was in close agreement with the photographic survey.

1.3 Previous Genetic Studies on the Yellow-throated Martens

Recently, *Martes flavigula* has received attention in molecular studies such as the species' phylogenetic status and complete mitochondrial genome. In 2002, Stone and Cook investigated the phylogeny of all extant species of *Martes* to infer evolutionary relationships and characteristics using complete sequences of mitochondrial cytochrome *b* gene and partial sequences of the nuclear aldolase C gene (241 bp). In 2011, Hosoda et al. studied phylogenetic relationships among species of the family Mustelidae using the combined nucleotide sequences of the three mitochondrial genetic loci (cytochrome *b* gene, 1140 bp; ND2 1044 bp; D-loop 540 bp). In 2013, Li et al. also examined evidence of molecular adaptation in the mitochondrial DNA cytochrome *b* gene in the subgenus *Martes*. Moreover, Li (2014) reported phylogenetic relationships within the subgenus *Martes* and the timing of gulonine divergences. In 2013, Xu et al. confirmed the mitogenome sequence of *M. flavigula*. The genome of *M. flavigula* was 16,549 bp in length and contained 13 protein-coding genes, 2 ribosomal RNA genes 22 transfer RNA genes and 1 control region.

In South Korea, Jang and Hwang (2014) determined complete mitochodrial sequences of *Martes flavigula*. The genome is 16,533 bp in length and control region 1078 bp was located between the tRNAPro and tRNAPhe genes. However, no previous studies have performed microsatellite analysis of *M. flavigula*. Thus, in order to aid in conservation management of *M. flavigula*, this study used microsatellite loci as the genetic marker to analyze yellow- throated marten populations in South Korea.

1.4 Goals of the Study

In populations estimation studies, hair trap is widely adopted for collecting hair samples; however it has certain concern regarding the origin of collected hairs (i.e. whether the collected hairs belong to the same or different individuals). DNA was extracted from single hair, which was obtained using specially designed hair trap, to prevent mixing of genotypes. By using multiplex panel of microsatellites, we obtained individual specific genetic profiles and these were used to estimate population abundance and to understand social structure (relationships among individuals). This study were aimed to (a) investigate genetic diversity of *Martes flavigula* using cross-species microsatellite markers developed from other mustelid species, (b) estimate the population size of *Martes flavigula* in intensive study area, and (c) examine the social structure based on genetic

relatedness among individuals in Jirisan National Park.

Materials and Methods

2.1 Study Area and Sampling

Jirisan National Park (N35 20′ E127 25′), part of the Baekdu Daegan mountain range, was designated Korea's first National Park on December 29th, 1967. It is also one of the 20 largest national parks with the area of 471.758 km² (Korea National Park Service, 2012). The sampling was conducted on the ridge of four different locations; Kojae (N35 17′ 26.52 E127 30′ 40.07), Jongsukdae (N35 17′ 48.89 E127 30′ 46.66), Siamjae (N35 18′ 06.11 E127 29′ 41.02) and Goribong (N35 18′ 50.41 E127 30′ 45.90) in Jirisan National Park (Figure 9). These locations were subjects of previous ecological study in which yellow-throated martens were captured alive and recorded by camera trapping (Woo, 2014). Sampling was conducted from December, 2014 to February, 2015. Hair samples were collected the day after bait was placed and/or up to 30 days.

We designed the hair trap made of wooden hexahedron (300 x 300 x 300 mm) with bottom and front panels removed. Three metal springs (15 mm in diameter and 300 mm long) were used as hair traps on the entrance where the front panel was removed (Figure 2, 3). Mixture of honey and raisins was used as bait and placed inside the hexahedron. When hair strands were captured on metal springs,

hair samples were collected by forceps, stored in paper bags, labeled, and then stored in room temperature until DNA extraction (Taberlet and Luikart, 1999; Murphy et al., 2000). After each sample collection, three springs and forceps were lighted with fire to prevent mixing of genotypes and cross-contamination.

Figure 2. Photographs of a designed hair trap used for non-invasive sampling of *M. flavigula* hair in each location of Jirisan National Park. Distance between the three springs are 50 mm each.

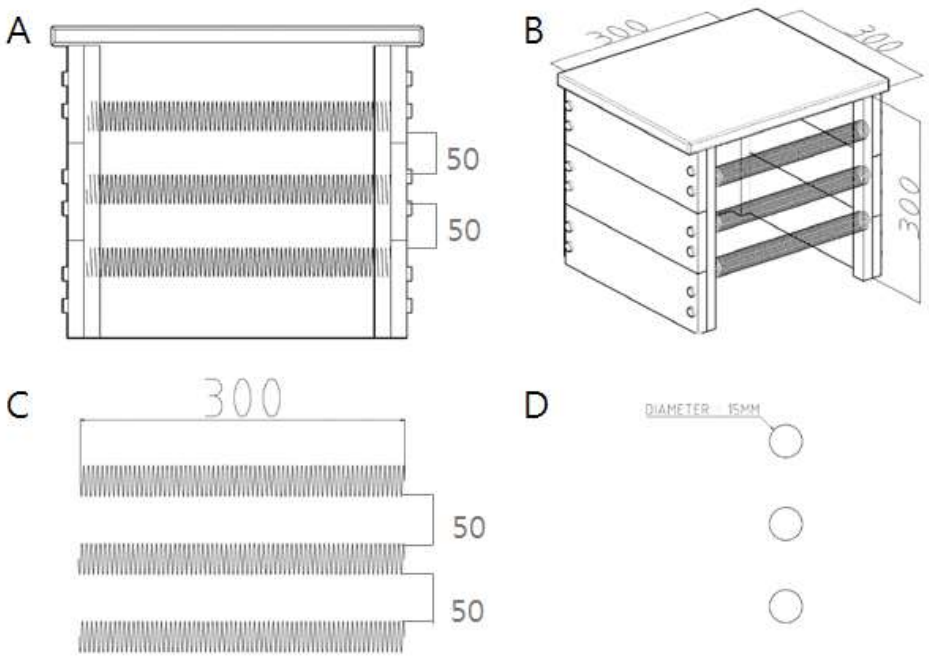

Figure 3. Illustration of hair trap designed in this study. A and B, designed hair trap made of wood in cube formation (300 mm x 300 mm x 300 mm) with bottom and front side opened. C, three metal springs attached in the front side of wooden trap. Each springs were 300 mm in length and set 50 mm apart from each other. D, each metal springs 15 mm in diameter.

Two motion sensor cameras (PC800 Hyperfire Professional Semi-Covert IR, Reconyx, USA and Natureview HD Essencial, Bushnell, USA) were placed at different positions, no more than 5 m apart from the trap, and were positioned facing towards the trap (Figure 4, 5). We followed two rules while sampling; first, species identification was only conducted when yellow-throated marten (*Martes flavigula*) and the same family Mustelidae (Mammalia, Carnivora) entered the hair trap. Second, in order to prevent overestimation, we compared the number of individuals from genotype data and the maximum number of individuals that entered the hair trap from camera traps. The data was deleted if the latter was higher than the former.

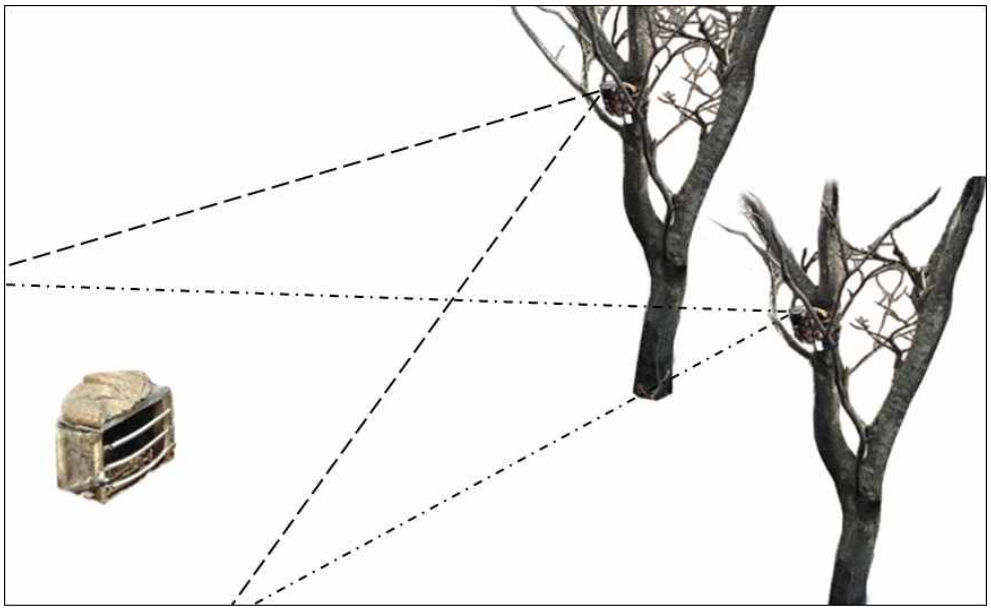

Figure 4. Mimetic diagram of sampling location with a hair trap and two camera traps in Jirisan National Park.

Figure 5. Photographs of the hair trap at the same location (Siamjae) by two camera traps.

For genetic background analysis, 12 tissue and blood samples of *M. flavigula* were collected across a wide geographic area in Korean peninsula during the years from 2007 to 2014 (Table 1). All samples were provided by the Conservation Genome Resource Bank for Korean Wildlife (CGRB; www.cgrb.org).

Table 1. Information of 12 individuals used for genetic background samples

CGRB ID	Locality	Type of samples	Year collected
4078	Gangwon	Tissue	2007
4096	Jeonbuk	Tissue	2007
9539	Chungbuk	Blood	2009
15210	Gyeongbuk	Tissue	2013
15211	Jeonnam	Tissue	2013
15212	Gyeonggi	Tissue	2013
15213	Jeonnam	Tissue	2013
15215	Gangwon	Tissue	2013
15216	Chungnam	Blood	2013
15218	Jeonnam	Tissue	2014
15219	Jeonnam	Tissue	2014
15220	Jeonnam	Tissue	2014

2.2 DNA Extraction

DNA was extracted from hair roots of single hair strand by cutting 2-cm portion from the root end and placing it in 200 ml of a 10% Chelex-100 (Bio - Rad) suspension. Samples were boiled at 99°C for 20 minutes in heat block, and then chilled in ice for 2 minutes. Samples were spun down using centrifuge at maximum speed (14,000 rpm) for 5 minutes. Only the supernatant was used for polymerase chain reaction (PCR). To check whether or not DNA was properly extracted, a single microsatellite marker (Ma-1) was amplified.

For genetic background samples, DNA was extracted from tissue and blood using the DNeasy Blood and Tissue Kit (Qiagen Inc., Cat. No. 69506, USA) following the manufacturer's protocol.

2.3 PCR Amplification and Genotyping

46 microsatellite markers developed from other mustelid species were tested for cross-amplification and analysis of DNA polymorphism (Table 2, 3). Microsatellite amplification was performed in a total volume of 15 μL with 2 μL template DNA, 10X buffer, 0.2 mM dNTPs, 1 μM each primer, 1 μM fluorescent (HEX, Applied Biosystems), M13 sequence primer and 1 U of *taq* polymerase (Qiagen, Valencia CA). Touch - down PCR conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 20 cycles of 30

seconds at 94°C, 30 seconds at 60 – 50°C, 30 seconds at 70°C, decreasing the annealing temperature by 0.5°C for 20 cycles and a final extension at 70°C for 7 minutes, then stored at 4°C. Alleles were determined using an ABI Prism 3730 XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) with the GENESCAN-500 ROX size standard, and GeneMapper v3.7 (Applied Biosystems, Foster C5ity, CA, USA).

Table 2. Details of the ten microsatellite loci from mustelid species used in this study

Locus	Primer sequence (5′ – 3′)	Repeat Motif	Product size (bp)
Mf3.2	F: TGTTAGCTTGCCCTATGC R: GGACCCATGAAAAACAGT	$(TTTC)_{12}$	165 – 177
Mar08	F: CCCTTTAGTTGGCACAGTCC R: CTTTGGCATGAGTCATTTGG	$(CA)_{22}$	144 – 158
Mar36	F: TGAGTTTGGTGGGAGAGAGG R: TTCACTGCCAATATTATCTTCTCAAG	$(CA)_{24}$	218 – 248
Mar43	F: CTTGTCACCCCAGGAGAGG R: CCTAAGCCCAAATCTAAGTGC	$(CA)_{26}$	123 – 169
Mar56	F: TCTGCACTTAACCCCTCTCC R: AGGGCCATTTGTCTCTTGC	$(CA)_{21}$	209 – 217
Ma-1	F: ATTTTATGTGCCTGGGTCTA R: TTATGCGTCTCTGTTTGTCA	$(TG)_4TA(TG)_{19}$	206 – 219
Tt-1	F: AACGGCTTCTAACCACTCCA R: CCCCGCTTTTCATTTCTTTA	$(TG)_{20}$	152 – 172
Tt-4	F: GGTGAGACCCTGGAAATAGAAA R: GCTAACCAAACCTAGCAATGAT	$(TG)_{18}$	172 – 188
Mvi4062	F: CCAGTGATCCGTGAAAACCT R: GCACCATTTGAAAAATGTTAAGC	$(TG)_{12}$	234 – 240
Mvi4066	F: GAAGCCCTGATGGTAATGGA R: CCTGGTTTTCAAGGTGAGGA	$(AC)_{19}$	316 – 330

M13 primer sequence is used for each forward primer sequence.

For microsatellite genotyping with ten markers, PCR experiments of single hair were repeated independently at least five times to minimize individual over- and under-estimation by allelic dropout and false allele by following the multiple-tube approach proposed by Taberlet et al. (1996).

To confirm whether the extracted genomic DNA was from a *M. flavigula*, we used the primers, L15775 (5′-ACATGAATTGGA GGACAACCAGT-3′) and H15915 (5′-GGAATTCATCTCTCCGGTTT ACAAGAC-3′), which sequence partial cytochrome *b* gene (140 bp) of most mammals (Irwin et al., 1991). Total mixture volume was of 30 μL with 2 μL template DNA, 10X buffer, 0.2 mM dNTPs, 1 μM each primer, and 1 U of *taq* polymerase (Takara, Valencia CA). PCR conditions consisted of an initial denaturation at 94°C for for 4 minutes, followed by 35 cycles 45 seconds at 94°C, 60 seconds at 50°C, 80 seconds at 72°C and a final extension at 72°C for 7 minutes, then stored at 4°C. PCR products were than sequenced on ABI3730XL DNA sequencer (Applied Biosystems, USA) at the National Instrumentation Center for Environmental Management (NICEM, Korea). BLAST was conducted by the NCBI on web site: http//www.ncbi.nlm.nih.gov.

2.4 Data Analysis

Multilocus matches analysis was used for identifying the same

and different genotypes in GeneAlex v6.5 (Peakall and Smouse, 2005). The allelic diversity along with observed (H_O) and expected (H_E) heterozygosities for each microsatellite locus were computed using the same program. GeneAlex v6.5 was further used to estimate the probability of identity ($P_{(ID)}$) and the probability of identity among siblings ($P_{(ID)sib}$), which are widely used as an indication for distinguishing two individuals (Peakall and Sydus, 1996). The program also was used to calculate pairwise relatedness. The specified relationships based on the *r*-values and genotypes of the individuals and population allele frequencies were also calculated. In the Queller and Goodnight method, *r*-value close or equal to 1 indicates zygotic twins, a value close or less to 0.5 indicates a full sibling (parents and offspring or brothers and sisters that share the same parents), a value close to 0.25 indicates half sibling (brothers and sisters that share only one parent), and a value close 0.0 or a negative value indicates unrelated individuals (Queller and Goodnight, 1989). In the Lynch and Ritland method, *r*-values around 0.5, 0.25, 0.125 and 0 correspond to identical, full siblings, half siblings and unrelated individuals, respectively (Lynch and Ritland, 1999).

Micro Checker 2.2.3 (Van Oosterhout et al., 2004) was used to detect occurrence of null alleles and to estimate null allele frequencies. Linkage disequilibrium between loci was tested using the FSTAT 2.9.3.2 (Goudet, 1995) and deviations from Hardy-Weinberg equilibrium were assessed using the GENEPOP v4.3 (Raymond and Rousset, 1995).

For the purpose of population estimation, the entire sampling efforts were divided in to eight occasions. As we collected multiple hairs from hair trap per occasion, samples of same individual (based on multilocus genotyping) in one sampling occasion were excluded for abundance estimation. Yellow-throated martens encounter histories were compiled and analyzed using program Mark (White and Burnham, 1999).

Results

3.1 Genetic Diversity and Equilibrium

Ten polymorphic microsatellite markers out of 46 microsatellite markers were used for cross-amplification and analysis of DNA polymorphism in *M. flavigula*. The mustelid species include *M. foina* (Mf3.2), *M. martes* (Mar08, Mar36, Mar43 and Mar56), *M. americana* (Ma-1), *Taxidae taxus* (Tt-1 and Tt-4), and *Mustela vison* (Mvi4062 and Mvi4066) (Table 2). 21 microsatellite markers out of 46 microsatellite markers were not amplified, 12 microsatellite markers were monomorphic and three microsatellite markers were not scored (Table 3).

Table 3. Information of 36 microsatellite markers developed from other mustelid species were tested for cross-amplification and analysis of DNA polymorphism

Locus	Primer sequence (5′ – 3′)	Repeat motif	Product size	Reference
Gg-7[@]	F: GTTTTCAATTTTAGCCGTTCTG R: GTTTATCTCCCTCTTCCTACCC	(TG)20(T)2(TG)5	154 – 172	Davis & Strobeck, (1998)
Gg-14*	F: ACTGTGAGAGCAGTGGGAG R: GATCTCTCTCTCTGTCCAATAAAT	(TG)16(A)2(GA)9	132 – 142	Davis & Strobeck, (1998)
Ma-8[@]	F: GTTTTCTAATGTTTCGTGTG R: CAGTGGTTGACTACAAGAAA	(TG)21	120 – 130	Davis & Strobeck, (1998)

Table 3. continued

Ma-9[$]	F: GGGTCAGCTGTATATCTATT R: GATTCTCTCCCTCTTTCTCT	(T)14(TG)4	139 – 142	Davis & Strobeck, (1998)
Ma-10*	F: GGTGCCCCATATTGACTATT R: TCTTTTCCTCTCCCTCTTCC	(T)11(TG)11	169 – 176	Davis & Strobeck, (1998)
Ma-18*	F: TGGGTGGGTGTATTTGTGTAT R: TACTCAGTGGGGAATCTGCT	(TG)4(TA)12	151 – 165	Davis & Strobeck, (1998)
Ma-19*	F: AAGGCTTATGGATACCACAT R: GATCATTTGGTATTTGTCTTTC	(TG)16	201 – 211	Davis & Strobeck, (1998)
Mar02*	F: CCCTCCTTTTCTTTTTCTTTCC R: CCGTTCTCTGAGTGAAATGC	(CA)17	150 – 156	Natali et al., (2010)
Mar06*	F: TGTTCAAACCAGGATTACAGC R: CAAACATTCCCCCAGACG	(CA)14(CT)13	219 – 227	Natali et al., (2010)
Mar14[@]	F: GGATTCATGCAGTCAAGAACAG R: CTCTGGGTTGTGAGATCAAGC	(TC)11AT(CA)15	223 – 229	Natali et al., (2010)
Mar19[@]	F: GAAGTAGTCCAAGTGTCCATCG R: TTGTCTTTCCCTGACTTATTTGG	(CT)15(CA)8	185 – 197	Natali et al., (2010)
Mar21*	F: ACATGCATACCTCCCAGACC R: TTTGCTTCCTCCATCTCTCC	(CA)24	159 – 183	Natali et al., (2010)
Mar53*	F: TCTCCAGCATTTACCTTTACCC R: GAACAGCCAACCCCATACC	(CA)18	238 – 254	Natali et al., (2010)
Mar58*	F: GTCCCAAATGTTGCACTGG R: CAAAAGACAGGGAGGTGTGG	(CA)12G(CA)7	231 – 257	Natali et al., (2010)
Mar64[@]	F: GGCCCCAAAGTCTTACAGTTC R: CGTTTTGAATCATGCTGTGG	(CA)21	171 – 191	Natali et al., (2010)
Mf1.3[$]	F: TTGCTGGAGGTGACCTTG R: CTGGGATTGAGCCTTGCA	(TTTC)8	221 – 237	Basto et al., (2010)
Mf6.5*	F: TCTTTTGGCTTTATCAGT R: CTCACATGGGAAATAGTC	(TTTC)13	249 – 265	Basto et al., (2010)
Mf8.7[@]	F: AGTCACTATCTCATAGCT R: GCATAGGACATTGGACTG	(TCTA)11	167 – 179	Basto et al., (2010)
Mf8.8[$]	F: GGAAGAGGTGATTTCTGA R: TCAGCTGGCGATCAGAGT	(CTTT)13	241 – 273	Basto et al., (2010)
MP0084[@]	F: GCTGGACCTGATGCTTGTAGA R: GAATCCAAAACCAACGTGCT	(GT)5GC(GT)14	130 – 170	Jordan et al., (2007)
MP0175[@]	F: CAGACCAAATGGACCCAATC R: TTCTACATTCATACGTGAGTAA AAGC	(CTTT)11(CCTT)3	150 – 220	Jordan et al., (2007)

Table 3. continued

MP0234[@]	F: CAACATGCAAAGGTGATGCT R: TTTTCCATTGCACTCAGGAA	(TGTT)7	100 – 160	Jordan et al., (2007)
MP0247*	F: GCATTGTGCACCAGCATAAC R: TTCCTTGCCTTTGCCTCA	(GAAA)3(GA)8(GAAA)11	120 – 170	Jordan et al., (2007)
Mvi4014*	F: CGTCCTGCTGACACCTTTATC R: ATCTTGGGGTCCTGGGATG	(GA)13	240 – 252	Anistoroaei et al., (2006)
Mvi4015*	F: AAAGAACTGAGCGGTGGTTG R: TTTCTCTCAATGGTGGCATCT	(CA)17	207 – 220	Anistoroaei et al., (2006)
Mvi4023*	F: TCATCAGCGCAGTGGTATTC R: CTTGGATAACCACCCACCAC	(TG)18	196 – 212	Anistoroaei et al., (2006)
Mvi4031[@]	F: GCCTTACCTCAGGCAATGTT R: CACTTAACCAGAGGCCATCA	(TG)12	282 – 289	Anistoroaei et al., (2006)
Mvi4037[@]	F: CAATGGATAAGCAACTGGTTTG R: TCCAGGTTTACATATCAGGCTTT	(CA)14	293 – 300	Anistoroaei et al., (2006)
Mvi4042*	F: TTGTTTCTGTCTGCCGTCTG R: CCCACCACAAATACCCTTGA	(GT)17	241 – 256	Anistoroaei et al., (2006)
Mvi4059*	F: GTCCTGGTCCAGAGTCCTGA R: CATCCCCAGACAGCTAGAGG	(TG)16	212 – 214	Anistoroaei et al., (2006)
Mvi4061*	F: TCACGAATGTTTCAGGACCA R: CAAATGAGAACCCCACCAGT	(CA)15	218 – 227	Anistoroaei et al., (2006)
Mvi4068[@]	F: CAAATGAGAACCCCACCAGT R: CTGCACAGCAAAGGAATCAG	(AG)9	200 – 204	Anistoroaei et al., (2006)
Mvi4077*	F: AGACGTGAGCCGTAGCATTT R: ACACGCAGCTTCTCTGGAAT	(AC)15	208 – 222	Anistoroaei et al., (2006)
Mvi4079*	F: CCGCAGGTCCTACTTTACCA R: TTGGAGACCCACATTCAGTG	(AC)12	375 – 378	Anistoroaei et al., (2006)
Mvi4090*	F: GGATCGAGTCCCACATCG R: TGTGTCAACAGTTACTTCTTTCTTTG	(TC)12	288 – 300	Anistoroaei et al., (2006)
Tt-3*	F: AGTCTGCTTGGGATTTTCTC R: TTTTGCTCTATGATTACACC	(TG)23	158 – 176	Davis & Strobeck, (1998)

* Not amplified; @ Monomorphic; $ Not scored.

A total of 98 *M. flavigula* individuals entered into the hair trap, identified by camera traps, and a total of 223 samples were collected from the four locations in the Jirisan National Park (Figure 6, 7, Table 4). Mean collection samples were 2.28 per individual. The PCR products of a single microsatellite marker (Ma-1) were detectable in 105 (47%) of single hairs and 50 (48%) DNA samples were successfully genotyped from ten microsatellite markers (Table 5). 21 individuals were identified from 50 genotype analyzed multilocus matches.

Table 4. Information of hair samples collected in Jirisan National Park

Locality	N	NE	NM	NI
Kojae	58	29	10	6
Jongsukdae	79	36	16	7
Siamjae	79	37	23	10
Goribong	7	3	1	1
Total	223	105(47%)	50(48%)	21

N, number of samples; NE, number of extracted DNA; NM, number of samples amplified for ten microsatellite; NI, number of individuals.

Table 5. Details of hair sample collected in Jirisan National Park

Locations	Place Baits Date	Maximum Martens	Sampling Date	No. Samples	Genotyping	No. Individuals
Kojae	December 18th. 2014	3	January 11th. 2015	9	0	0
	January 13th. 2015	5	January 28th. 2015	6	0	0
	January 28th. 2015	3	February 10th. 2015	4	2	2
	February 10th. 2015	5	February 17th. 2015	5	2	2
	February 17th. 2015	8	February 24th. 2015	34	6	5
Jongsukdae	January 13th. 2015	10	January 28th. 2015	12	1	1
	January 28th. 2015	8	February 10th. 2015	12	3	2
	February 10th. 2015	8	February 17th. 2015	19	8	4
	February 17th. 2015	10	February 24th. 2015	36	4	2
Siamjae	December 19th. 2014	3	December 31th. 2014	8	2	2
	December 31th. 2014	4	January 9th. 2015	7	3	2
	January 13th. 2015	3	January 28th. 2015	12	8	2
	January 28th. 2015	7	February 11th. 2015	12	0	0
	February 11th. 2015	5	February 16th. 2015	15	8	5
	February 16th. 2015	4	February 25th. 2015	25	2	2
Goribong	January 13th. 2015	6	January 28th. 2015	3	0	0
	February 16th. 2015	6	February 25th. 2015	4	1	1
Total	17 days	98	17 days	223	50	21
Mean		5.76 ± SD 2.39		13.12 ± SD 10.03		

Maximum martens indicate the number of individuals entered in hair trap by camera traps.

Figure 6. Photograph of *M. flavigula* hairs collected in spring.

Figure 7. Photographs of yellow-throated martens entering into the hair trap at the same location (Jongsukdae) by two camera traps.

From these 21 individuals, ten microsatellite loci were polymorphic with the number of allele per locus ranging from two to five, with an average of 2.9 alleles per locus. The averages of observed and expected heterozygosity were 0.51 (SE = 0.07) and 0.44 (SE = 0.05), respectively. Genetic background samples of 12 were also polymorphic and the mean number of alleles per locus was slightly higher with the value of 3.3 (range, 2-7) compared to the hair sample. The averages of observed and expected heterozygosity were 0.42 (SE = 0.06) and 0.45 (SE = 0.08), respectively (Table 6). All pairs of loci were not found to be in linkage disequilibrium and departure of observed from expected heterozygosity was not significant (Hardy-Weinberg equilibrium). No null alleles were found from ten microsatellite loci.

Table 6. Characteristics of polymorphic mustelid microsatellite for 21 *Martes flavigula* individuals from Jirisan National Park and genetic background samples of 12 *M. flavigula* individuals in Korea peninsula

Locus	Size range (bp)	Jirisan National Park (n=21)			Genetic background samples (n=12)			Species	Reference
		Na	H_O	H_E	Na	H_O	H_E		
Mf3.2	141-157	4	0.62	0.55	4	0.58	0.71	*M. foina*	Basto et al., (2010)
Mar08	159-167	2	0.10	0.09	2	0.08	0.08	*M. martes*	Natali et al., (2010)
Mar36	239-249	3	0.52	0.48	4	0.67	0.68	*M. martes*	Natali et al., (2010)
Mar43	154-164	3	0.67	0.47	4	0.42	0.46	*M. martes*	Natali et al., (2010)
Mar56	223-229	3	0.76	0.54	3	0.33	0.29	*M. martes*	Natali et al., (2010)
Ma-1	216-220	2	0.29	0.44	2	0.33	0.38	*M. americana*	Davis & Strobeck, (1998)
Tt-1	177-191	5	0.76	0.60	7	0.67	0.79	*T. taxus*	Davis & Strobeck, (1998)
Tt-4	197-199	2	0.43	0.34	2	0.30	0.22	*T. taxus*	Davis & Strobeck, (1998)
Mvi4062	255-257	2	0.48	0.36	2	0.33	0.28	*M. vison*	Anistoroaei et al., (2006)
Mvi4066	341-345	3	0.48	0.49	3	0.50	0.59	*M. vison*	Anistoroaei et al., (2006)
Mean		2.9	0.51	0.44	3.3	0.42	0.45		
SE		0.31	0.07	0.05	0.50	0.06	0.08		

Na, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

When ten microsatellite loci were combined into a set of marker loci, the probability of identity ($P_{(ID)}$) and the probability of identity among sibling ($P_{(ID)sib}$) of hair samples were 5.8×10^{-5} and 9.0×10^{-3}, respectively. $P_{(ID)}$ and $P_{(ID)sib}$ of genetic background samples were 9.6×10^{-6} and 6.2×10^{-3}, respectively (Figure 8). Therefore, estimates of $P_{(ID)}$ and $P_{(ID)sib}$ in this study were reliable for identifying unrelated individuals and siblings ($P_{(ID)}$ 0.01) (Taberlet and Luikart 1999; Mills et al., 2000).

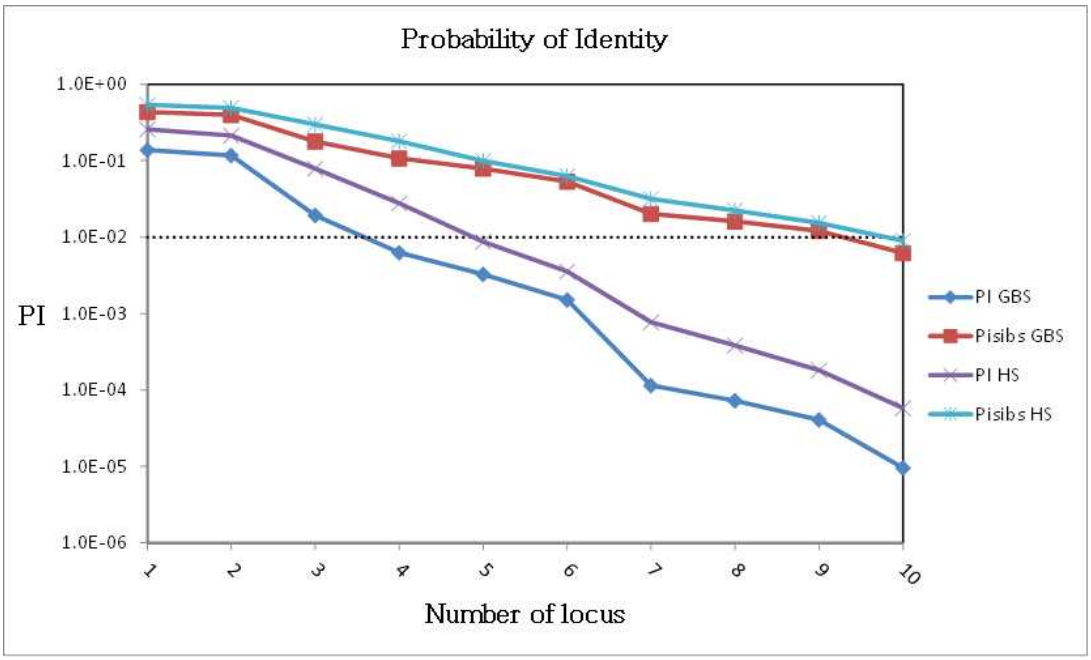

Figure 8. Relationship among the probability of identity (PI), PI among siblings (Pisibs) and the number of loci. GBS indicates genetic background samples (n = 12) and HS indicates hair samples (n = 21) in Jirisan National Park. The horizontal line indicates a PI of 0.01.

Social Structure and Genetic Relatedness

Among the completed 50 genotypes from hair samples, we found 21 genetic profiles. We identified a total of 21 individuals ranging from one to ten in all four locations in Jirisan National Park; six in Kojae (K), seven in Jongsukdae (J), ten in Siamjae (S), one in Goribong (G). Among the identified 21 individuals, both Ymt-10 and Ymt-12 were both identified in Kojae and Jongsukdae. Ymt-2 was identified in Jongsukdae and Siamjae. Ymt-3 and Ymt-7 were identified twice with different dates in Kojae. Ymt-8 and Ymt-9 were identified twice with different dates in Siamjae. No individual was found in more than three locations and other individuals were identified only once (Figure 9, Table 7).

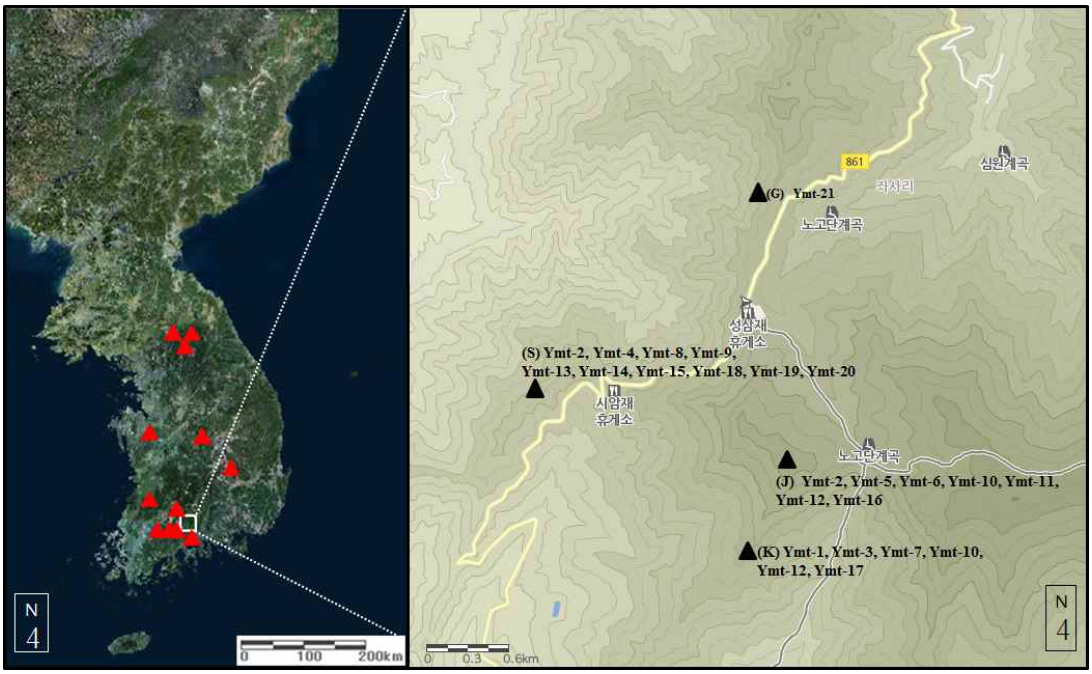

Figure 9. Map of collection sites of the genetic background samples from CGRB (left map) and individuals identified from four hair trap locations in Jirisan National Park (right map): red triangles indicate location of genetic background sample, white square indicates the region where hair traps were set in Jirisan National Park (left). Black triangles represent; Jongsukdae (J), Kojae (K), Siamjae (S) and Goribong (G) (right), where each hair traps were positioned. www.ngii.go.kr

Table 7. Information of 21 *M. flavigula* individuals used for non-invasive hair samples in Jirisan National Park

ID	No. Samples	Sampling date	Location
Ymt-1	2	February 24, 2015	K
Ymt-2	2	February 10, 2015	J
	2	February 24, 2015	
	1	February 25, 2015	S
Ymt-3	1	February 10, 2015	K
	1	February 24, 2015	
Ymt-4	4	January 28, 2015	S
	3	February 16, 2015	
Ymt-5	2	February 17, 2015	J
Ymt-6	3	February 17, 2015	J
Ymt-7	1	February 10, 2015	K
	1	February 24, 2015	
Ymt-8	1	January 9, 2015	S
	1	February 25, 2015	
Ymt-9	4	January 28, 2015	S
	2	February 16, 2015	
Ymt-10	1	February 17, 2015	J
	1	February 24, 2015	K
Ymt-11	2	February 24, 2015	J

Table 7. continued

Ymt-12	1	January 28, 2015	J
	2	February 17, 2015	
	1	February 17, 2015	K
	1	February 24, 2015	
Ymt-13	2	January 9, 2015	S
Ymt-14	1	December 31, 2014	S
Ymt-15	1	December 31, 2014	S
Ymt-16	1	February 10, 2015	J
Ymt-17	1	February 17, 2015	K
Ymt-18	1	February 16, 2015	S
Ymt-19	1	February 16, 2015	S
Ymt-20	1	February 16, 2015	S
Ymt-21	1	February 25, 2015	G

K, Kojae; J, Jongsukdae; S, Siamjae; G, Goribong.

The *r*-values for all 210 pairwise combinations were estimated for among 21 individuals using Queller and Goodnight method and Lynch and Ritland method. Mean relatedness among yellow-throated martens was generally low. Mean relatedness of 21 martens using Queller and Goodnight method and Lynch and Ritland method were −0.05 ± SD 0.42 (SE = 0.03) and −0.05 ± SD 0.27 (SE = 0.02), respectively (Figure 10). The mean relatedness of individuals from three locations (Kojae, Jongsukdae and Siamjae) also showed low *r*-values and mean relatedness in Goribong was not estimated due to small sample size (one individual) (Figure 10). Although mean relatedness of two methods among 21 individuals showed low r-values, relatedness *r*-values of 37 pairs among 21 individuals using Queller and Goodnight method showed kin relationship and 36 pairs using Lynch and Ritland method showed kin relationship (Table 8).

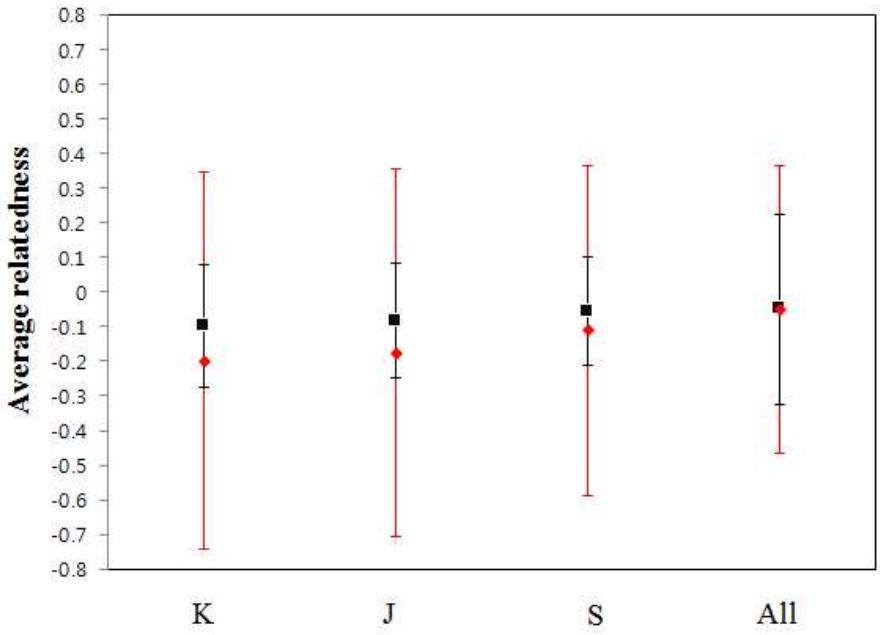

Figure 10. Coefficient of relatedness (mean r ± SD) for all martens identified in Kojae (K), Jongsuckdae (J), Siamjae (S), and r-value for all martens revealed in this study (n=21). Goribong (G) was not estimated due to small population size (n=1). Red indicate the Queller and Goodnight method and Black indicate the Lynch and Ritland method. Error bars are standard deviation.

Table 8. Relatedness analysis among 21 *M. flavigula* in Jirisan National Park using the Queller and Goodnight method (below diagonal) and the Lynch and Ritland method (above diagonal)

	Ymt-4	Ymt-14	Ymt-15	Ymt-13	Ymt-8	Ymt-12	Ymt-9	Ymt-7	Ymt-3	Ymt-16	Ymt-17	Ymt-6	Ymt-5	Ymt-10	Ymt-18	Ymt-19	Ymt-20	Ymt-1	Ymt-11	Ymt-21	Ymt-2
Ymt-4	0	0.153	-0.044	-0.456	0.186*	0.058	0.844*	-0.460	-0.222	-0.459	-0.218	0.099	0.125	-0.127	0.340*	0.504*	0.309*	-0.270	0.049	-0.283	-0.311
Ymt-14	0.584*	0	0.542*	-0.249	-0.021	-0.461	-0.048	0.009	0.026	-0.371	-0.515	-0.243	-0.047	0.558*	0.387*	0.245*	0.040	-0.410	0.395*	0.123	-0.463
Ymt-15	0.333	0.801*	0	-0.281	-0.118	-0.280	-0.184	0.005	0.016	-0.323	-0.330	-0.306	-0.117	0.232*	0.244*	0.025	-0.149	-0.217	0.124	0.649*	-0.238
Ymt-13	-0.688	-0.309	-0.567	0	0.116	0.282*	-0.271	-0.065	-0.298	0.939*	0.476*	-0.146	-0.176	-0.091	-0.367	-0.177	-0.326	-0.003	-0.199	0.106	-0.172
Ymt-8	0.188	0.044	-0.213	-0.401	0	-0.222	0.298*	-0.112	-0.265	0.055	0.056	-0.227	-0.156	-0.064	0.154	-0.160	-0.173	-0.217	-0.161	-0.171	0.183
Ymt-12	-0.538	-0.668	-0.575	0.623*	-0.542	0	0.237*	-0.304	-0.348	0.311*	0.325*	-0.202	-0.089	-0.096	-0.312	0.280*	0.272*	0.030	0.040	-0.047	-0.275
Ymt-9	0.611*	0.376	-0.037	-0.227	0.397*	-0.253	0	-0.363	-0.360	-0.299	-0.101	-0.068	0.008	0.008	0.201*	0.349*	0.475*	-0.332	0.184	-0.353	-0.373
Ymt-7	-0.264	0.396*	0.425*	-0.255	-0.234	-0.281	-0.056	0	0.842*	-0.136	-0.112	-0.057	-0.165	0.182	0.217*	-0.495	-0.194	-0.183	0.079	-0.067	0.044
Ymt-3	0.257	0.661*	0.670*	-0.578	-0.240	-0.428	-0.080	0.843*	0	-0.328	-0.110	0.170	-0.024	-0.035	0.227*	-0.274	-0.191	-0.096	-0.132	-0.099	0.124
Ymt-16	-0.772	-0.645	-0.747	0.798*	-0.519	0.421*	-0.630	-0.578	-0.725	0	0.495*	-0.025	-0.212	-0.196	-0.359	-0.054	-0.437	0.165	-0.287	0.154	-0.187
Ymt-17	-0.093	-0.443	-0.368	0.252	-0.068	0.709*	0.115	-0.190	-0.215	0.187	0	0.086	-0.022	-0.380	-0.364	0.048	-0.128	0.223*	-0.483	-0.309	-0.036
Ymt-6	0.178	0.155	-0.001	-0.474	-0.106	-0.666	-0.399	-0.016	0.313	-0.263	-0.051	0	0.171	-0.485	-0.041	0.207*	0.121	0.053	-0.307	-0.074	0.197*
Ymt-5	0.426*	0.562*	0.369	-0.601	-0.216	-0.451	-0.008	0.134	0.482*	-0.845	-0.142	0.296	0	-0.087	-0.221	0.054	0.227*	-0.110	0.033	-0.193	-0.014
Ymt-10	0.044	0.508*	0.361	0.123	-0.166	-0.079	0.432*	0.522*	0.403*	-0.285	-0.321	-0.359	0.321	0	0.020	-0.299	0.412*	-0.305	0.852*	0.017	-0.406
Ymt-18	0.392*	0.440*	0.468*	-0.781	-0.118	-0.456	0.046	0.460*	0.682*	-0.616	-0.273	0.071	-0.002	-0.064	0	0.027	-0.430	-0.210	-0.089	-0.031	-0.111
Ymt-19	0.494*	0.376	0.215	-0.101	-0.080	-0.127	0.105	-0.402	-0.043	-0.028	0.176	0.151	0.078	-0.333	0.275	0	0.081	-0.048	-0.129	-0.242	-0.334
Ymt-20	0.066	0.311	0.118	0.080	0.090	-0.122	0.455*	0.338	0.257	-0.459	0.047	0.022	0.474*	0.716*	-0.480	-0.442	0	-0.332	0.607*	-0.182	-0.268
Ymt-1	-0.551	-0.514	-0.430	-0.295	-0.885	0.146	-1.161	-0.274	-0.119	0.284	0.262	0.045	-0.464	-0.359	-0.157	-0.120	-0.533	0	-0.374	-0.020	0.140
Ymt-11	0.044	0.508*	0.361	0.123	-0.166	-0.079	0.432*	0.522*	0.403*	-0.285	-0.321	-0.359	0.321	1.000*	-0.064	-0.333	0.716*	-0.359	0	-0.076	-0.292
Ymt-21	-0.378	0.105	0.322	-0.077	-0.360	-0.517	-0.800	0.139	0.230	-0.004	-0.389	0.300	-0.158	-0.018	-0.104	-0.403	0.005	0.062	-0.018	0	-0.201
Ymt-2	-0.595	0.020	0.077	-0.994	-0.308	-0.492	-1.212	0.433*	0.519*	-0.887	-0.336	0.014	-0.077	-0.083	0.148	-0.544	-0.127	-0.202	-0.083	0.030	0

*Full sibling relationship.

Population Abundance

Among the completed 50 genotypes from hair samples, 19 were found to be repeat sampling (i.e. hair of same individual from same trap at same time) and thus were excluded from abundance analysis. The model M(o) was selected for abundance estimation as it showed the highest explanatory criteria. Using M(o) model in program Mark, abundance estimate for yellow-throated martens in intensive study area was 32 ± 6.8397 (95% CI; 25 – 54) with the estimated probability of capture (p-hat) 0.12 (Table 9).

Table 9. Abundance estimates for *M. flavigula* population of Jirisan National Park, South Korea by capture-recapture sampling using DNA collected hair samples

Parameters	Program Mark
Individuals caught M_{t+1}	21
Total Captures	31
Average per sample capture probability (p-hat)	0.12
Population estimates (M_o model)	32 ± 6.8397
Approximate 95 percent confidence interval	25 to 54 individuals

Discussion

Collection of non-invasive hair samples for genotyping is a prerequisite for ecological genetics in this study. The results showed that designed hair traps, placed on mountain ridges, generate an effective non-invasive method of collecting hair samples of martens for genotyping. We successfully collected 213 hair samples using the designed hair trap and 105 (49%) single hairs were used in DNA extraction (Table 4). From these, 50 (48%) genetic profiles were achieved. Although success rate of obtained genetic profiles was relatively lower than previous studies using single hair (Sloane et al., 2000; Banks et al., 2002, 2003; Frantz et al., 2003, 2004; Scheppers et al., 2007), genetic profiles of yellow-throated marten were conservative because we employed double quality controls. Avoidance or minimization of individual over- and under-estimation due to false allele and allele dropout is the key issue in using non-invasive samples (Taberlet et al., 1996). We applied genotyping via multiple-tube approach and also camera trap for quality control. We believe that these quality controls provided reliable profiles, increasing the degree of reliability of genetic data. Until now, combining camera trap and non-invasively collected samples have not been used much. However, using camera trap while sampling has several benefits; first, camera trap allows identification of each species that visit the hair trap through visual observation that renders genetic species

identification process unnecessary. It means that using camera trap saves time and cost, and also simplifies laboratory procedures. Second, the number of maximum individuals that entered hair trap using camera trap can be measured against the number of genetic profiles obtained from genotyping for quality control. Therefore, for future study, we encourage to use more camera traps while sampling.

Ten polymorphic microsatellite loci were found to be polymorphic for yellow-throated martens, which were also effective for genotyping non-invasive samples. Genetic diversity of genetic background samples and hair samples showed similar level (H_E = 0.45 and 0.44) (Table 6). This means that there is no evidence of errors from null allele. Genetic diversity cannot be compared directly because no microsatellite data of yellow-throated martens were available from previous studies. However, when the genetic diversity indirectly compared to other marten species that used different panel of cross-species microsatellite markers developed for mustelid species, *M. flavigula* from this study showed moderate genetic diversity, similar to that of *M. melampus* from Japan (H_E = 0.48) (Kamada et al., 2013). Whereas, the diversity was relatively higher than that of *M. foina* from Spain (H_E = 0.38) (Ruiz-González et al., 2013) and Ireland (H_E = 0.35) (Mullins et al., 2010) and lower than *M. martes* from Spain (H_E = 0.59) (Ruiz-González et al., 2013) and France (H_E = 0.59) (Mullins et al., 2010), and *M. zibellina* from Japan (H_E = 0.58) (Nagai et al., 2012). When the genetic diversity was compared to other mustelid species that used the same panel of species specific

microsatellite markers, *M. flavigula* showed lower values in eight microsatellite markers than that of other species (Mf3.2, H_E = 0.68; Mar08, H_E = 0.83; Mar36, H_E = 0.86; Mar43, H_E = 0.57; Mar56 H_E = 0.72; Ma-1, H_E = 0.86; Tt-1, H_E = 0.83; Tt-4, H_E = 0.86); Mvi4062 and Mvi4066 were excluded from this comparison because no evaluation of genetic diversity was given. When the genetic diversity compared to genus *Martes* that used same panel of four cross-species microsatellite markers (Mar08, Mar36, Mar43, Mar56), 21 individuals from Jirisan National Park (H_E = 0.40) and 12 genetic background samples (H_E = 0.38) showed similar values to that of *M. melampus* from Japan (H_E = 0.41) (Kamada et al., 2013). Whereas, the diversity was lower than *M. zibellina* from Japan (H_E = 0.62) (Nagai et al., 2012). Although the results cannot be directly comparable due to difference in sampling size, number of samples, number of markers and cross-species marker used for analysis, these results discovered that moderate level of genetic diversity of *Martes flavigula* in Korean peninsula compared to other martens.

We identified 21 individuals out of 50 genotypes analyzed ($P_{(ID)}$ 0.01) (Figure 9) with an abundance estimate of 32 ± 6.8397 (95% CI; 25 – 54) (Table 9). As our sampling efforts were limited and sampling was carried out in winter (extremely low temperature with limited food resource), the animals have moved longer distances in search of food (Taitt, 1981; Lindstedt et al., 1986; Sturtevant and Bissonette, 1997; Woo, 2014), thus many new individuals were captured. Therefore abundance results should be taken with caution

and there is need for long-term study with extensive sampling for reliable population estimates at Jirisan National Park. Nevertheless our findings (abundance) were in agreement with field observation where in at least 5 different marten groups (2-3 individuals) were identified visually.

The mean genetic relatedness among 21 individuals using Queller and Goodnight method and Lynch and Ritland method were −0.05 ± SD 0.42 (SE = 0.03) and −0.05 ± SD 0.27 (SE = 0.02), respectively (Figure 10). Results suggest that the mean relatedness of yellow-throated martens show that kinship does not play a significant role in shaping social structure. Also the low degree of relatedness among population means that high degree of dispersal can be predicted (Blundell et al., 2004). Dispersal of most mammals occurs to avoid resource competition and inbreeding (Gompper, 1996; Gompper and Wayne, 1996). River otters (*Lontra kanadensis*) consisted of highly social but non-related population (Blundell et al., 2004). San Joaquin kit foxes (*Vulpes macrotis mutica*) were monogamous in which pair-mates were not closely related (Ralls et al., 2001). In contrast in several carnivores, relatedness of social structure is high especially when dispersal is limited (Amos, 1993; Gompper, 1996; Gompper and Wayne 1996; Gompper et al., 1997; Clutton-Brock, 2002; Blundell et al., 2004). Relatedness *r*-values among martens revealed full sibling relations that were expected to be parent-offspring relationship when considering the sampling period (Table 8). During the sampling period of this study, yellow-throated martens might

have consisted of breeding groups as suggested by the previous study that individuals form reproductive units from August to April of next year (Woo, 2014).

In March, 2014, we tested the validity of designed hair trap for collecting hair samples in Goribong. DNA was successfully extracted from single hairs and genotyped by ten microsatellite markers. Ymt-4 was also identified in Goribong.

Female juvenile yellow-throated marten was fitted with radio-collars and tracked using ground telemetry for ecological study in Jirisan National Park from December, 2011 until May, 2012 (Woo, personal communication, 2014). This juvenile *M. flavigula* was roadkill in May, 2012 at Mt.Jogye in Suncheon. Although this was a single case of one individual, the dispersal of female *M. flavigula* juvenile was approximately 40km. This case coincides with our result of species dispersal.

Previous studies of *M. flavigula* reported that yellow-throated martens may hunt together in groups but the number of individuals in a group is different. Sathyakumar (1999) stated seven yellow-throated marten pair sightings in the western Himalaya. Woo (2014) reported two to six individuals in a group at Jirisan National Park in Korea peninsula but during the sampling period of this study, two to three individuals of yellow-throated marten were observed by our camera traps. However, it is unknown if these individuals were related, non-related, male-female pairs, or fusion-fission groups. Therefore, further research is needed on the group compositions of

yellow-throated marten for conservation.

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국문초록

지리산에 서식하는 담비의 microsatellite 좌위 분석을 이용한 생태유전학적 연구 : 개체식별, 혈연관계 그리고 개체수 측정

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혈연관계를 통한 사회구조의 이해와 개체수 측정은 담비와 같이 사회적 동물의 효율적인 보전방법의 발전을 위해 필수적이다. 혈연관계를 통한 사회구조는 생활방식 즉, 행동, 수렵, 교배양식 그리고 분산에 영향을 미치지만, 눈에 잘 띄지 않으며 전통적인 생태적 연구방법의 제약 때문에 담비의 사회구조는 연구되지 않았다. 본 연구에서는 담비와 유사종에서 개발된 10개의 microsatellite 마커를 이용하여 비 침습적 샘플을 통해 유전적 다양성과 혈연관계 그리고 개체수를 측정하였다. 지리산 국립공원에서 헤어트랩을 통해 수집된 223개의 담비 털 시료로부터 50개의 유전자형을 확인하였으며, 이로부터 21개체의 유전적 정보를 얻었다. 담비 12개체의 유전적 기초 샘플은 한반도 전역으로부터 얻었다. 지리산 국립공원에서 확인된 담비 21개체와 유전적 기초 샘플 12개체의 평균 기대 이형접합도는 각각 0.45와 0.44로 나타났다. 이와 같은 유전적 다양성 수치는 같은 담비속에 있는 다른 4 종과 비교하였을 때 중등의 값이다. 또한 지리산 국립공원에서 확인된 21개체의 평균 혈연수치는 각각 −0.05 ±

0.42 와 −0.05 ± 0.27 로 나타났다. 이 수치는 혈연관계가 사회구조를 형성하는데 중요한 역할을 하지 않았으며, 낮은 평균 혈연수치는 담비의 분산을 예측할 수 있었다. 표시 방류법을 통한 지리산국립공원내 연구지역에서의 개체수는 32 ± 6.8397 (95% CI; 25 – 54) 로 나타났지만 지리산국립공원 전 지역의 개체수를 파악하기엔 연구지역이 너무 제한적이었다. 본 연구결과에 따르면 다형성을 가진 10개의 microsatellite 마커는 지리산국립공원 연구지역내 담비집단의 개체식별, 혈연관계 그리고 개체수 측정을 가능하게 해주었다.

주요어 : 담비, 혈연관계, 개체수 측정, microsatellite, 지리산국립공원

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