## A preliminary study of genetic structure and relatedness analysis of Nutria (*Myocastor coypus*) in Upo Wetland

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Nutria *Myocastor coypus* is one of a well known invasive riparian mammal found species around world from North America to Eurasia and Africa. In South Korea, feral nutrias inhabit areas from the Nakdonggang and Namgang (River) to their tributaries and Upo Wetland where they have had devastating effects on environment. Nevertheless, there has been little research about nutrias in Korea. This study is to analyze the genetic structure of the nutria population in the Upo Wetland and identify the origin of the source populations. Twenty individuals from the Upo Wetland were genotyped using 25 microsatellite loci. When compared with another introduced population, that of the Blackwater Nation Wildlife Refuge in U.S., the Upo population contains considerable genetic variations. Tests for Hardy-Weinberg equilibrium and Bayesian clustering analysis suggest the Upo population is genetically structured and has at least two source populations. This preliminary study presents the need for further in-depth studies about this species which should combine genetic and ecological studies.

Keywords: genetic structure, Korea, Myocastor coypus, Nutria, Upo Wetland

Except for the indigenous habitat in South America, Nutria *Myocastor coypus* is one of well known invasive riparian mammal species found around world from North America to Eurasia and Africa (Gosling and Baker, 1991). IUCN (the International Union for Conservation of Nature) has listed Nutria on 100 of world's worst invasive alien species (Lowe *et al.*, 2000). In South Korea, nutria was first imported in 1985 for fur farming (Kim *et al.*, 2006). Since the economic value of nutria in Korean market has been decreased, this foreign mammal has become a feral species in Korea (Han, 2004; Bang *et al.*, 2007). As feral nutria populations have increased, the Korean Ministry of Environment designated nutria as an invasive alien species in 2009 (Ministry of Environment, 2009).

In spite of sparse occurrences all over South Korea, most feral nutrias have disappeared except for the Nakdonggang (River) populations, which ranges from the Nakdonggang and Namgang (River) to their tributaries and Upo Wetland (Kim *et al.*, 2006; Ministry of Environment, 2009). Although the Upo Wetland was designated as an Ecosystem Conservation Area in 1999, a Ramsar site in 1998, and a Wetland Protection Area in 1999 (Kang, 2003), the wetland is one of main habitats for the Korean nutria population (Ministry of Environment, 2009). In 2009, 91 nutrias were removed from the Upo Wetland, nevertheless, 109 nutrias were caught at the Upo Wetland in 2010 according to the data of the Nakdonggang River watershed management office. Despite continuous effort, the nutria population of the Upo Wetland has not yet extirpated.

Even though there is enough awareness about nutria being a major threat to native riparian ecosystems and consensus on extensive management of the invasive nutrias, there has been little research into nutrias in Korea. For the management of this invasive species, a genetic analysis is the basic information needed for making strategies about extirpation or reduction of the target species (Lee, 2002; Roux and Wieczorek, 2009).

This preliminary study is to analyze the genetic structure of the nutria population in the Upo Wetland and identify the origin of the source population. The information will provide a better foundation for the development of management strategies for the Korean nutria population and conservation of the Upo Wetland.

Twenty individual nutria were collected from the Upo Wetland (Fig. 1,  $35^{\circ} 33' 34.22'' S 128^{\circ} 24' 48.46'' E)$  using beaver traps (Leg hold trap, Bridger #5 Long Spring Trap) in March and November of 2009 and January and May of 2010. Trapping and handling followed guidelines of the American Society of Mammalogists (Gannon *et al.*, 2007). Samples were taken from the thigh muscle of each animal while making a skin study and stored at  $-70^{\circ}C$  until DNA extraction. In the laboratory, DNAs were extracted from the muscle tissue of the specimens using the



Fig. 1. Maps denoting Upo Wetland where nutrias were collected.

Table 1. Summary	v statistics of	of Upo r	opulation	of nutria.
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Locus	Size range	AR	Ho	$H_{\rm E}$	$HW^{\dagger}$	NE-1P	NE-2P	NE-PP	NE-I	NE-SI
McoA04	228-238	4	0.750	0.529	**	0.865	0.761	0.635	0.323	0.573
McoA10	227-233	4	0.750	0.553		0.848	0.703	0.545	0.269	0.548
McoB17	287-290	2	0.150	0.501	**	0.881	0.815	0.722	0.381	0.601
McoC103	214-222	2	0.550	0.481		0.890	0.821	0.729	0.392	0.614
McoC116	179-187	4	0.550	0.606	**	0.820	0.685	0.533	0.243	0.515
McoC118	158-174	4	0.650	0.556		0.851	0.752	0.627	0.310	0.556
McoC124	240-248	3	0.600	0.565		0.848	0.736	0.604	0.291	0.547
McoC203	207-211	2	0.050	0.050		0.999	0.976	0.954	0.906	0.952
McoC213	223-227	2	0.200	0.185		0.984	0.918	0.856	0.689	0.832
McoC220	214-222	3	0.300	0.417	*	0.917	0.787	0.651	0.388	0.644
McoC227	176-192	3	0.150	0.550	***	0.856	0.715	0.567	0.274	0.550
McoD10	216-244	4	0.300	0.459		0.894	0.743	0.581	0.334	0.610
McoD35	221-253	8	0.600	0.745		0.663	0.480	0.281	0.107	0.414
McoD48	178-182	2	0.000	0.185	**	0.984	0.918	0.856	0.689	0.832
McoD55	234-242	3	0.050	0.540	***	0.862	0.761	0.638	0.320	0.567
McoD59	248-252	2	0.150	0.358	*	0.939	0.856	0.771	0.485	0.697
McoD60	241-249	2	0.150	0.142		0.990	0.935	0.883	0.751	0.868
McoD67	220-228	3	0.150	0.381	**	0.931	0.810	0.685	0.429	0.672
McoD69	177-213	5	0.400	0.451	*	0.898	0.758	0.607	0.350	0.617
McoD212	184-204	5	0.700	0.710	**	0.739	0.578	0.412	0.156	0.443
McoD214	202-218	5	0.750	0.610	**	0.813	0.675	0.517	0.237	0.512
McoD215	199-221	6	0.750	0.774		0.650	0.471	0.289	0.100	0.397
McoD217	164-184	3	0.400	0.344		0.944	0.834	0.721	0.474	0.701
McoD219	203-207	2	0.100	0.262	*	0.967	0.889	0.814	0.588	0.769
McoD228	251	1	0.000	0.000	—	1.000	1.000	1.000	1.000	1.000

<sup>†</sup>, significance level: \*=significant at the 5% level, \*\*=significant at the 1% level, \*\*\*=significant at the 0.1% level.

AR, allelic richness;  $H_0$ , observed heterozygosity;  $H_E$ , expected heterozygosity; HW, significance of deviation from Hardy-Weinberg equilibrium; NE-1P, non-exclusion probability of first parent; NE-2P, non-exclusion probability of second parent; NE-PP, non-exclusion probability of parent pair; NE-I, non-exclusion probability of identity; NE-SI, non-exclusion probability of sib identity.

Qiagen DNeasy tissue kit (Qiagen, Inc., Valencia, CA) according to the manufacture's protocols.

Twenty five microsatellite loci developed by Callahan *et al.* (2005) were amplified under the following conditions. The reaction mixtures (total  $25 \,\mu$ L) were composed of 0.5  $\mu$ L of template DNA, 0.7  $\mu$ L of each primer (10  $\mu$ M), 1.0  $\mu$ L of dNTP solution (10 mM), 2.0  $\mu$ L of MgCl<sub>2</sub> (25 mM), 2.5  $\mu$ L of *Taq* buffer (10X), 0.2  $\mu$ L of *Taq* DNA

polymerase (Promega co., Madison, WI), and  $17.4 \mu L$  of DW. PCR reactions were carried out on GeneAmp 9700 (Applied Biosystems Inc., Foster City, CA) and included the following steps: an initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at a set temperature depending on each locus for 30s, elongation at 72°C for 30 s, and final extension at 72°C for 30 min. PCR products were determined using the Gen

Fig. 2. A bar plot representing the estimated membership coefficients of nutria individuals (K=2).

etic Analyzer 3730 (Applied Biosystems Inc.), and analyzed by GENESCAN 3.7 and GENOTYPER 3.7 (Applied Biosystems Inc.).

Summary statistics of genotype data such as allelic richness (number of alleles), observed and expected heterozygosities, and non-exclusion probability of first and second parents, parent pair, identity and sib-identity with unrelated individual were calculated with CERVUS 3.0 (Kalinowski et al., 2007). Non-exclusion probability of unrelated individuals indicates marker's usefulness in parentage, relatedness and identity analyses. Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using GENE-POP 4.0.10 (Raymond & Rousset, 1995). Genetic structure of nutria population from Upo Wetland was investigated using Bayesian clustering analysis which was performed by STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2007). The posterior density of K was analyzed using prior distribution of K in the range of 1-5. Markov chain Monte Carlo (MCMC) simulation was executed for each K with 10,000 steps of burn-in and 500,000 steps of Markov chain.

From the results of the genotyping 25 microsatellite loci, the mean number of allele per locus is 3.36. Allelic richness ranges between 1 at the McoD228 locus and from 8 at the McoD35 locus (Table 1). We compared our data with that of Callahan et al. (2005) who investigated microsatellite genetic variation on 64 individuals from the Blackwater National Wildlife Refuge (BNWR), an introduced population in U.S. Though allele size range of 16 loci of the Upo population is narrower than the BNWR population, 7 loci of the Upo population contain alleles that were not present in the BNWR population. Moreover the Upo population carries more allele than the BNWR population at 7 loci. Mean expected heterozygosity is 0.4382. Expected and observed heterozygosity of loci range between 0 and 0.774 and between 0 and 0.750, respectively (Table 1). At 15 loci, observed heterozygosity of the Upo population is larger than the BNWR population. The Upo population also shows larger expected hetrozygosity at 16 loci than the BNWR population. Consi-

**Table 2.** Estimated natural logarithm of probability of data according to *K* (the number of population).

K	$\operatorname{Ln}\operatorname{Pr}\left(\mathbf{X} \mathbf{K}\right)$		
1	-801.3		
2	-769.3		
3	-869.7		
4	-895.4		
5	-859.6		

dering that specimens from the Upo are far smaller than those from BNWR, these results indicate that the Upo population contains considerable genetic variation, and therefore implicates that the nutria population introduced to South Korea might be larger than expected.

Non-exclusion probabilities of loci are listed in Table 1. Non-exclusion probabilities of first parent (NE-1P), second parent (NE-2P) and parent pair (NE-PP) range between 0.650 and 1.000, between 0.471 and 1.000, and between 0.281 and 1.000, respectively (Table 1). Combined NE-1P, NE-2P and NE-PP over all loci are 0.03657281, 0.00114302, and 0.00001013, respectively. Non-exclusion probabilities of identity (NE-I) and sib identity (NE-SI) range from 0.100 to 1.000 and from 0.397 to 1.000, respectively (Table 1). And combined non-exclusion probability of identity and sib identity over all loci are 8.37E-12 and 0.00000739, respectively. The non-exclusion probability in parentage analysis is the probability of not excluding a single unrelated candidate parent or parent pair from parentage of a given offspring at one locus. And the non-exclusion probability in identity analysis is the probability that the genotypes at a single locus do not differ between two randomly-chosen individuals. Therefore low non-exclusion probabilities ensure the reliability of these microsatellite genotypes in parentage, relatedness and identity analyses.

Tests for Hardy-Weinberg equilibrium and Bayesian clustering analysis represent that the Upo population may be genetically structured. As a result of the HWE test, 13 loci deviate significantly from HWE (Table 1). Deviation from HWE may be caused mostly by recent admixture of populations. Estimated natural logarithm of probability of data was highest when the number of the population (K) is 2 (Table 2). Nutria individuals from the Upo Wetland seemingly consist of individuals from two populations and their hybrids (Fig. 2).

In summary, the nutria population of the Upo Wetland is genetically structured, and contains considerable genetic variations which might enable them to adapt to the Korean environment. Thus this preliminary study presents the need for in-depth studies about this introduced species. These further studies should combine population genetic and ecological studies which would be useful in managing the nutria population in this country.

## ACKNOWLEDGEMENTS

We thank Mr. Yeong-Hak Jo and Dr. Gi-Cheol Oh (Nadong-river Watershed Management office) for giving us samples of Nutrias. Professor Won Kim and the Laboratory of systematic and molecular evolution in Seoul National University provided help for experiments. 3 anonymous reviewers provided valuable comments that significantly improved the manuscript.

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Submitted: January 16, 2012, Accepted: February 9, 2012