

Variation of Mitochondrial DNA in Two Subspecies of Striped Field Mice, *Apodemus agrarius coreae* and *Apodemus agrarius chejuensis*, from Korea

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Samples of two subspecies of striped field mice, *Apodemus agrarius coreae* & *Apodemus agrarius chejuensis*, from four localities in Korea were used for the analyses of mitochondrial DNA fragment patterns resulted from the digestion with eight restriction enzymes.

A total of 31 fragments were recognized and 15 clones were revealed. The 15 clones were grouped into four major subgroups. One sample from Cheongju was distinct, and formed one of the four major subgroups: the mean divergence with other subgroups was 4.6 per cent, and extensive analyses using samples from various sites are necessary to clarify the taxonomic status of the subgroup.

Samples from Cheju island constituted another subgroup, and they should be named as *Apodemus chejuensis*. Samples from Wan island composed still another subgroup, and they seemed to be another population of *Apodemus chejuensis*: further analyses are needed for the classification of *Apodemus chejuensis*.

In the last subgroup composed of six of seven samples from Cheongju and four samples from Haenam, two samples from Cheongju and one sample from Haenam were identical in their mitochondrial genotypes, indicating that these striped field mice from Cheongju and Haenam have close maternal relationship.

KEY WORDS: Systematics, mtDNA, *Apodemus agrarius*, Korea

Mitochondrial DNA (mtDNA) studies are important to infer maternal lineages among closely related species and infraspecific populations (Avice, 1986; Moritz *et al.*, 1987). The analysis of Restriction Fragment Length Polymorphisms (RFLPs) is simple and it is possible to analyze more loci per individual by RFLPs analysis than by sequencing (Hillis and Moritz, 1990).

Apodemus agrarius Pallas (striped field mice), inhabiting from West Germany to Korea, is a distinct species regarded as the sole member of the

subgenus *Apodemus* (Corbet, 1978). Thomas (1906) described striped field mice from the Korean peninsula and Cheju island as a single subspecies, *Apodemus agrarius coreae*. Jones and Johnson (1965) reported that four subspecies of *A. agrarius* were recognized in Korea: *manchuricus* in the extreme northern part, *pallescens* in the coastal lowlands of southern and southwestern Korea; *coreae* throughout the major portion of the peninsula, and *chejuensis* in Cheju island. Koh (1986) concluded that *A. agrarius pallescens* is a synonym of *A. agrarius coreae* and Koh (1987) reported that *A. agrarius chejuensis* and *A. agrarius coreae* are distinct in their morphometric characters with each other, although they are similar in

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karyotypes. Morphometric characters of eight subspecies of striped field mice in Asia (subspecies *agrarius*, *ningpoensis*, *pallidior*, *chevrieri*, *insulaemus*, *manchuricus*, *coreae*, and *chejuensis*) were analyzed and it is concluded that *chejuensis* appeared to be a distinct species, although molecular analyses may be necessary (Koh, 1991).

The purpose of this paper is to analyze RFLPs of mtDNA in the samples of two subspecies of striped field mice (*Apodemus agrarius coreae* at Cheongju, Haenam, and Wan island; *A. agrarius chejuensis* at Cheju island) in order to determine their mtDNA genotypes, their degree of differentiation, and the subspecific status of the subspecies *chejuensis*.

Materials and Methods

Twenty one samples of two subspecies of striped field mice (*Apodemus agrarius coreae* and *A. agrarius chejuensis*) from four localities in Korea were utilized (see Table 1).

The mtDNA was visualized from southern blots (Southern, 1975) of total cellular DNA. DNA isola-

tion from liver, electrophoresis, Southern transfer, and hybridization were described by Davis (1986). All samples were individually digested using the following eight restriction endonucleases: *EcoR* I, *Pvu* II, *Dra* I, *Hind* III, *Bam*HI, *EcoR* V, *Pst* I, and *Stu* I. The mtDNA probe used for all hybridization was pPld, which contains 10 Kb *Pst* I fragment of *Peromyscus leucopus* mitochondrial genome in the vector pUC (courtesy of Dr. S. K. Davis in Texas A & M University).

In mtDNA analysis, each different restriction fragment produced by a particular enzyme is given a different (but arbitrary) number. Sequence divergence in base substitutions per nucleotide (p in per cent) was estimated from the restriction site data by the method of Upholt (1977). Phenogram was constructed from this data by Unweighted Pair Group Method with Arithmetic means, UP-GMA (Sneath and Sokal, 1973).

Results

A total of 31 fragments were recognized and 15 clones were revealed, as shown in Table 1. In

Table 1. Specimens, mitochondrial DNA genotypes, and clones in two subspecies of striped field mice, *Apodemus agrarius coreae* and *A. agrarius chejuensis*, from four localities in Korea. Mitochondrial genotypes are based on the fragment patterns resulted from the digestion with eight restriction enzymes, *EcoR* I, *Pvu* II, *Dra* I, *Hind* III, *Bam*HI, *EcoR* V, *Pst* I, and *Stu* I in order.

Subspecies	Specimen no.	Locality	mtDNA genotype	mtDNA clone	*Size
<i>coreae</i>	421	Cheongju, Chungbuk	1 1 1 1 1 1 1 1	1	small
	460, 466	"	1 1 2 1 2 1 1 1	2	"
	461, 478	"	1 1 2 2 1 1 2 1	3	"
	465	"	2 2 2 2 1 2 2 2	4	"
	479	"	1 1 2 1 1 3 1 1	5	"
	473	Haenam, Cheonnam	1 1 3 1 2 1 1 1	6	"
	480	"	1 1 2 2 1 1 2 1	3	"
	481	"	1 1 2 1 2 1 2 1	7	"
	482	"	1 1 2 2 1 1 1 1	8	"
	483	Wan island, Cheonnam	1 1 2 2 1 3 2 3	9	large
	485	"	1 1 2 1 1 3 1 3	10	"
	486	"	1 1 2 1 1 3 2 3	11	"
	487	"	1 1 2 1 2 1 1 3	12	"
	<i>chejuensis</i>	493, 494, 497	Yeongsil, Cheju	1 3 2 1 1 3 3 1	13
495, 498		"	1 3 2 1 1 3 1 1	14	"
496		"	1 3 2 1 1 3 2 1	15	"

*According to morphometric analyses by Koh (1989)

clone 3, two samples (461, 468) from Cheongju and one sample (480) from Haenam are identical in their fragment patterns, or their mtDNA genotypes. In *EcoR* I, *Pvu* II, *EcoR* V, and *Stu* I genotypes, clone 4 (465 from Cheongju) appeared to be quite different from the other 14 clones. Clones 9 to 12 (Wan island) were distinct in *Stu* I genotype, whereas clones 13 to 15 (Cheju island) differ in *Pvu* II genotype. In the frequency of *EcoR* V genotype, genotype 1 is high (9 of 10 samples) in clones 1, 2, 3, 5, 6, 7, and 8 (Cheongju and Haenam) and genotype 3 is high (9 of 10 samples) in clones 9 to 15 (Wan and Cheju island).

The nucleotide-sequence divergences (per cent p) among 15 mtDNA clones are given in Table 2. Fig. 1 shows the grouping of 15 clones by UPGMA. Four subgroups were revealed (I, clones 1, 2, 3, 5, 6, 7, and 8; II, clone 9 to 12; III, clone 13 to 15; IV, clone 4). The mean divergence value between subgroup I and subgroup II is 1.9 per cent, whereas that between subgroups I and II and subgroup III is 2.6 per cent. Subgroup IV was quite distinct from other three subgroups with the mean value of 4.6 per cent. Fig. 2 shows the representative fragment patterns of mtDNA in clones 2, 4, 8, 9, and 13 with *Pvu* II, *EcoR* I, and *Stu* I.

In summary (see Fig. 1 and Tables 1 & 2), *Apodemus agrarius chejuensis* from Cheju island

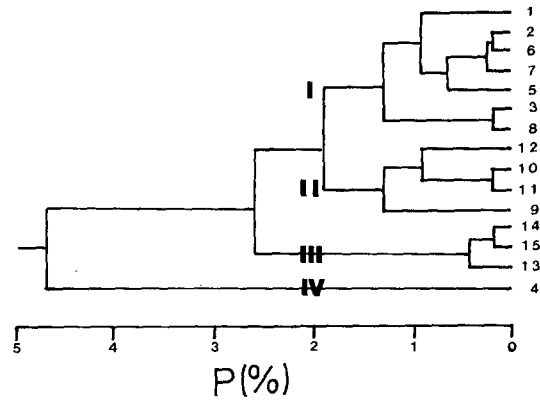


Fig. 1. Grouping of 15 mtDNA clones in two subspecies of striped field mice, *Apodemus agrarius coreae* and *chejuensis*, in Korea. The nucleotide-sequence divergences, given in Table 2, were used for UPGMA cluster analysis. Numerals refer to the clones in Table 1. Roman numerals indicate subgroups.

(III, clone 13 to 15) appeared to be different in its mtDNA genotype. In *A. agrarius coreae*, samples from Wan island (II, clone 9 to 12) differed from samples at the Korean mainland. Among the 11 samples from the mainland, one sample at Cheongju (IV, clone 4) was distinct from the other 10 samples at Cheongju and Haenam (I; clone 1, 2, 3, 5, 6, 7, and 8). Moreover, great divergence also revealed between one sample from Cheongju

Table 2. Nucleotide-sequence divergence among 15 mtDNA clones in two subspecies of striped field mice, *Apodemus agrarius coreae* and *A. agrarius chejuensis*, from Korea. For locality and subspecies name of each clone see Table 1.

Clones	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.7													
3	1.6	1.2												
4	6.8	5.1	2.9											
5	1.0	0.5	1.3	4.8										
6	0.9	0.2	1.3	5.2	0.7									
7	1.0	0.2	0.9	4.8	0.7	0.3								
8	1.3	0.9	0.2	3.1	1.2	1.1	1.2							
9	3.7	2.7	1.2	3.2	2.0	2.9	2.5	1.3						
10	2.1	1.5	2.5	5.4	0.8	1.6	1.8	2.1	1.1					
11	2.6	1.8	2.3	5.3	1.1	2.0	1.5	2.0	0.9	0.2				
12	2.0	0.7	2.2	4.8	1.5	0.9	1.5	2.6	2.0	0.8	1.1			
13	3.3	2.3	3.7	4.3	1.6	2.5	2.8	3.2	5.0	3.1	3.6	4.0		
14	2.3	1.6	2.7	4.7	1.0	1.8	2.0	2.3	3.7	2.1	2.6	3.0	0.4	
15	2.8	2.0	2.5	4.5	1.3	2.1	1.8	2.7	3.5	2.6	2.3	3.5	0.7	0.2

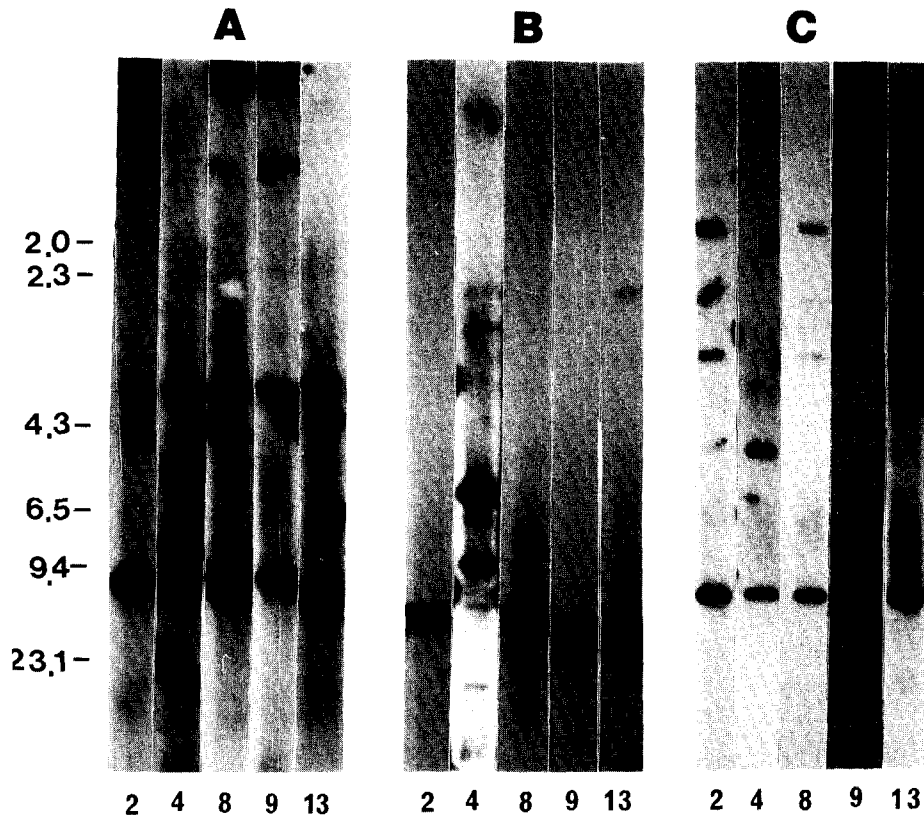


Fig. 2. Representative restriction fragment patterns of mtDNA in five of 15 clones of striped field mice, *Apodemus agrarius coreae* and *A. agrarius chejuensis*, from Korea. Numerals refer to clones given in Table 1. The lane at the far left contains size markers indicated in kilo base pairs. A, *Pvu* II. B, *EcoR* V. C, *Stu* I.

and the other 20 samples from the four localities studied.

Discussion

The greatest intraspecific sequence divergence among mtDNA clones of *Apodemus sylvaticus* was 1.4 per cent and that of *A. flavicollis* was 1.0 per cent (Tegelstrom and Jaarola, 1989). Sequence divergence estimated from *Rattus norvegicus* ranged from 0.2 to 0.8 per cent (Brown and Simpson, 1981). The maximum sequence divergence in *Clethrionomys rutilus* was 0.89 per cent (Tegelstrom *et al.*, 1988). On the other hand, sequence divergence between *Peromyscus maniculatus* mtDNA clones from continental North America was as high as 7 per cent and seven assemblages were revealed (Lansmann *et al.*, 1983).

In this study with 21 samples of *Apodemus agrarius* in Korea (Fig. 1 and Tables 1 & 2), a distinct mtDNA subgroup IV (clone 4, one specimen from Cheongju) was recognized and the average divergence between it and the other three subgroups (I, clones 1, 2, 3, 5, 6, 7, and 8 from Cheongju and Haenam; II, clone 9 to 12 from Wan island; III, clone 13 to 15 from Cheju island) was 4.6 per cent: the subgroup IV seemed to separate 2.3 million years ago, assuming a 2% rate of divergence per million years (Brown *et al.*, 1979).

Furthermore, RFLPs have been invaluable for the analysis of matrilineal relationships between distinct forms in the pocket gopher, *Geomys* (Sites and Davis, 1989). It seemed that the subgroup IV from Cheongju have a maternal lineage, which differs from samples at Cheongju, Haenam, Wan island, and Cheju island: but only one sample

from Cheongju formed the subgroup IV and the other three localities are in the areas of southern coasts and islands. Therefore, to trace maternal lineage in order to clarify the taxonomic status of the subgroup IV, extensive analyses using additional samples from various sites including Cheongju appeared to be necessary.

Cheju island was formed by series of volcanic activities at the end of Tertiary period in Cenozoic era (Park, 1985), and as one of continental islands it is connected to the mainland during the Pleistocene and became isolated 10,000 years ago, although isolation time have been variable (Wilcox, 1978). The mtDNA sequence divergence between Santa Catalina island and mainland harvest mice, *Reithrodontomys megalotis*, was less than 0.2 per cent (Ashley, 1989). On the other hand, the divergence between Channel islands and mainland deer mice, *Peromyscus maniculatus*, was as high as 1 per cent, and it was suggested that colonization of islands occurred fairly recently, probably within the last 500,000 years (Ashley and Willis, 1987). In the present study (see Fig. 1 and Tables 1 & 2), six samples of *A. agrarius chejuensis* formed one of four subgroups, subgroup III, and the mean divergence between the subgroup III and the subgroup II (samples from Cheongju and Haenam) was 2.3 per cent, indicating that Cheju island samples have been isolated since about 1.1 million years ago.

Corbet (1978) noted that *Apodemus agrarius chejuensis* from Cheju island is rather large, but are not very distinctive. In morphometric analyses, *A. agrarius chejuensis* was different from *A. agrarius coreae* in the Korean mainland (Koh, 1986) and it also differed from six subspecies in Asia (Koh, 1991). In this study (see Fig. 1 and Tables 1 & 2), *Apodemus agrarius chejuensis* is distinct in its mtDNA genotypes (subgroup III), and it should be a species, *Apodemus chejuensis* Jones and Johnson.

Wan island is a typical continental-island near southwestern coasts in Korea. In morphometric analyses with samples of *Apodemus agrarius coreae* from eight localities in southwestern coasts and islands, Wan island samples were a large-size group with samples of *A. agrarius chejuensis* from Cheju island, whereas the mainland and Jin island samples was a small-size group: it is suggested

that small-size population in Wan island were extinct and replaced by large-size population immigrated by Cheju island by humans (Koh, 1989). In the present study (see Fig. 1 and Tables 1 & 2), samples from Wan island formed the subgroup II and the mean divergence between the subgroup II and the subgroup I from the mainland was 1.9 per cent, indicating that samples from Wan island have been isolated since about 1 million years ago. Wan island samples (subgroup II) also differed from Cheju island samples (subgroup III) with the mean divergence of 3.3 per cent, and it is concluded that striped field mice in Wan island is another population of *Apodemus chejuensis*, although further analyses are necessary for the classification of *A. chejuensis*.

Samples of striped field mice from Cheongju and Haenam (*Apodemus agrarius coreae*) are a small-size group (Koh, 1986). In this study (see Fig. 1 and Tables 1 & 2), samples from Cheongju and Haenam formed the mtDNA subgroup I. Moreover, two of six samples from Cheongju and one of four samples from Haenam were identical in their mtDNA genotypes, indicating that samples from two localities have close maternal relationship.

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References

- Ashley, M. V., 1989. Absence of differentiation in mitochondrial DNA of island and mainland harvest mice, *Reithrodontomys megalotis*. *J. Mamm.* **70**: 383-386.
- Ashley, M. V. and C. Willis, 1987. Analysis of mitochondrial DNA polymorphism among Channel island deer mice. *Evolution* **41**: 845-853.
- Avise, J. C., 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. R. Soc. Lond.* **312**: 325-342.
- Brown, G. G. and M. V. Simpson, 1981. Intra- and interspecific variation of the mitochondrial genome in

- Rattus norvegicus* and *Rattus rattus*: restriction enzyme analysis of variant mitochondrial DNA molecules and their evolutionary relationships. *Genetics* **97**: 125-143.
- Brown, W. M., M. George, and A. C. Wilson, 1979. Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* **76**: 1967-1971.
- Corbet, G. B., 1978. The mammals of the Palaearctic region: a taxonomic review. British Museum (Nat. Hist.), Cornell Univ. Press. London.
- Davis, S. K., 1986. Evolutionary relationships in *Geomys*: an analysis using mitochondrial DNA. Ph.D. Diss. Washington Univ., St. Louis, Mo.
- Hillis, D. M. and C. Moritz, 1990. Molecular systematics. Sinauer Associates, Inc., Mass.
- Jones, J. K. and D. H. Johnson, 1965. Synopsis of the lagomorphs and rodents of Korea. *Univ. Kansas Publ. Mus. Nat. Hist.* **16**: 357-407.
- Koh, H. S., 1986. Geographic variation of morphometric characters among three subspecies of striped field mice, *Apodemus agrarius* Pallas (Muridae, Rodentia) from Korea. *Korean. J. Zool.* **29**: 272-282.
- Koh, H. S., 1987. Systematic studies of Korean rodents: III. Morphometric and chromosomal analyses of striped field mice, *Apodemus agrarius chejuensis* Jones and Johnson, from Cheju-Do. *Korean. J. Syst. Zool.* **3**: 24-40.
- Koh, H. S., 1989. Systematic studies of Korean rodents: V. Morphometric and chromosomal analyses in island populations of striped field mice (*Apodemus agrarius coreae*) in Southwestern coasts of the Korean peninsula. *Korean. J. Syst. Zool.* **5**: 1-12.
- Koh, H. S., 1991. Multivariate analysis with morphometric characters of samples representing eight subspecies of striped field mice, *Apodemus agrarius* Pallas (Rodentia, Mammalia), in Asia: the taxonomic status of subspecies *chejuensis* at Cheju island, Korea. *Korean. J. Syst. Zool.* **7**: 179-188.
- Lansmann, R. A., J. C. Avise, C. F. Aquadro, J. F. Shapira, and S. W. Daniel, 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of deer mice, *Peromyscus maniculatus*. *Evolution* **37**: 1-16.
- Moritz, C., T. E. Dowling, and W. B. Brown, 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.* **18**: 269-292.
- Park, D. W., 1985. Geology of coasts and mountain in Cheju-Do. Cheju-Do Study II: 321-322 (in Korean).
- Sites, J. W., Jr. and S. K. Davis, 1989. Phylogenetic relationships and molecular variability within and among six chromosome races of *Sceloporus grammicus* (Sauria, Iguanidae), based on nuclear and mitochondrial markers. *Evolution* **43**: 296-317.
- Sneath, P. H. A. and R. R. Sokal, 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco.
- Southern, E. M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Evolution* **98**: 503-517.
- Tegelstrom, H. and M. Jaarola, 1989. Genetic divergence in mitochondrial DNA between the wood mice (*Apodemus sylvaticus*) and the yellow necked mouse (*A. flavicolis*). *Hereditas* **111**: 49-60.
- Tegelstrom, H., P. Wyoni, H. Gelter, and M. Jaarola, 1988. Concordant divergence in proteins and mitochondrial DNA between two vole species in the genus *Clethrionomys*. *Biochem. Genetics* **26**: 223-237.
- Thomas, O., 1906. The Duke of Bedford's zoological exploration in eastern Asia: III. List of small mammals from Korea and Quelpart. *Proc. Zool. Soc. London* : 858-865.
- Upholt, W. P., 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. *Nucleic Acids Res.* **4**: 1257-1265.
- Wilcox, B. A., 1978. Supersaturated island faunas: a species-age relationships for lizards on post-Pleistocene land-bridge islands. *Science* **199**: 996-998.

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한국산 등줄쥐 2아종(*Apodemus agrarius coreae* and *Apodemus agrarius chejuensis*)의
미토콘드리아 DNA의 변이

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한국의 4개 지역에서 채집한 등줄쥐 2아종(*Apodemus agrarius coreae* and *Apodemus agrarius chejuensis*)을 사용하여, 표본들의 미토콘드리아 DNA를 8가지 제한효소로 소화시켰으며, 미토콘드리아 절단단편들의 양상에 관한 분석을 수행하였다.

전부 31개의 단편들이 나타났고, 15개의 mtDNA clone이 밝혀졌다. 이들 15 mtDNA clone들은 4개 mtDNA군을 형성하였다. 청주에서 채집한 한 마리는 특이하여, 4개 mtDNA군중의 한 군을 형성하였으며, 다른 mtDNA군과의 평균발산값은 4.6 퍼센트였다. 이 군의 분류학적 위치를 파악하기 위하여 여러지역의 표본들을 사용한 집중적인 연구가 필요하다.

제주도 표본들은 다른 한 군을 형성하였으며, 이들은 *Apodemus chejuensis*로 명명되어야 한다. 완도 표본들도 또 다른 군을 형성하였고, *Apodemus chejuensis*의 다른 집단으로 판단된다: *A. chejuensis*의 완전한 분류를 위하여 후속 연구가 요구된다.

청주에서 채집한 일곱 마리 중에서의 여섯 마리와 해남에서 채집한 네 마리는 마지막 mtDNA 군을 형성하였으며, 청주에서의 두 마리와 해남에서의 한 마리는 미토콘드리아 유전형에 있어서도 똑 같았으므로, 이들 청주와 해남 등줄쥐들은 모계적으로 근연함이 밝혀졌다.