한국동물분류학회지 제9권 제2호
The Korean Journal of Systematic Zoology
Vol. 9, No. 2: 171-179 (December 1, 1993)

Variation of Mitochondrial DNA in Striped Field Mice, *Apodemus agrarius coreae Thomas* (Mammalia, Rodentia), from the Korean Peninsula

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한반도산 등줄쥐 *Apodemus agrarius coreae* Thomas(포유강, 설치목)의 미토콘드리아 DNA의 변이

고흥선 · 유상규 · *김상복 · **유병선
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적요

한반도의 8개 지역에서 채집한 39마리의 등줄쥐(*Apodemus agrarius coreae* Thomas)의 미토콘드리아 DNA(mtDNA)를 8가지의 제한효소로 digestion시켰으며, 이들 mtDNA 절단단편들을 분석하였다.

전부 31개의 절단단편들이 나타났고, 7개의 mtDNA clone이 밝혀졌으며, 전체 8개 지역의 39마리 중에서 8개 지역의 32마리(속초, 1중에 1; 치악산, 5중에 4; 월악산, 3중에 3; 속리산, 2중에 2; 덕유산, 2중에 2; 지리산, 4중에 3; 해남, 4중에 2; 청주, 18중에 15)가 동일한 한 clone에 속했다. 또한 이들 7개 mtDNA clone들 간의 nucleotide-sequence divergence (p)의 범위는 0.2%-2.3%였으며, 이들 clone들은 뚜렷한 차이를 보이는 subgroup으로 구분되지 않았다.

한반도산 등줄쥐는, mtDNA genotype에 있어서도 뚜렷한 차이를 보이는 집단

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*The research was supported by a grant from Korea Science and Engineering Foundation in 1992.

으로 나뉘어지지 않았으므로, 단일 아종인 *Apodemus agrarius coreae*임을 확인하게 되었다.

Key words: Systematics, MtDNA, *Apodemus agrarius coreae*, Korea

INTRODUCTION

The genus *Apodemus* composed of 14 species is confined to the Palaearctic and northern part of the Oriental region: *A. agrarius* Pallas (striped field mice) is a sole member of the subgenus *Apodemus* and inhabits from central Europe to China and Korea (Corbet & Hill, 1991). The subclassification of *A. agrarius*, however, is in confusion. Corbet (1978) noted that most of subspecies of *A. agrarius* were designated by differences in pelage colour and/or mean body size and he summarized 23 nominal subspecies into three subspecies (*agrarius*, a western subspecies including 12 named subspecies; *ningpoensis*, an eastern subspecies including *coreae*, *chejuensis*, and six named subspecies; *chevrieri*, a southern Chinese subspecies). Wang (1985) performed the analyses with penile bones, papilla lingualis, and serum proteins of three subspecies of *A. agrarius* in Sichuan, China, and concluded that *A. chevrieri* (= *A. agrarius chevrieri*) is a species which is distinct from *A. agrarius ningpoensis* and *A. agrarius pallidior.*

The methods of numerical taxonomy based on equal weighting and overall similarity seemed inapplicable in defining higher categories above the species level (Farris, 1966). On the other hand, Flake & Turner (1968) stated that the numerical approach offers potential for the resolution of taxonomic problems for populations at infraspecific level. Koh (1987) carried out multivariate analyses with external and cranial characters of two subspecies of *A. agrarius* in Korea and noted that *A. agrarius chejuensis* from Cheju island is distinct from *A. agrarius coreae* from the Korean peninsula (the former is a large-size group and the latter is a small-size group). In morphometric analyses with eight subspecies of striped field mice, *A. agrarius*, in Asia (Koh, 1991), it is confirmed that one subspecies of *A. agrarius* (a large-size form, *A. agrarius chevrieri*) from southern China is a species, *A. chevrieri*, and another subspecies (a largest-size form, *A. agrarius chejuensis*) from Cheju island is also a distinct species, *A. chejuensis*. Moreover, it is revealed that *A. agrarius coreae* from the Korean peninsula is a medium-size form, whereas other five subspecies (*agrarius, ningpoensis, pallidior, insulaemus*, and *manchuricus*) from Turkey, China, Manchuria, and northern Korea is a small-size form.

Mitochondrial DNA (mtDNA) studies are important to infer maternal lineages among closely related species and infraspecific populations (Avise, 1986). The analyses of Restriction Fragment Length Polymorphisms (RFLPs) are simple and it is possible to analyze more loci per individual by RFLPs analysis than by sequencing (Dowling et al., 1990). In the analyses of mtDNA between the samples of two subspecies of striped field mice, *Apodemus agrarius coreae* and *A. agrarius chejuensis*, from Korea (Koh & Yoo, 1992), it is revealed that *A. agrarius chejuensis* (= *A. chejuensis*) from Cheju island is distinct in its mtDNA genotype from *A. agrarius coreae* from two localities in the Korean peninsula and that in *A. agrarius coreae*, one sample from Cheongju is different in its mtDNA genotype from other ten samples from Cheongju and Haenam.

In this paper, we added 28 samples from eight localities and analyzed mtDNA of 39 samples of *A. agrarius coreae* from eight localities in the Korean peninsula in order to clarify the taxonomic status of one sample with distinct mtDNA genotype.

MATERIALS AND METHODS

Thirty nine samples of striped field mice (*Apodemus agrarius coreae*) from eight localities (OTUs) in the Korean peninsula were utilized (see Table 1 and Fig. 1). The mtDNA was visualized from southern blots (Southern, 1975) of total cellular DNA. DNA isolation from liver, electrophoresis, Southern transfer, and hybridization were described by Davis (1986). All samples were individually digested using the following eight restriction endonucleases: Pvu II, Stu I, Pst I, EcoR V, BamH I, Hind III, Dra I, and EcoR I.

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 cluster analyses of single, average, and complete linkage methods (Sneath & Sokal,1973).

Table 1. Locality, mitochondrial DNA genotype, and clone of samples of striped field mice, *Apodemus agrarius coreae*, from eight localities (OTUs) in the Korean peninsula. Mitochondrial genotypes are based on the fragment patterns resulted from the digestion with eight restriction enzymes, Pvu II, Stu I, Pst I, EcoR V, BamH I, Hind III, Dra I, and EcoR I in order.

Locality	OTU	Specimen No..	MtDNA genotype	MtDNA clone
Sogcho	1	444	1 1 1 1 1 1 1 1	1
Mt. Chiak	2	576, 577, 578, 581	1 1 1 1 1 1 1 1	1
〃	〃	582	1 1 1 2 1 1 1 1	2
Mt. Weolak	3	579, 580, 583	1 1 1 1 1 1 1 1	1
Mt. Sogri	4	554, 556	1 1 1 1 1 1 1 1	1
Mt. Deokyoo	5	587, 588	1 1 1 1 1 1 1 1	1
Mt. Jiri	6	549, 550, 551	1 1 1 1 1 1 1 1	1
〃	〃	552	1 1 1 1 1 1 2 1	3
Haenam	7	473, 480	1 1 1 1 1 1 1 1	1
〃	〃	481	1 2 1 1 1 1 1 1	4
〃	〃	482	2 2 1 1 1 1 1 1	5
Cheongju	8	460, 461, 466, 478, 479, 501, 502, 503	1 1 1 1 1 1 1 1	1
〃	〃	504, 506, 518, 521, 523, 524, 526	〃	〃
〃	〃	421	1 1 1 1 1 1 2 1	3
〃	〃	465	2 2 1 2 2 1 1 2	6
〃	〃	522	1 1 1 1 1 1 1 2	7

Fig. 1. A map showing eight localities (OTUs) of samples of striped field mice, *Apodemus agrarius coreae*, from the Korean peninsula. Numerals indicate OTU number and locality of each OTU is given in Table 1.

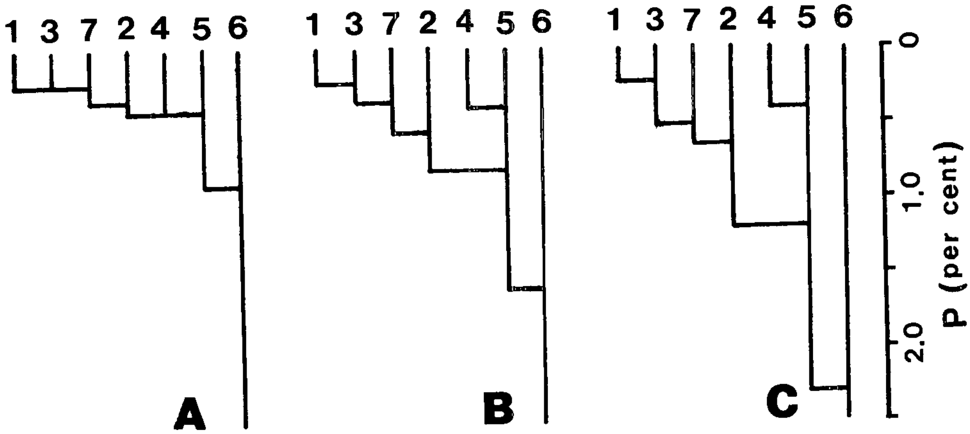

Fig. 2. Groupings of seven mtDNA clones in striped field mice, *Apodemus agrarius coreae*, from the Korean peninsula. The nucleotide-sequence divergences, given in Table 2, were used for cluster analyses of single (A), average (B), and complete (C) linkage methods. Numerals refer to the clones in Table 1.

Table 2. Nucleotide-sequence divergence (p in per cent) among seven mtDNA clones in striped field mice, *Apodemus agrarius coreae*, from eight localities in the Korean peninsula. For locality and specimen number of each clone, see Table 1.

Clone	1	2	3	4	5	6
2	0.34					
3	0.22	0.65				
4	0.40	0.78	0.65			
5	0.78	1.19	1.05	0.40		
6	1.87	1.33	2.31	1.41	0.92	
7	0.22	0.65	0.52	0.65	1.05	1.57

RESULTS

A total of 31 fragments were recognized and seven clones were revealed, as shown in Table 1. The clone 1 consisted of 32 among 39 samples from eight localities (1 of 1 from Sogcho, 4 of 5 from Mt. Chiak, 3 of 3 from Mt. Weolak, 2 of 2 from Mt. Sogri, 2 of 2 from Mt. Deokyoo, 3 of 4 from Mt. Jiri, 2 of 4 from Haenam, and 15 of 18 from Cheongju), indicating that most striped field mice have identical mtDNA genotype. One of 5 samples from Mt. Chiak and 1 of 4 ones from Mt. Jiri was clones 2 and 3, respectively. Among 4 samples from Haenam, one was found to be clone 4 and another was clone 5. Among 18 specimens from Cheongju, one was clone 6 and another was clone 7.

The nucleotide-sequence divergences (p in per cent) among seven clones are given in Table 2. The divergence value ranged from 0.22% (between clones 1 and 3) to 2.31% (between clones 3 and 6). Fig. 2 shows the groupings of seven mtDNA clones by cluster analyses of single (A), average (B), and complete (C) linkage methods. The clone 6 (one sample from Cheongju) appeared to be somewhat diverged from other six clones, but it was not distinctly different enough to form a subgroup. Representative fragment patterns of mtDNA in clones 1, 5, and 6 with Hind III, Pvu II, and EcoR V are shown in Fig. 3.

In summary, as shown in Fig. 2 and Tables 1 and 2, seven mtDNA clones were revealed in the analyses with 39 samples of *Apodemus agrarius coreae* from eight localities in the Korean peninsula and they appeared to be clustered into a single group, although the clone 6 (one sample from Cheongju) was somewhat divergent to other six clones (38 samples from eight localities).

DISCUSSION

Thomas (1906) described striped field mice from the Korean peninsula and Cheju island as a single subspecies, *Apodemus agrarius coreae* Thomas. Jones and Johnson (1965) reported that four subspecies of *A. agrarius* were recognized in Korea: *manchuricus* in the extreme northern part,

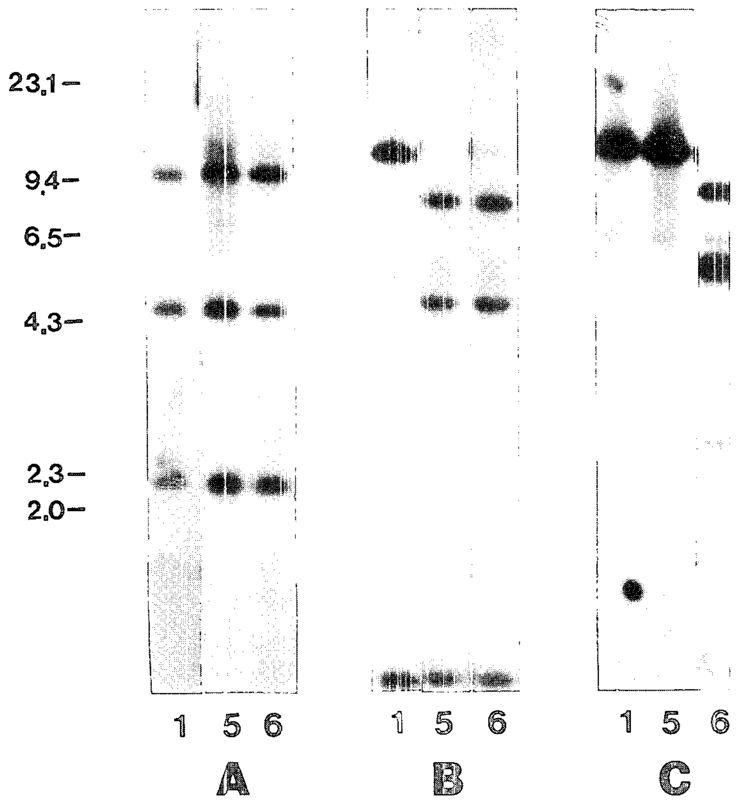

Fig. 3. Representative restriction fragment patterns in three mtDNA clones of striped field mice, *Apodemus agrarius coreae*, from the Korean peninsula. Numerals refer to the clones in Table 1. The lane at the far left contains size markers indicated in kilo base pairs. A, Hind III. B, Pvu II. C, EcoR V.

pallescence 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 karyotypes. In morphometric analyses with eight subspecies of *A. agrarius* in Asia (Koh, 1991), it is confirmed that one subspecies of *A. agrarius* (*A. agrarius chevrieri*) from southern China is a species, *A. chevrieri*, and another subspecies (*A. agrarius chejuensis*) from Cheju island in Korea is also a distinct species, *A. chejuensis*. Moreover, it is revealed that *A. agrarius coreae* in the Korean peninsula is different in morphometric characters from other five subspecies (*agrarius, ningpoensis, pallidior, insulaemus,* and *manchuricus*).

Geographic variation within species, hybridization, and discovery of cryptic species are all effectively studied with restriction site analysis (Hillis & Moritz, 1990). The nucleotide-sequence divergence values for eight detected mtDNA variants of *Rattus norvegicus* ranged from 0.2% to 1.8% and for eight *R. rattus* variants, from 0.2% to 9.6% (Brown & Simpson, 1981). The largest divergence observed between any of 22 mtDNA clones of *Peromyscus maniculatus* was 7% (Lansman *et al.*, 1983). The greatest sequence divergence found between eight mtDNA clones within *Apodemus sylvaticus* was 1.4% and that in *A. flavicolis* was 1.0% (Tegelstrom & Jaarola, 1989).

Restriction fragment length polymorphisms have been invaluable for the analysis of matrilineal relationships between distinct forms in the pocket gopher, *Geomys* (Davis, 1986). In *Clethrionomys glareolus,* the Finnish mtDNA clones differed from all Swedish lineages, with a mean sequence divegence of 2.58% (Tegelstrom *et al.,* 1988). In *Cryptomys hottentotus,* average divergence among four distinct mtDNA clones was 1.6% with a maximum divergence of 2.8%, and nucleotide-sequence divergence between two mtDNA clones of *C. h. natalensis* was 1.7% (Honeycutt *et al.,* 1987). In the mtDNA analysis with *Apodemus agrarius coreae* from two localities in the Korean peninsula, Koh & Yoo (1992) found a distinct mtDNA subgroup (one sample from Cheongju) among 11 samples from Cheongju and Haenam, it is concluded that further analysis with additional samples from the Korean peninsula are necessary to clarify the taxonomic status of the distinct mtDNA subgroup.

In this study with 39 samples of *Apodemus agrarius coreae* from eight localities in the Korean peninsula, 32 samples were found to have a identical genotype (see Table 1): the nucleotide-sequence divergence values among seven mtDNA clones ranged from 0.22% to 2.31% (see Table 2): and the clone 6 (one sample from Cheongju) appeared to be somewhat diverged from other six clones (38 samples from eight localities), but it was not distinctly different enough to form a subgroup (see Fig. 2).

Developments in the areas of molecular, cyto-, and numerical taxonomy are enormous and there have been a conflict between biologists and morphologists about the merits of their data (Maxon & Wilson, 1975; Ferguson, 1980). However, modern molecular techniques have not yet pushed comparative morphology into the shadows (Patterson, 1987). It was advocated that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied (Crovollo, 1969; Mayr & Ashlock, 1991). Therefore, it is concluded that the variability among the mtDNA clones of striped field mice from the Korean peninsula can not justify the separation of the distinct mtDNA clone into a different population and it is confirmed that striped field mice from the Korean peninsula are a single subspecies of *Apodemus agrarius* (*A. agrarius coreae* Thomas), as noted by Koh (1991).

ABSTRACT

Thirty nine samples of striped field mice (*Apodemus agrarius coreae* Thomas) from eight localities in the Korean peninsula were used for the analyses of mitochondrial DNA (mtDNA) fragment patterns resulted from the digestion with eight restriction enzymes.

A total of 31 fragments were recognized and seven mtDNA clones were revealed: one clone consisted of 32 among 39 samples from eight localities (1 of 1 from Sogcho, 4 of 5 from Mt. Chiak, 3 of 3 from Mt. Weolak, 2 of 2 from Mt. Sogri, 2 of 2 from Mt. Deokyoo, 3 of 4 from Mt. Jiri, 2 of 4 from Haenam, and 15 of 18 from Cheongju). The nucleotide-sequence divergences (p) among seven mtDNA clones ranged from 0.2% to 2.3% and distinct subgroups were not resulted from the grouping of these clones.

It is confirmed that striped field mcie from the Korean peninsula is a single subspecies of *Apodemus agrarius* (*A. agrarius coreae*) because they were not divided into separate subgroups in their mtDNA genotypes.

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RECEIVED: 4 October 1993
ACCEPTED: 21 October 1993