

Variation of Mitochondrial DNA Restriction Fragments within One Subspecies of Korean Field mice, *Apodemus peninsulae peninsulae* Thomas (Mammalia: Rodentia), from Korea

Hung Sun Koh, Tae Young Chun, Sang Kyu Yoo, Young Ki Kim, Young Ju Song

(Department of Biology, Chungbuk National University, Cheongju 360-763, Korea)

ABSTRACT

Samples of Korean field mice (*Apodemus peninsulae peninsulae* Thomas) from six localities in Korea were used for the analyses of mitochondrial DNA (mtDNA) fragment patterns resulted from the digestion with eight restriction enzymes.

A total of 29 fragments were recognized and seven mtDNA clones were revealed. The nucleotide-sequence divergences (p) among the seven mtDNA clones ranged from 0.42% to 2.01%. Moreover, the seven clones were grouped into three major subgroups with the mean divergence value of 1.52% among them. One subgroup was composed of three clones of 18 samples from three localities (16, Cheongju; 1, Mt. Sobaek; 1, Mt. Weolak); another subgroup, three clones of eight samples from four localities (2, Cheongju; 2, Mt. Weolak; 2, Mt. Gaya; 2, haenam); and the last subgroup, one clone of two samples from Cheongju.

Three subgroups were also distinct with one another in their mtDNA genotypes of *Stu* I and the former two subgroups differed from the last subgroup in their genotypes with *Pvu* II. Further analyses with additional samples from various localities in Korea appeared to be necessary in order to clarify the taxonomic status of the distinct mtDNA subgroups.

Key words: Systematics, mtDNA, *Apodemus peninsulae peninsulae*, Korea

INTRODUCTION

The genus *Apodemus* composed of 14 species is confined to the Palaearctic and northern part of the Oriental regions (Corbet and Hill, 1991). Seven species (*A. agrarius*, *A. argenteus*, *A. chevrieri*, *A. draco*, *A. latronum*, *A. speciosus*, and *A. peninsulae*) inhabit in eastern Asia and two species (*A. agrarius* and *A. peninsulae*) are distributed in Korea.

Korean field mice, *A. peninsulae* Thomas 1906, are distributed over much of Siberia, China, Manchuria, Korea, and Hokkaido (Corbet and Hill, 1991), and the type locality of *A. peninsulae* is Mungyong, 110 miles southeast of Seoul, Korea (Jones, 1956). *A. peninsulae* was considered as a subspecies of *A. speciosus* (Thomas, 1906; Vinogradov and Argiropulo, 1941; Tate, 1947; Woon, 1967), but Vorontsov *et al.* (1977) claimed on the basis of karyological and morphological analyses with samples of boreal regions of eastern Asia that all the eastern Asian forms of *A. speciosus* should be transferred to the species, *A. peninsulae*. Corbet (1978) summarized eight subspecies (*peninsulae*, *major*, *majusculus*, *praetor*, *rufulus*, *tscherga*, *giliacus*, and *sowerbyi*) of *A. peninsulae* into three ones (subspecies *peninsulae* including five other subspecies; *sowerbyi*; and *giliacus*). Therefore, the taxonomy of *A. peninsulae* is still in doubt, as noted by Xia (1985).

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 and Turner (1968) stated that the numerical approach offers potential for the resolution of taxonomic problems for populations at infraspecific level.

In morphometric and chromosomal analyses it is confirmed that the Korean field mice from Korea are not *A. speciosus peninsulae*, but *A. peninsulae peninsulae* (Koh, 1986). Four external and 27 cranial characters of Korean field mice (*Apodemus peninsulae*) from nine localities in eastern Asia, representing five subspecies, were analyzed by multivariate statistical methods (Koh and Lee, 1994): it was confirmed that *Apodemus peninsulae* could be classified into two subspecies (*peninsulae* in Korea, Manchuria, and E. Siberia and *sowerbyi* in central China) within the distribution range in the continent of Asia, as noted by Corbet (1978).

Mitochondrial DNA (mtDNA) studies are important to infer maternal lineages in order to determine the heterogeneity in molecular level among closely related species and infraspecific populations (Avice, 1986): the analyses of Restriction Fragment Length Polymorphisms (RFLPs) are both simple and fast, and it is possible to analyze more loci per individual by RFLPs analyses than by sequencing (Dowling *et al.*, 1990).

The objective of this paper is to analyze the range and pattern of variation of mtDNA restriction fragments in Korean field mice (*Apodemus peninsulae peninsulae*) from Korea in order to confirm its taxonomic status.

MATERIALS AND METHODS

Twenty eight samples of Korean field mice (*Apodemus peninsulae peninsulae*) from six localities

in Korea were utilized (see Table 1 and Fig. 1).

Mitochondrial DNA 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: *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 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 cluster analyses of single, average, and complete linkage methods (Sneath and Sokal,1973).

RESULTS

A total of 29 fragments were recognized and eight mtDNA clones were revealed, as shown in Table 1 and Fig. 1. Clone 1 is composed of five samples from two localities (4 from Cheongju, 1 from Mt. Sobaek): clone 2, four samples from four localities (1, Cheongju; 1, Mt. Weolak; 1, Mt. Gaya; 1, Haenam): clone 3, six samples from Cheongju: clone 4, two samples from two localities (6, Cheongju; 1, Seolak): clone 5, three samples from three localities (1, Cheongju; 1, Mt. Weolak; 1,

Table 1. Locality, specimen number, mitochondrial DNA genotype, and clone of Korean field mice, *Apodemus peninsulae peninsulae*, from six 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.

Locality	Specimen no.	mtDNA genotype	Clone
Cheongju,Chungbuk	626, 649, 713, 786	1 1 1 1 1 1 1 1	1
	714	1 1 1 1 1 1 1 2	2
	607, 652, 672, 675, 679, 683	1 1 1 2 1 1 1 1	3
	610, 648, 653, 684, 686, 688	1 1 1 3 1 1 1 1	4
	685	1 1 1 2 1 1 1 2	5
	687, 696	1 2 1 1 1 1 1 3	6
Mt. Weolak,Chungbuk	475	1 1 1 1 1 1 1 2	2
	467	1 1 1 2 1 1 1 2	5
Mt. Sobaek, Kangwon	762	1 1 1 1 1 1 1 1	1
Mt. Seolak, Kangwon	448	1 1 1 3 1 1 1 1	4
Mt. Gaya, Kyoungnam	759	1 1 1 1 1 1 1 2	2
	758	1 1 1 2 1 1 1 2	5
Haenam, Chennam	472	1 1 1 1 1 1 1 2	2
	474	1 1 1 3 1 1 1 2	7

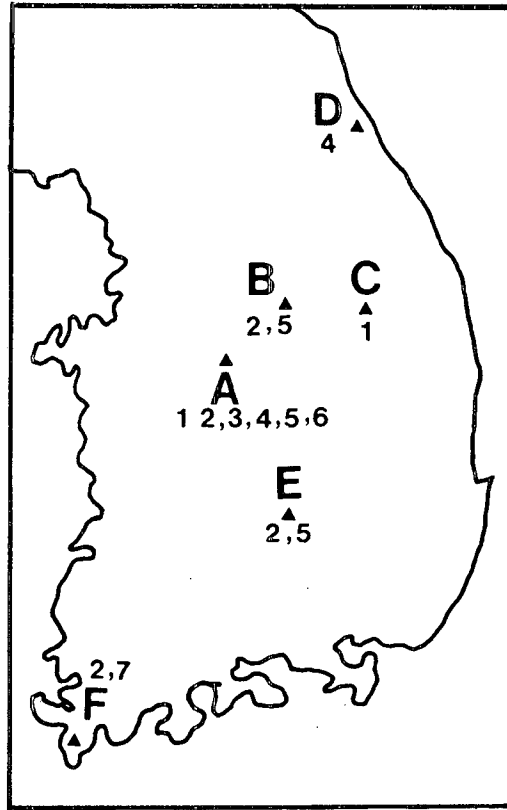


Fig. 1. A map showing six localities of Korean field mice, *Apodemus peninsulae peninsulae*, in Korea, with their mitochondrial DNA clones. Alphabets indicate locality; Cheongju (A), Mt. Weolak (B), Mt. Sobaek (C), Mt. Seolak (D), Mt. Gaya (E), and Haenam (F). Numerals indicate mtDNA clones in Table 1.

Table 2. Nucleotide-sequence divergences (p in per cent) among seven mitochondrial DNA clones in Korean field mice, *Apodemus peninsulae peninsulae*, from six localities in Korea. For locality and specimen number of each clone see Table 1.

Clone	1	2	3	4	5	6
2	0.92					
3	0.42	1.41				
4	0.62	1.74	0.77			
5	1.41	0.56	0.87	1.89		
6	1.11	1.11	1.63	2.01	1.63	
7	1.74	0.62	1.89	1.03	0.77	2.01

Mt. Gaya): clone 6, two samples from Cheongju; and clone 7, one sample from Haenam.

The nucleotide-sequence divergences (p) among seven mtDNA clones ranged from 0.42% to 2.01% (Table 2). Moreover, the seven clones were grouped into three major subgroups with the mean divergence value of 1.52% among them (Fig. 2). One subgroup was composed of three clones

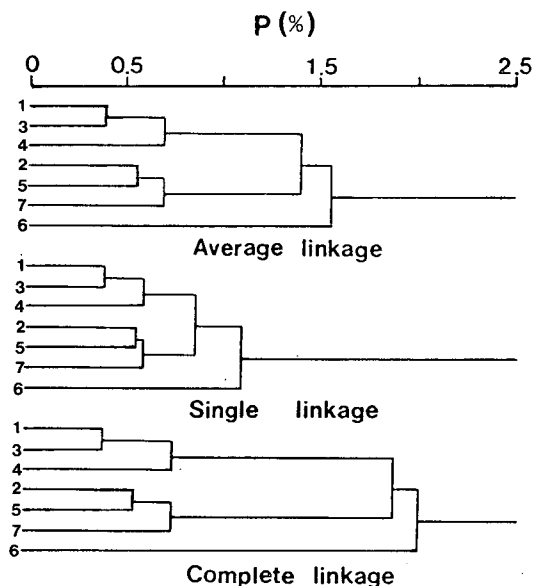


Fig. 2. Groupings of seven mitochondrial DNA clones in Korean field mice, *Apodemus peninsulae peninsulae*, from Korea. The nucleotide-sequence divergences (p in per cent), shown in Table 2, were used for cluster analyses of average (A), single (B), and complete (C) linkage methods. Numerals indicate mtDNA clones in Table 1.

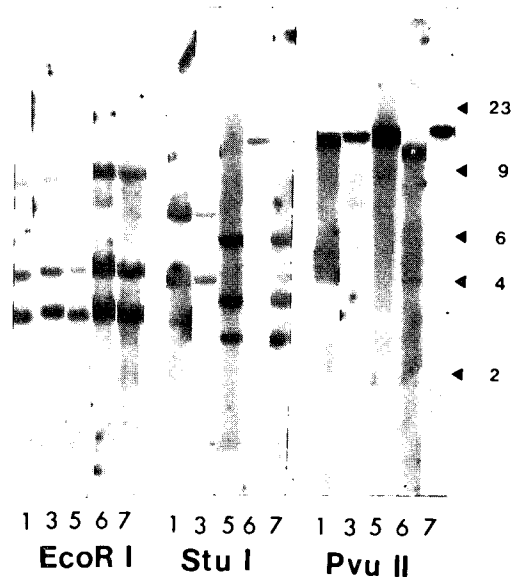


Fig. 3. Representative fragment patterns of five mitochondrial DNA clones in Korean field mice, *Apodemus peninsulae peninsulae*, from Korea. Numerals indicate mtDNA clones and the lane far left contains size markers indicated in kilo base-pairs.

of 18 samples from three localities (16 from Cheongju, 1 from Mt. Sobaek, 1 from Mt. Seolak): another subgroup, three clones of eight samples from four localities (2 from Cheongju, 2 from Mt. Weolak, 2 from Mt. Gaya, 2 from Haenam): and the last subgroup, one clone of two samples from Cheongju.

The three subgroups were also distinct with one another in their mtDNA genotypes of *Stu I*: the former two subgroups differed from the last subgroup in their genotypes with *Pvu II* (see Table 1). The representative fragment patterns of mtDNA in clones 1, 3, 5, 6, and 7 with *EcoR I*, *Stu I* and *Pvu II* are shown in Fig. 3.

DISCUSSION

Apodemus peninsulae was considered as a subspecies of *A. speciosus* (Thomas, 1906). Vorontsov *et al.* (1977) reported that all the Far Eastern forms of *A. speciosus* should be transferred into *A. peninsulae*, and Corbet (1978) summarized eight subspecies of *A. peninsulae* into three subspecies: subspecies *peninsulae* including five nominal subspecies (*major*, *majuculus*, *praetor*, *rufulus*, and *tscherga*) from Korea, Manchuria, and Siberia, *sowerbyi* from central China, and *giliacus* from Hokkaido, Japan. Kobayashi (1985) noted that "there still remains the important

question that either Vorontsov's *peninsulae* is homologous with the Thomas's *peninsulae*, or he made a mistake in terms of the international naming code."

Geographic variation within species, hybridization, and discovery of cryptic species are all effectively studied with restriction site analysis (Hillis and Moritz, 1990). In *Mus musculus*, nucleotide-sequence divergence between *M. m. domesticus* and *M. m. bactrinus* was 7.1% (Yonekawa *et al.*, 1981), and for eight *R. rattus* variants in the United States and Asia, the divergences varied from 0.2% to 9.6% (Brown and Simpson, 1981): The largest nucleotide-sequence divergence observed between any of 22 mtDNA clones of *Peromyscus maniculatus* from continental North America was 7% (Lansmann *et al.*, 1983).

On the other hand, the maximum divergence in *Clethrionomys rutilus* was 0.89% and in *Clethrionomys glareolus*, the Finnish mtDNA clones differed from all Swedish lineages, with a mean sequence divergence of 2.58% (Tegelstrom *et al.*, 1988). The divergences among eight detected mtDNA variants of *R. norvegicus* in the United States and Japan ranged from 0.2% to 1.8% (Brown and Simpson, 1981).

In the species of *Apodemus*, the greatest sequence divergence revealed between eight mtDNA clones of *Apodemus sylvaticus* was 1.4% and that in *A. flavicolis* was 1.0% (Tegelstrom and Jaarola, 1989).

In this study (Table 2) the nucleotide-sequence divergences (p) among seven mtDNA clones ranged from 0.42% to 2.01%. Moreover, the seven clones were grouped into three major subgroups with the mean divergence value of 1.52% among them (Fig. 2). Assuming a 2% rate of divergence per million years (Brown *et al.*, 1979), they have separated with one another since 0.76 million years ago.

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 and Wilson, 1975; Fergusson, 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 (Crovillo, 1969; Mayr and Ashlock, 1991).

Mayr (1969) noted that a subspecies is an aggregate of phenotypically similar populations of a species, differing taxonomically from other populations. In *Cryptomys h. 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 *Apodemus agrarius coreae* from eight localities in the Korean peninsula, nucleotide-sequence divergences among seven mtDNA clones ranged from 0.2% to 2.3% (Koh *et al.*, 1993).

Infrasubspecific heterogeneity was found in *Mus musculus bactrinus* (0.4%) and *M. m. castaneus* (0.3%), but *M. m. domesticus* and *M. m. molossinus* revealed no infrasubspecific heterogeneity (Yonekawa *et al.*, 1981): they concluded that the mtDNA cleavage patterns provide support for many of the subspecies that have been described within the species *Mus musculus*.

Koh and Lee (1994) carried out morphometric analyses with five subspecies of Korean field mice, *Apodemus peninsulae*, and concluded that two subspecies, *peninsulae* and *sowerbyi*, should be recognized in continental regions of distribution and that subspecies *peninsulae* inhabits in Korea.

However, in this paper with Korean field mice (*A. peninsulae peninsulae*) from Korea (Tables 1 and 2; Figs. 1-3), the seven mtDNA clones were grouped into three major subgroups: the three subgroups were also distinct with one another in their mtDNA genotypes of *Stu* I: the former two subgroups differed from the last subgroup in their genotypes with *Pvu* II. Further analyses with additional samples from various localities seemed to be necessary to perform in order to clarify the taxonomic status of the distinct mtDNA subgroups.

REFERENCES

- 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*. *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 Palearctic region: A taxonomic review, British Mus. (Nat. Hist.), Cornell University Press, London.
- Corbet, G.B. and J.E. Hill, 1991. A world list of mammalian species, Oxford University Press, Oxford.
- Crovillo, T.J., 1969. Effects of change of characters and number of characters in numerical taxonomy. *Am. Midl. Nat.*, **81**: 68-86.
- Davis, S.K., 1986. Evolutionary relationships in *Geomys*: An analysis using mitochondrial DNA. Ph. D. Diss. Washington Univ., St. Louis, Mo.
- Dowling, T.E., C. Moritz, and J.D. Palmer, 1990. Nucleic acids II: restriction site analyses, In: *Molecular systematics* (Hillis, D.M. and C. Moritz, eds.). Sinauer Associates, Inc., Mass. p. 250.
- Farris, J.S., 1966. Estimation of conservatism of characters by constancy within biological populations. *Evolution*, **22**: 260-270.
- Fergusson, A., 1980. *Biochemical systematics and evolution*. John Wiley and Sons, New York.
- Flake, R.H. and B.L. Turner, 1968. Numerical classification for taxonomic problems. *J. Theoret. Biol.*, **20**: 587-591.
- Hillis, D.M. and C. Moritz, 1990. *Molecular systematics*. Sinauer Associates, Inc., Mass.
- Honeycutt, R.L., S.V. Edwards, K. Nelson, and E. Nevo, 1987. Mitochondrial DNA variation and the phylogeny of African mole rats (Rodentia: Bathyergidae). *Syst. Zool.*, **36**: 280-292.
- Jones, J.K., 1956. Comments on the taxonomic status of *Apodemus peninsulae*, with description of a new subspecies from north China. *Univ. Kansas Publ. Mus. Nat. Hist.*, **9**: 337-346.
- Kobayashi, T., 1985. Taxonomical problems in the genus *Apodemus* and its allies, In: *Contemporary mammalogy in China and Japan* (Kawamichi, T., ed.), Mamm. Soc. Japan, pp. 80-82.
- Koh, H.S., 1986. Systematic studies on Korean rodents: II. A chromosome analysis in Korean field mice, *Apodemus peninsulae peninsulae* Thomas (Muridae, Rodentia), from Munkyeong, with the comparison of morphometric characters of these Korean field mice to sympatric striped field mice, *A. agrarius coreae* Thomas. *Kor. J. Syst. Zool.*, **2**: 1-10.
- Koh, H.S., S.K. Yoo, S.B. Kim, and B.S. Yoo, 1993. Variation of mtDNA in striped field mice, *Apodemus*

- agrarius coreae* Thomas, from the Korean peninsula. Kor. J. Syst. Zool., **9**: 171-179.
- Koh, H.S. and W.J. Lee, 1994. Geographic variation of morphometric characters in five subspecies of Korean field mice, *Apodemus peninsulae* Thomas (Rodentia, Mammalia), in eastern Asia. Kor. J. Zool., **37**: 33-39.
- 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.
- Maxon, L.R. and C. Wilson, 1975. Albumin evolution and organismal evolution in tree frogs (Hylidae). Syst. Zool., **24**: 1-15.
- Mayr, E., 1969. Principles of systematic zoology. McGraw-Hill, Inc., New York.
- Mayr, E. and P.D. Ashlock, 1991. Principles of systematic zoology. McGraw-Hill, Inc., New York.
- Patterson, C., 1987. Introduction, In: Molecules and morphology in evolution (Patterson, C., ed.), Cambridge Univ. Press, Cambridge, pp. 1-22.
- 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.
- Tate, G.H.H., 1947. Mammals of eastern Asia, Macmillan Co., New York.
- Thomas, F.R.S., 1906. The Duke of Bedford's zoological exploration in eastern Asia. II. List of small mammals from Korea and Quelpart. Proc. Zool. Soc., London: 858-865.
- Tegelstrom, H. and M. Jaarola, 1989. Genetic divergence in mitochondrial DNA between wood mice (*Apodemus sylvaticus*) and the yellow necked mouse (*A. flavicollis*). 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.
- Upholt, W.P., 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic Acids Res., **4**: 1257-1265.
- Vinogradov, B.S. and A.I. Argiropulo, 1941. Fauna of the U.S.S.R., Mammals, Israel Program for Scientific Translations, Ltd., Jerusalem.
- Vorontsov, N.N., T.S. Bekasova, B. Kral, K.V. Korobitsina, and E. Yu. Ivanitskaya, 1977. On specific status of Asian wood mice of the genus *Apodemus* (Rodentia, Muridae) from Siberia and Far East. Zool. Zh., **56**: 437-449.
- Woon, P. H., 1967. Illustrated encyclopedia of fauna and flora of Korea, Vol. 7, Mammals. Samhwa Publishing Co., Seoul.
- Yonekawa, H., K. Moriwaki, O. Gotoh, J. Hayashi, J. Watanabe, N. Miyashita, M. L. Petras, and Y. Tagashira, 1981. Evolutionary relationships among five subspecies of *Mus musculus* based on restriction enzyme cleavage patterns of mitochondrial DNA. Genetics, **98**: 801-816.
- Xia, W., 1985. A study on Chinese *Apodemus* and its relation to Japanese species. In: Contemporary mammalogy in China and Japan (Ed., T. Kawamichi), pp. 76-79. Mamm. Soc. Japan.

RECEIVED: 17 March 1995

ACCEPTED: 2 June 1995

한국에 서식하는 흰넓적다리붉은쥐 한 아종 *Apodemus peninsulae peninsulae*
Thomas(포유강: 설치목)의 미토콘드리아 DNA 절단단편의 변이

고 홍 선·천 태 영·유 상 규·김 영 기·송 영 주
(충북대학교 생물학과)

적 요

한국의 6개 지역에서 채집한 흰넓적다리붉은쥐(*Apodemus peninsulae peninsulae*)를 사용하여, 8개 제한효소로 절단한 미토콘드리아 DNA(mtDNA)의 단편들을 분석하였다.

총 29개의 절단단편들이 나타났고, 7개 mtDNA의 clone이 밝혀졌다. 이들 mtDNA clone간의 nucleotide-sequence divergence(p)는 0.42-2.01%였으며, 이들은 평균 divergence가 1.52%인 3개 소군으로 나뉘어졌다. 한 소군은 3개 clone으로써 3개 지역의 18마리(청주 16, 소백산 1, 설악산 1)였고, 다른 한 소군은 3개 clone으로 4개 지역의 8마리(청주 2, 월악산 2, 가야산 2, 해남 2)였다. 나머지 한 소군은 1개 clone으로 청주의 2마리였다. 뿐만아니라, 이들 3개 소군은 *Stu* I의 genotype에 있어서 서로 뚜렷한 차이를 보였고, 전자의 2개 소군과 마지막 소군과는 *Pvu* II의 genotype이 달랐다.

이들 뚜렷한 mtDNA 소군의 분류학적 위치를 규명하기 위하여 한국내 여러지역의 더 많은 표본들을 사용한 계속적인 연구가 필요하다고 본다.