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# Variation of Mitochondrial DNA Restriction Fragments of Common Rats, *Rattus norvegicus caraco* Pallas (Mammalia, Rodentia), from Cheongju, Korea

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## ABSTRACT

Forty samples of common rats (*Rattus norvegicus caraco*) from Cheongju, Korea, were used for the analyses of mitochondrial DNA (mtDNA) fragment patterns resulted from the digestion with eight restriction enzymes. A total of 36 fragments were recognized and six mtDNA clones were revealed. The nucleotide-sequence divergences (p) among six mtDNA clones ranged from 0.35% to 2.73%. Moreover, the six clones were grouped into three major subgroups: the first, second, and third subgroup were composed of 29 samples of three clones, ten samples of two clones, and one sample of one clone, respectively. The second and third subgroups were different in their mtDNA genotype of *Pvu* II from the first subgroup, and the third subgroup differed in the genotype of *Dra* I from other two subgroups. Furthermore, the maximum divergence among common rats from Korea in this study is greater than that among common rats from the United States and Japan by Brown and Simpson (1981). Further analyses with additional samples from other localities in Korea appeared to be necessary in order to clarify the taxonomic status of the distinct mtDNA subgroups.

Key words: Systematics, mtDNA, *Rattus norvegicus caraco*, Cheongju, Korea

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## INTRODUCTION

Genus *Rattus* is a large, confuging taxon composed of 61 species (Corbet and Hill, 1991). Schwartz and Schwartz (1967) included *Rattus norvegicus* as one of subspecies of *R. rattus*, but Corbet (1978) treated *R. norvegicus* as a good species with world-wide distribution: he also classified the common rats from western Palaearctic region as *R. n. norvegicus* Berkenhaut and eastern Asian form of common rat as *R. n. caraco* Pallas. In Korea, Jones and Johnson (1965) described Korean common rat as a single subspecies, *R. n. caraco*, but Won (1967) recognized two subspecies, *R. n. caraco* in the extreme northern part and *R. n. norvegicus* in most region of the Korea.

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. Koh (1992) carried out morphometric analyses with common rats, *Rattus norvegicus caraco*, from seven localities in Korea and concluded that samples were more or less similar with one another, i.e., a single subspecies.

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 (Avise, 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 analysis than by sequnecing (Dowling *et al.*, 1990). In the analysis of mtDNA RFLPs with samples of *Rattus norvegicus* from United States and Japan, the maximum divergence value of 1.8%, which is four to five times lower than that observed in *R. rattus*, was revealed among the eight detected variants (Brown and Simpson, 1981).

The objective of this paper is to analyze mtDNA restriction fragments in common rats (*R. norvegicus caraco*) from Cheongju in order to determine the range and pattern of their variation.

## MATERIALS AND METHODS

Forty samples of common rats (*Rattus norvegicus caraco*) from Cheongju, Korea, were utilized (see Table 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: *Eco*R I, *Pvu* II, *Dra* I, *Hind* III, *Bam*H I, *Eco*R V, *Pst* I, and *Stu* 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 and Sokal,1973).

## RESULTS

A total of 36 fragments were recognized and six mtDNA clones were revealed, as shown inTable 1. Among 40 samples studied, 26 samples were identical in their mtDNA genotypes, clone 1, and nine samples also had the same mtDNA genotype, clone 2.

The nucleotide-sequence divergences (p) among six mtDNA clones ranged from 0.35% to 2.73% (Table 2). The six mtDNA clones were grouped by single, average, and complete linkage methods, as shown in Fig. 1. Three subgroups appeared to be evident. One subgroup is composed of 29 samples of clones 1, 3, and 4: another subgroup, ten samples of clones 2 and 6: still another subgroup, one sample of clone 5. The second and third subgroups were distinct in their mtDNA genotype of *Pvu* II, and the third subgroup was in the genotype of Dra I.

Fig. 2 shows the representative fragment patterns of mtDNA in clones 1, 2, 3, and 5 with *Bam*H I (A), *Pvu* II (B), and *Dra* I (C).

**Table 1.** Specimen number, mitochondrial DNA genotype, and clone of common rats, *Rattus norvegicus caraco*, from Cheongju, Korea. Mitochondrial genotypes are based on the fragment patterns resulted from the digestion with eight restriction enzymes. *Eco*R I, *Pvu* II, *Dra* I, *Hin*d III, *Bam*H I, *Eco*R V, *Pst* I, and *Stu* I, in order.

| Specimen no.(total) | mtDNA genotype | Clone |
|---|---|---|
| 490,491,615,628-631,669, 719,730,731,734,736-740, 760,761,774,777-781, 791 (26) | 1 1 1 1 1 1 1 1 | 1 |
| 462-464,721,725-729 (9) | 1 2 1 1 1 1 1 1 | 2 |
| 614,677 (2) | 1 1 1 2 1 1 1 1 | 3 |
| 735 (1) | 1 1 1 1 1 1 1 2 | 4 |
| 613 (1) | 1 2 2 1 1 1 1 1 | 5 |
| 722 (1) | 1 2 1 1 1 2 1 1 | 6 |

**Table 2.** Nucleotide-sequence divergences (p in per cent) among six mitochondrial DNAclones in common rats, *Rattus norvegicus caraco*, from Cheongju, Korea. Specimen number of each clone see Table 1.

| Clone | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 0.58 | | | | |
| 3 | 0.60 | 1.32 | | | |
| 4 | 0.58 | 0.98 | 1.03 | | |
| 5 | 1.63 | 0.91 | 2.73 | 2.20 | |
| 6 | 0.98 | 0.35 | 1.85 | 1.44 | 1.39 |

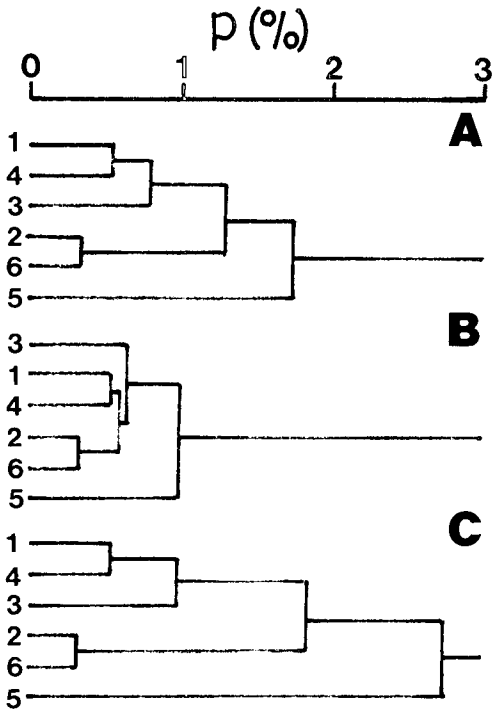


**Fig. 1.** Grouping of six mitochondrial DNA clones in common rats, *Rattus norvegicus caraco*, from Cheongju, Korea. The nucleotide-sequence divergences (p in per cent), shown in Table 2, were used for cluster analyses of average (A), single (B), and complete (C) linkage methods. Numerals indicate mtDNA clones in Table 1.

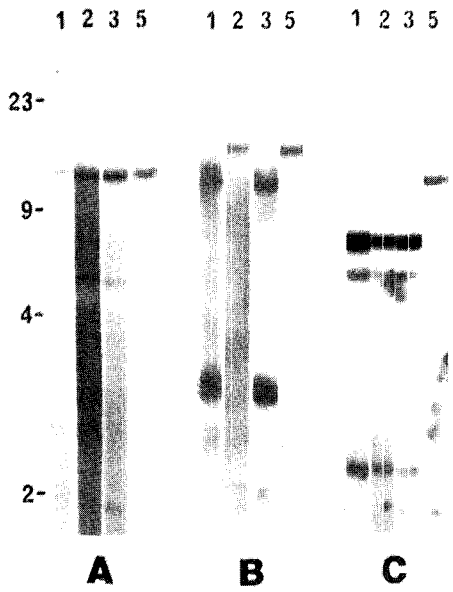


**Fig. 2.** Representative fragment patterns of four mitochondrial DNA clones in common rats, *Rattus norvegicus caraco*, from Cheongju, Korea. Numerals indicate mtDNA clones and the lane far left contains size markers indicated in kilo base-pairs. A, *Bam*H I. B, *Pvu* II. C, *Dra* I.

## DISCUSSION

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* were 7.1% (Yonekawa *et al.*, 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 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). The maximum divergence in *Clethrionomys rutilus* was 0.89% (Tegelstrom *et al.*, 1988).

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 divergence of 2.58% (Tegelstrom *et al.*, 1988).

In the species of *Rattus*, the extent of mtDNA sequence-divergence of *R. norvegicus* was only 1%, but that of *R. rattus* was 8% (Yosida, 1980). And the divergences among eight detected mtDNA variants of *R. norvegicus* in the United States and Japan ranged from 0.2% to 1.8%, and for eight *R. rattus* variants in the United States and Asia, from 0.2% to 9.6% (Brown and Simpson, 1981): they concluded that the isolation from the geograhpic origin by major barriers like oceans resulted in the high divergence value.

In this study with 40 samples of *R. norvegicus caraco* from Cheongju, Korea, 26 samples were identical in their mtDNA genotypes, clone 1, and nine samples also had the same mtDNA genotype, clone 2 (Table 1). However, the nucleotide-sequence divergences among six mtDNA clones ranged from 0.35% to 2.73% (Table 2) and the six mtDNA clones were grouped into three subgroups (Fig. 1). One subgroup is composed of 29 samples of clones 1, 3, and 4: another subgroup, ten samples of clones 2 and 6: still another subgroup, one sample of clone 5. The second and third subgroups were distinct in their mtDNA genotype of *Pvu* II, and the third subgroup was in the genotype of *Dra* I (Fig. 2). Moreover, assuming a 2% rate of divergence per million years (Brown *et al.*, 1979), the third subgroup with the mean divergence value of 1.75% separated from the first and second subgroups 0.88 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 (Crovollo,1969; Mayr and Ashlock, 1991).

Mayr (1968) 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 *M. musculus*. Koh (1992) carried out morphometric analyses with common rats, *Rattus norvegicus caraco*, from seven localities in Korea and concluded that samples were more or less similar with one another, enough to be classified into a single subspecies: he also performed chromosomal analyses with *R. norvegicus caraco* from two localities in Korea and concluded that *R. norvegicus* is uniform in its chromosomal karyotype.

However, in this study with the analyses of mtDNA RFLPs with *R. norvegicus caraco* from Cheongju, Korea, three distinct subgroups were revealed (see Tables 1 and 2; Figs. 1 and 2): it is concluded that further analyses with additional samples from other localities are necessary to perform in order to clarify the taxonomic status of the distinct mtDNA subgroups.

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# 청주에 서식하는 집쥐〔*Rattus norvegicus caraco* Pallas (설치목, 포유강)〕의 미토콘드리아 DNA 절단단편의 변이

고 홍 선 · 노 용 석 · 김 상 복* · 유 병 선**

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## 적 요

한국의 청주에서 채집한 집쥐(*Rattus norvegicus caraco*) 40마리를 사용하여, 8개 제한효소로 절단한 미토콘드리아 DNA(mtDNA)의 단편들을 분석하였다. 총 36개의 절단단편들이 나타났고, 6개 mtDNA의 clone이 밝혀졌다. 여섯 mtDNA clone간의 nucleotide-sequence divergence(p)는 0.35-2.73%였으며, 이들 6개 clone은 3개 소군으로 나뉘어졌다. 한 소군은 3개 clone의 29마리였고, 다른 한 소군은 2개 clone의 열마리였으며, 나머지 소군은 한 clone의 한마리였다. 둘째와 셋째 소군은 첫째 소군과 *Pvu* II의 genotype이 달랐으며, 셋째 소군은 나머지 두 소군과 *Dra* I에 있어서 뚜렷한 차이를 보였다. 뿐만아니라, 한국산 집쥐를 사용한 본 연구의 결과로 밝혀진 최대 divergence는 Brown과 Simpson(1981)의 미국과 일본의 집쥐를 사용해서 연구한 최대값보다 컸다. 이들 뚜렷한 mtDNA 소군의 분류학적 위치를 규명하기 위하여 한국내 다른 지역의 더 많은 표본들을 사용한 계속적인 연구가 필요하게 되었다.