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Systematic Studies on Korean Rodents: VI. Analyses of Morphometric Characters, Chromosomal Karyotypes and Mitochondrial DNA in Two Species of Genus *Rattus*

Koh, Hung Sun
(Department of Biology, Chungbuk National University, Chongju 360-763, Republic of Korea)

한국에 서식하고 있는 설치류의 계통분류학적 연구 : 6. 집쥐속 2종의 형태학적 형질, 염색체 핵형및 미토콘드리아 DNA의 분석

고 흥 선
(충북대학교 생물학과, 청주 360-763)

적 요

한국에 서식하고 있는 집쥐속 2종 (곰쥐, *Rattus rattus* Linnaeus ; 집쥐, *Rattus norvegicus* Berkenhaut)의 채집된 표본들의 31개 형태적 형질들을 통계적으로 분석하여 종내 지리적 변이와 종간 차이를 규명하였다. 염색체 G-bands와 C-bands도 비교하였으며, 미토콘드리아 DNA의 제한효소에 의한 절단 단편들의 분석도 하였다.

한국내 여섯지역의 곰쥐들은 형태적 형질에 있어서 서로 비슷하였다 : 두동장, 미장, 염색체 핵형과 C-bands에 있어서는 일본산 곰쥐인 *Rattus rattus tanezumi*와 유사하였다. 한국내 일곱지역의 집쥐들은 형태적 형질에 있어서 서로 비슷하였다 : 염색체 핵형은 동부 아시아산 집쥐인 *Rattus norvegicus caraco*와 유사하였다. 집쥐와 곰쥐는 형태적 형질뿐만아니라, 염색체 핵형과 미토콘드리아 DNA도 차이가 있었다.

한국에 서식하고 있는 곰쥐의 옳바른 학명은 *Rattus rattus tanezumi*

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Temminck이고, 집쥐의 학명은 *Rattus norvegicus caraco* Pallas이며, 집쥐는 곰쥐와 다른 종임이 확인되었다.

Key words: morphometrics, chromosome, mtDNA, *Rattus rattus*, *R. norvegicus*, Korea.

INTRODUCTION

Developments in the areas of molecular, cyto-, and numerical taxonomy are enormous and there have been a conflict between molecular 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).

Genus *Rattus* is a large, confuging taxon composed of 61 species (Corbet and Hill, 1986). Schwarz and Schwarz (1967) included *Rattus norvegicus* as one of subspecies of *R. rattus*, but Corbet (1978) treated *R. norvegicus* as a good species because of its coexistance with *R. rattus* in many regions. Won (1967) noted that two species of genus *Rattus* inhabit in Korea (black rat, *R. rattus rufescens*; common rat, *R. norvegicus caraco* in the extreme northern part of the peninsula and *R. norvegicus norvegicus* in most region of the peninsula). However, Jones and Johnson (1965) described Korean black rat as *R. rattus flavipectus* and Korean common rat as *R. norvegicus caraco*. Corbet (1978) stated that *R. rattus flavipectus* is the synonym of *R. rattus tanezumi* and that common rat from west Palaearctic region is *R. norvegicus norvegicus*, whereas eastern Asian form of common rat is *R. norvegicus caraco*.

In chromosomal studies of genus *Rattus*, Yosida (1980) reported four karyotype variants in *Rattus rattus* from the world. Moreover, he noted that pericentric inversions of chromosome pair nos. 1, 9 and 13 in *Rattus rattus* resulted in the karyotype of *Rattus norvegicus*. Kang and Kim (1963) studied the karyotype of *R. norvegicus norvegicus* from an unstated locality in Korea. Koh (1982) analyzed the conventional, G-banded, and C-banded karyotypes of *Rattus rattus rufescens* from Chongju and Mt. Palgong in Korea. In mitochondrial DNA (mtDNA) analysis, Brown and Simpson (1981) reported that *R. norvegicus* comprises a group taxonomically distinct from any of the *R. rattus* subspecies.

In this paper, morphometric characters, chromosomal karyotypes, and mtDNA restriction patterns of samples in two species of genus *Rattus* from Korea were used to determine the range of variation within each species and differences between them in order to solve the taxonomic problems related with Korean rats.

MATERIALS AND METHODS

Phenetic analyses

Forty samples of black rats from six localities and 62 specimens of common rats from seven localities were trapped and aged into one of six age classes (Koh, 1991): age variation was found to be significant and the largest sample size was found in young adults. Therefore, 20 young adults of black rats and 29 young common rats were used for further phenetic analyses. Number of samples from six locality in black

rats were 12 from Chongju (OTU 1), 3 from Jochiwon (2), 2 from Jinchon (3), 1 from Chunchon (4), 1 from Inchon(5), and 1 from Ochang (6). Number of common rats from seven locality were 14 from Chongju (OTU 1), 1 from Bugang (2), 2 from Jochiwon (3), 1 from Daejon (4), 5 from Inchon (5), 5 from Jeju (6) and 1 from Gori (7). For the analyses of geographic variation within each species, individual measurements of each OTU were used and principal component analyses were carried out by the program PCAS of ISP.

For the analyses of interspecific difference, 12 black rats and 14 common rats from Chongju were used, and principal component analysis and discriminant analysis were performed by the program DISCRIM of SPSS/PC+.

Chromosomal analyses

Among 20 black rats and 29 common rats, which were used for phenetic analyses, three black rats and two common rats from Chongju were utilized for chromosomal analyses. Moreover, one black rat from Miwon and one common rat from Chunchon were also used. The bone-marrow *in vivo* method of Ford

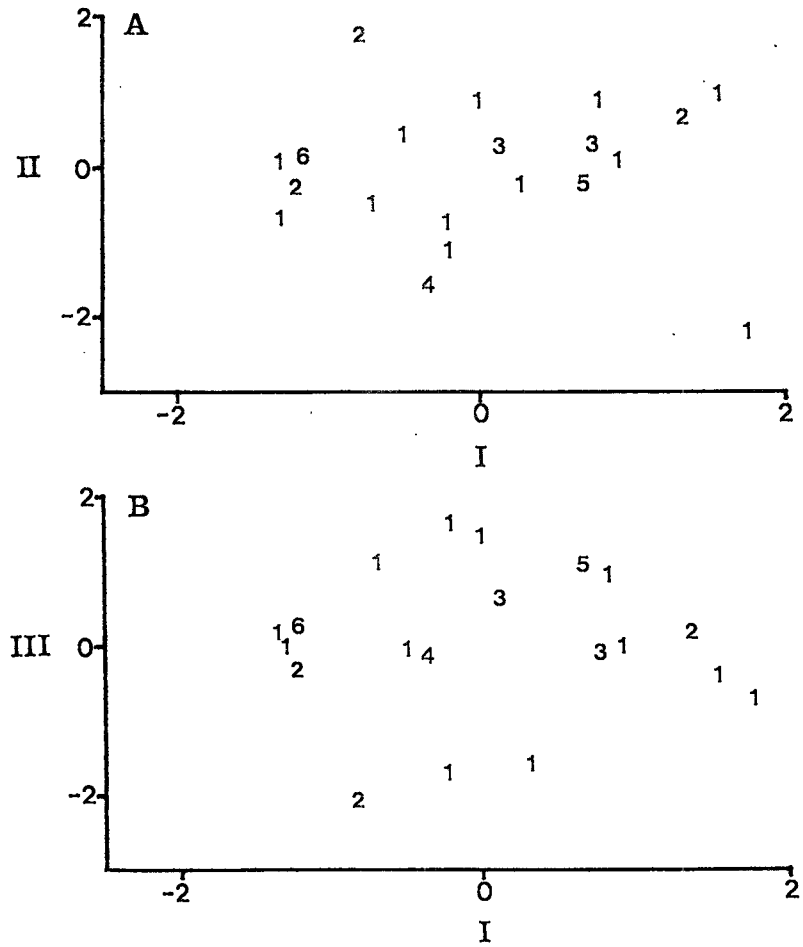

Fig. 1. Plottings of 20 young adults of black rats in Korea by principal component analysis in three dimensions. Numerals indicate samples of each OTU. Factors I, II and III represented 62, 12 and 7 per cent of the variance, respectively. A, samples ordinated with factor I vs. factor II. B, samples ordinated with factor I vs. factor III. For the locality of each OTU see Materials and Methods.

and Hamerton (1956) was used for the preparation of chromosome slides: Seabright's (1971) trypsin G-banding method to obtain G-banded chromosomes: and Sumner's (1972) method to get C-banded chromosomes [for details see Koh (1982)].

Mitochondrial DNA analyses

Three black rats and two common rats from Chongju, which were used for chromosomal analyses, were also utilized for mtDNA analyses. 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): eight restriction enzymes were *Stu* I, *Pst* I, *Eco*R V, *Bam*H I, *Hind* III, *Dra* I, *Pvu* II, and *Eco*R I. Sequence divergence in base substitutions per nucleoetide (p) in per cent were estimated by the method of Upholt (1977) and phenogram was constructed by Unweighted Pair Group Method with Arithmatic means, UPGMA (Sneath and Sokal, 1973).

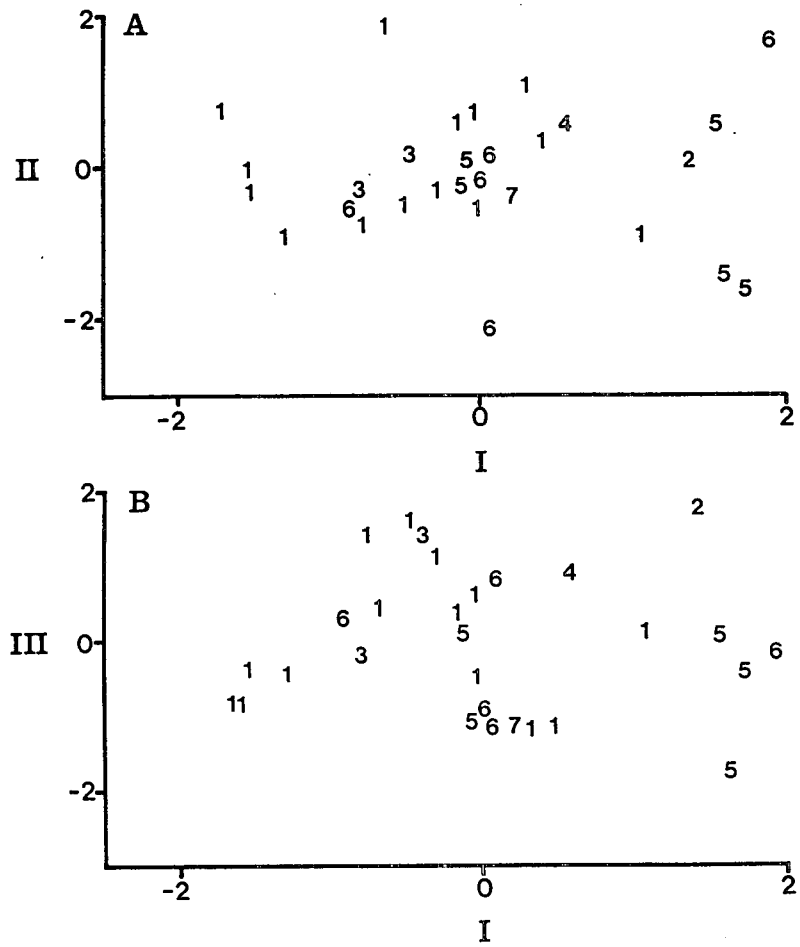

Fig. 2. Projections of 29 young adults of common rats in Korea by principal component analysis in three dimensions. Numerals indicate samples of each OTU. Factors I, II and III represented 72, 8 and 7 per cent of the variance, respectively. A, samples ordinated with factor I vs. factor II. B, samples ordinated with factor I vs. factor III. For the locality of each OTU see Materials and Methods.

RESULTS

Phenetic analyses

Two dimensional configurations from principal component analysis with 20 black rats are shown in Fig. 1. Samples of black rats from six localities are more or less similar with one another in their morphometric characters.

Two dimensional configurations from principal component analysis with 29 common rats are shown in Fig. 2. Five samples (1 of 1 from Bugang, OTU 2; 3 of 5 from Inchon, OTU 5; 1 of 5 from Jeju, OTU 6) are somewhat different from other 24 samples, but common rats from seven localities of Korea appeared to be similar with one another.

Two dimensional configurations from principal component analysis with 26 samples of two specis of

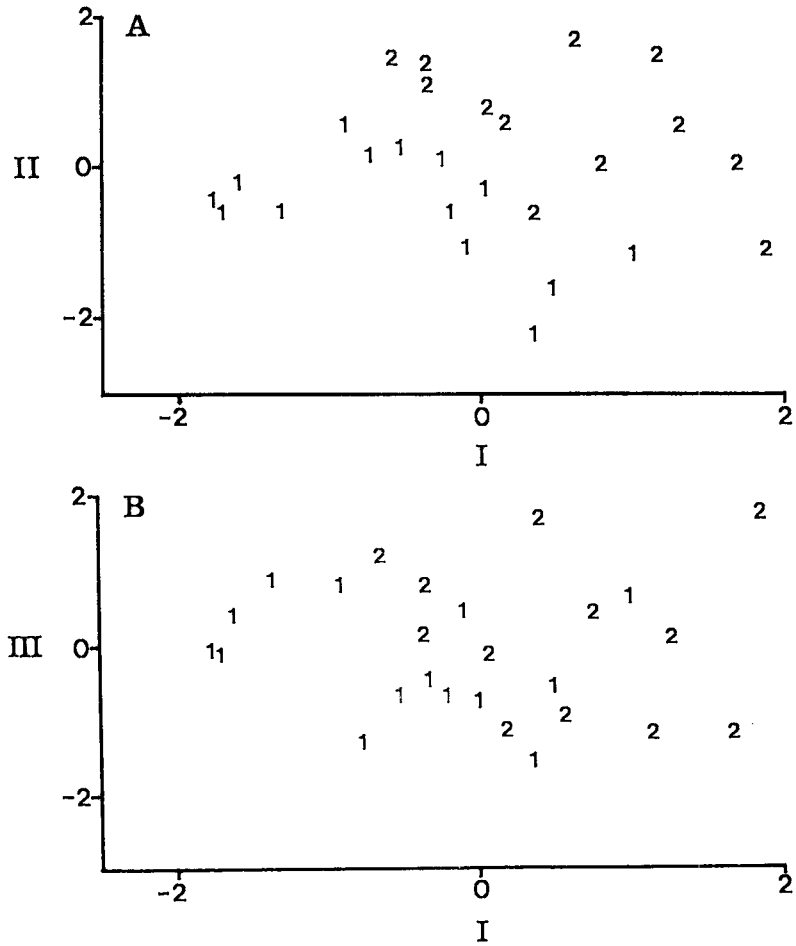

Fig. 3. Plottings with 26 young adults of two species of rats from Chongju by principal component analysis in three dimensions. Numerals 1 indicate 14 common rats and numerals 2 indicate 12 black rats. Factors I, II and III represented 68, 10 and 7 per cent of the variance, respectively. A, samples ordinated with factor I vs. factor II, B, samples ordinated with factor I vs. factor III.

rats from Chongju are shown in Fig. 3 (numerals 1 and 2 indicate 14 common rats and 12 black rats, respectively). Two dimensional plottings from discriminant analysis are shown in Fig. 4 (numerals 1 indicate 14 common rats and numerals 2 indicate 12 black rats). In summary, black rats seem to be larger than common rats.

Chromosomal analyses

Representative karyotype of black rat (K-120 from Chongju) is shown in Fig. 5 (pair nos. 1 to 13, acrocentric; nos. 14 to 20, metacentric; and X and Y, acrocentric). Pair no. 1 of the other three samples is also acrocentric. G- and C-banded patterns are the same as an author's former study (Koh, 1982) and the results are not shown here: the number of G-positive bands is 54 and C-positive regions near centromere are revealed in four of 13 acrocentric autosome pairs (nos. 1, 3, 9 and 12), all seven metacentric autosome pairs (nos. 14 to 20) and sex chromosomes.

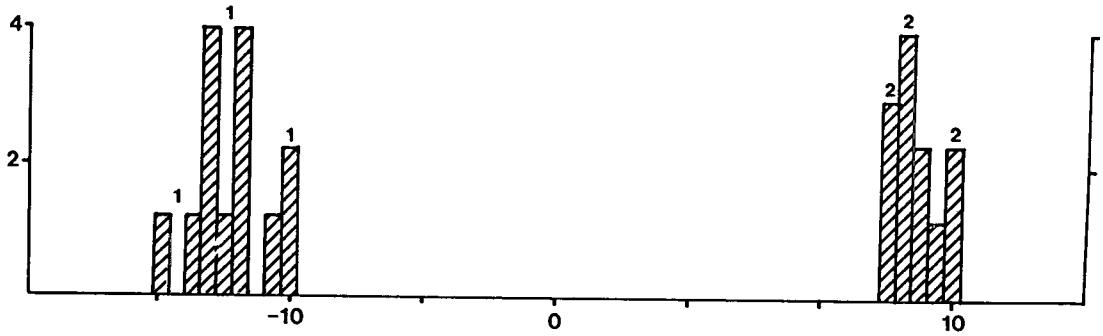

Fig. 4. Projections with 26 young adults of two species of rats from Chongju by discriminant analysis in one dimension. Factor I represented 100 per cent of the variance. Numerals 1 indicate 14 common rats and numerals 2 indicate 12 black rats.

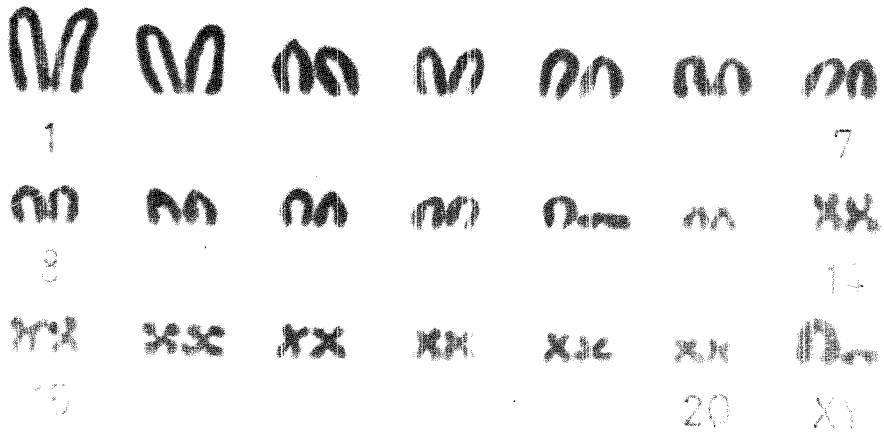

Fig. 5. Conventional karyotype of a black rat (K-120) from Chongju.

Representative karyotype of common rat (K-204 from Chongju) is shown in Fig. 6: G-banded karyotype (K-205 from Chongju) is in Fig. 7: and C-banded idiogram (K-309 from Chunchon) is in Fig. 8. Diploid chromosome number is 42 with the complement of three subtelocentric pairs (nos. 1, 9 and 13), ten acrocentric pairs (nos. 2 to 8 and 10 to 12) and seven metacentric pairs (nos. 14 to 20) and acrocentric X and Y chromosomes. Number of G-bands is 48 and C-positive regions near centromere are recognized in four of ten acrocentric pairs (nos. 7, 8, 11 and 12), three of seven metacentric pairs (nos. 14 to 16), two of three subtelocentric pair (nos. 9 and 13) and acrocentric X and Y chromosomes. Moreover, chromosome pair no.3 are polymorphic due to the addition of heterochromatin (K-204 from Chongju is acrocentric, but K-205 from Chongju and K-309 from Chunchon are acrocentric/subtelocentric).

It is also revealed that samples of black rats differ from those of common rats in pair nos. 1, 9 and 13 due to pericentric inversions, although samples of common rats are polymorphic in pair no. 3: they are different in C-bands.

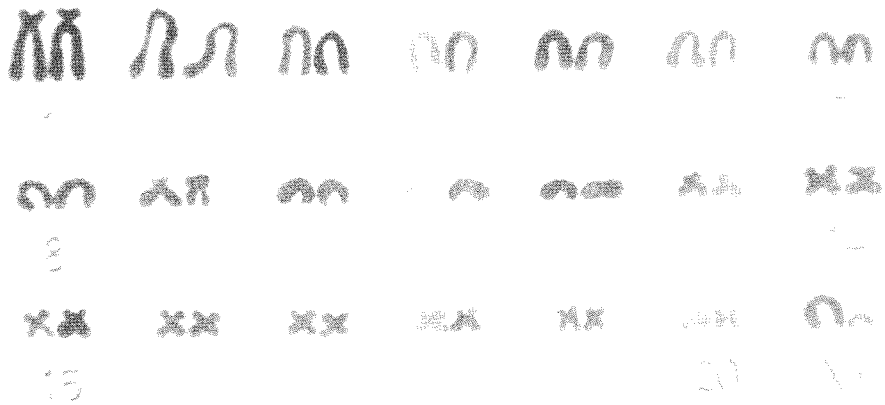

Fig. 6. Conventional karyotype of a common rat (K-204) from Chongju.

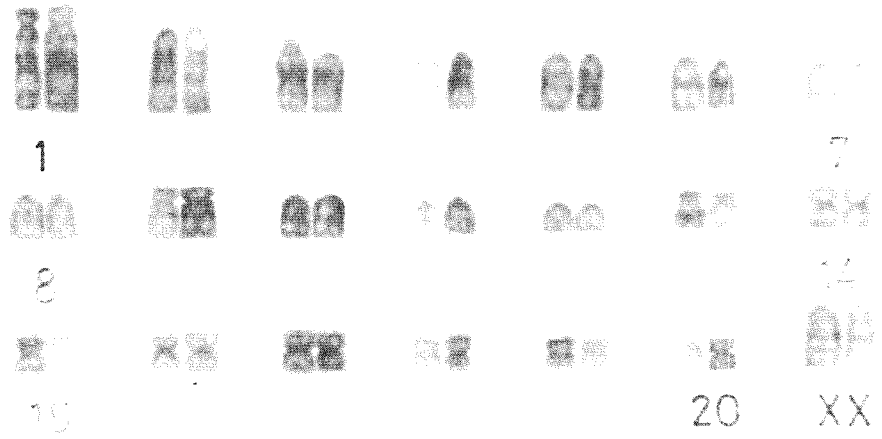

Fig. 7. G-banded chromosomes of a common rat (K-205) from Chongju.

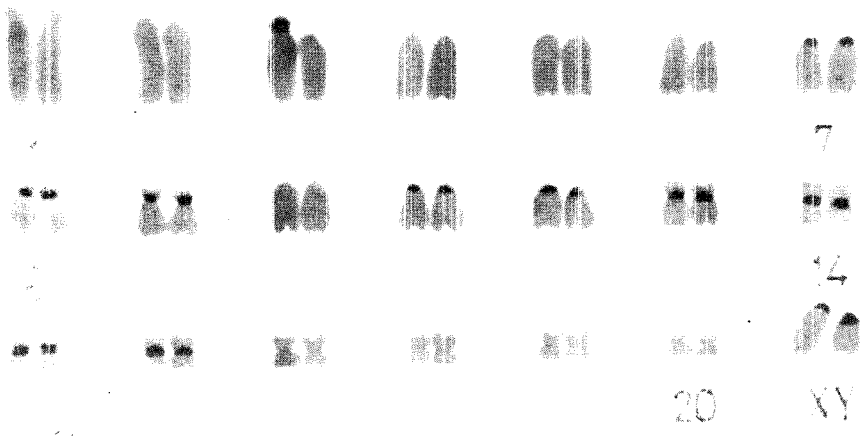

Fig. 8. C-banded chromosomes of a common rat (K-309) from Chunchon.

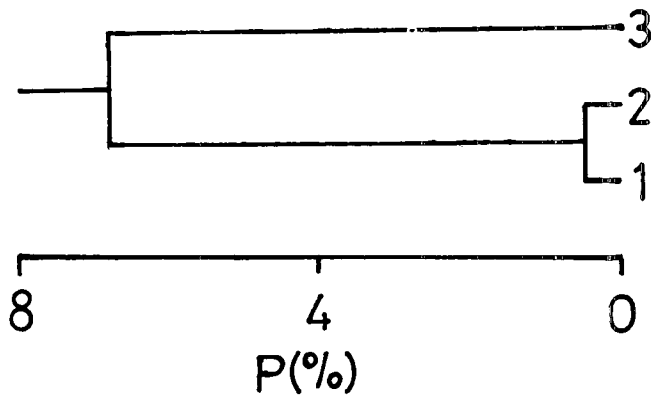

Fig. 9. Grouping of three mtDNA clones in two species of rats in Korea. The nucleotide-sequence divergences (p) in per cent were used for UPGMA cluster analysis. For the species name of each clone see Table 1.

Table 1. Specimens, mtDNA genotypes and mtDNA clones in two species of rats (*Rattus rattus* and *R. norvegicus*) from Chongju. Mitochondrial genotypes are based on the fragment patterns resulted from the digestion with eight restriction enzymes, *Stu* I, *Pst* I, *Eco*R V, *Bam*H I, *Hind* III, *Dra* I, *Pvu* II and *Eco*R I, in order.

Species	Specimen no.	mtDNA genotype	mtDNA clone
Rattus norvegicus	K-204	1 1 1 1 1 1 1 1	1
Rattus norvegicus	K-205	2 1 1 1 1 1 1 2	2
Rattus rattus	K-120, K-345, K-346	3 1 2 1 2 2 2 3	3

Mitochondrial DNA analysis

A total of 27 fragments were recognized and three clones were revealed, as shown in Table 1. Fig. 9 shows the grouping of three clones by UPGMA based on the nucleotide-sequence divergences. Clones 1 and 2 are samples of common rats from Chongju, whereas clone 3 is samples of black rats from Chongju: it is found that mean divergence value between two species is 6.7%. Fig. 10 shows the representative fragment patterns of the three mtDNA clones with *Stu* I and *Hind* III.

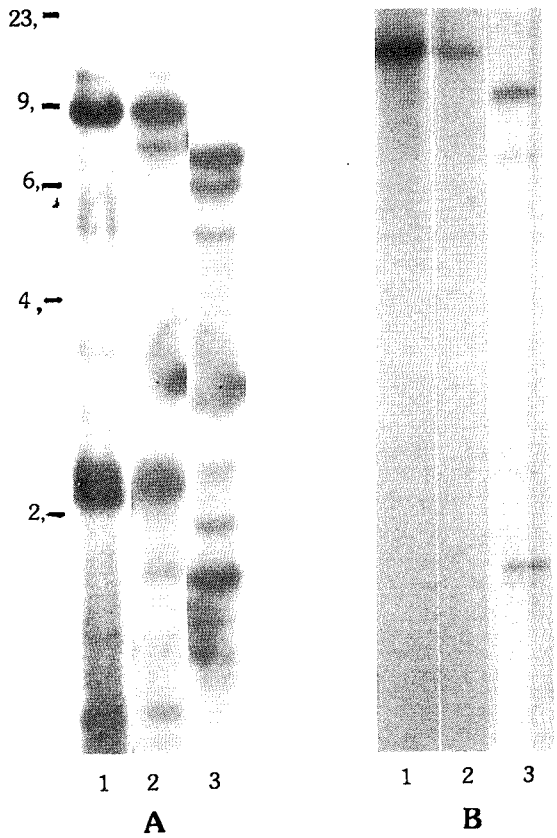

Fig. 10. Representative fragment patterns of three mtDNA clones in two species of rats from Chongju. Numerals indicate mtDNA clones: two species are black rat *Rattus rattus* (clone 3) and common rat, *R. norvegicus* (clones 1 and 2). The lane far left contains size markers indicated in base pairs. A, *Stu* I. B, *Hind* III.

DISCUSSION

Corbet (1978) clumped 29 nominal subspecies of black rats, *R. rattus*, into three ones (*rattus* in Mediterranean region to India, *tanezumi* in China and Japan, and *diardi* in E. Indies). Jones and Johnson (1965) described black rats in Korea as *R. rattus flavipectus*, but Tate (1947) noted that *R. rattus flavipectus* is the subspecies in Yunnan, Tibet and Laos. Moreover, Won (1967) wrote that Korean black rat is *R. rattus rufescens*, whereas it is the subspecies in India as stated by Jerdan (1989). On the other hand, Corbet (1978) stated that *R. rattus flavipectus* in south China is the synonym of *R. rattus tanezumi* in Japan.

Yosida (1980) compared standard external measurements of several subspecies of black rats, *R. rattus*. Head and body length (HBL) and tail length (TL) are 169.9±9.1 and 184.9±9.3 in *tanezumi* in Japan, 166.1±9.9 and 198.5±11.9 in *flavipectus* in Hong Kong and 166.7±3.7 and 213. 8±13.3 in *rufescens* in India, respectively. In chromosomal studies of black rats, Yosida and Sagai (1975) reported that diploid number of Indian black rats, *R. rattus rufescens*, is 38 and that all chromosome pairs showed a clear C-band near centromere. They also noted that diploid chromosome number in Hong-Kong black rats, *R. rattus rufescens* is 42, but pair no. 1 was always acrocentric and C-bands were observed in the centromere region of almost all of the chromosome pairs. On the other hand, karyotype of Japanese black rats, *R. rattus tanezumi*, consisted of 42 chromosomes with polymorphic pair no. 1: positive C-bands were revealed in nos. 3, 12 and 14 to 20: polymorphic C-bands in nos. 1, 4, 7, 9, 11 and 13: and negative C-bands

in 2, 5, 6, 8 and 10. Brown and Simpson (1981) detected the sequence divergence values of 0.2% to 9.6% among the eight mtDNA variants.

In this study with morphometric characters of black rats in Korea, 20 samples of black rats from six localities were more or less similar with one another in their external and cranial characters (Fig. 1). Head and body length and length of tail vertebrae of 20 black rats are 154.9 ± 3.2 and 167.3 ± 4.7, respectively. Moreover, Korean black rats from Chongju and Mt. Palgong showed pair no. 1 chromosome polymorphism (acrocentric/subtelocentric) due to pericentric inversion: C-banding analyses revealed positive bands in nos. 1, 3, 9, 12 and 14 to 20: and negative bands in nos. 2, 4, 5 to 8, 10, 11 and 13 (Koh, 1982; present study, Fig. 5). All three samples of black rats from Chongju are similar with one another in their mtDNA fragment patterns (Table 1). It is concluded that black rats from Korea are similar in morphometric characters and that they are comparable to *R. rattus tanezumi* in two external characters, conventional karyotype and C-banding patterns. Mayr and Ashlock (1991) noted that a subspecies is an aggregate of phenotypically similar populations of a species, differing taxonomically from other populations. It is confirmed that the subspecies name of black rats in Korea is *R. rattus tanezumi,* as already noted by Corbet (1978).

Corbet (1978) stated that common rats, *Rattus norvegicus,* distribute widely in the world and that two subspecies (*R. norvegicus norvegicus* in west Palaearctic and *R. norvegicus caraco* in eastern Asia) can be recognized. Won (1967) reported two subspecies of *R. norvegicus* in Korea (*norvegicus* in most region of Korean peninsular and *caraco* in the extreme northern part). But Jones and Johnson (1965) noted that only one subspecies, *caraco,* inhabits in entire peninsula. Kral (1971) reported that two subspecies of common rats (*R. norvegicus norvegicus, R. norvegicus caraco*) in Russia are uniform in their karyotype, i.e., seven metacentric, nine telocentric, four subtelocentric autosome pairs: the same karyotype was reported by Kang and Kim (1963) in *R. norvegicus norvegicus* from Korea. Yosida and Sagai (1973) stated that karyotype of *R. norvegicus caraco* in Japan is seven metacentric, ten telocentric and three subtelocentric autosome pairs. Yosida (1980) stated that C-bands in common rats were generally small in size: the bands of some pairs (nos. 4, 6 to 10, 12 and 14 to 16) were clear, while those of others (nos. 1 to 3, 5, 11, 13, 19 and 20) were indistinct. Brown and Simpson (1981) reported that sequence divergence values for eight detected mtDNA variants of *R. norvegicus* range from 0.2% to 1.8%.

In this study, it is found that in morphometric characters samples of common rats from seven localities appeared to be similar with one another enough to be recognized as a single subspecies (Fig. 2). Chromosomal karyotype of samples from Chongju and Chunchon are similar with each other, i.e., seven metacenetric, ten acrocentric and three subtelocentric autosome pairs (Fig. 6). C-bands of nine pairs (nos. 7 to 9, 11 to 16) are positive, whereas those of others (nos. 1 to 6, 10, and 17 to 20) are negative (Fig. 8) and sequence divergence of mtDNA between two samples from Chongju is 0.6% (Fig. 9). It is concluded that common rats in Korea is somewhat similar in their morphometric characters with one another and that in their conventional karyotype and C-bands they are comparable to *R. norvegicus caraco.* Therefore, it is confirmed that subspecies name of common rats in Korea is *R. norvegicus caraco,* as already stated by Jones and Johnson (1965).

Schwarz and Schwarz (1967) included *Rattus norvegicus* as one of subspecies of *R. rattus,* but Corbet (1978) noted that *R. norvegicus* is a distinct species; Yosida (1980) found that *R. rattus* and *R. norvegicus* are different in chromosome pair nos. 1, 9 and 13. Brown and Simpson (1981) reported in their mtDNA analyses that interspecific differences ranged from 13.7 to 18.4%. In this analyses with two species of *Rat-*

tus in Korea (*R. rattus tanezumi* and *R. norvegicus caraco*) from Korea, two subspecies are morphologically distinct (Figs. 3, 4). They differ in chromosome pair nos. 1. 9 and 12 (Figs. 5, 6), and mean sequence divergence between them is 7.0% (Fig. 9). And it is confirmed that *R. norvegicus* is distinct in its morphology, chromosomes and mtDNA from *R. rattus*.

ABSTRACT

Samples of two species of genus *Rattus* (black rat, *Rattus rattus* Linnaeus; common rat, *Rattus norvegicus* Berkenhaut) in Korea were trapped and their 31 morphometric characters were analyzed statistically in order to determine the range of geographic variation within each species and the interspecific differences. In addition, chromosomal G-bands and C-bands were compared and the fragment patterns of mtDNA resulted from the digestion with restriction enzymes were also analyzed.

Samples of black rats from six localities in Korea were similar with one another in their morphometric characters: in head and body length, length of tail vertebrae, conventional karyotype and C-bands, they are comparable to *Rattus rattus tanezumi* in Japan. Specimens of common rats from seven localities in Korea were similar with one another in their morphometric characters: in conventional karyotype, they are comparable to *Rattus norvegicus caraco* in eastern Asia. Common rats differ from black rats in their morphometric characters, chromosomal karyotypes and mtDNA.

It is confirmed that correct species name of black rat in Korea is *Rattus rattus tanezumi* Temminck: species name of common rat in Korea is *Rattus norvegicus caraco* Pallas: the common rat is a species, which is distinct from the black rat.

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