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G- and C-Banding Pattern Analyses of Korean Rodents. I. Chromosome Banding Patterns of Striped Field Mice (*Apodemus agrarius coreae*) and Black Rats (*R. rattus rufescens*).

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한국산 설치류의 G- 및 C-Banding Pattern의 분석 : I. 등줄쥐 (*Apodemus agrarius coreae*)와 곰쥐 (*R. rattus rufescens*)의 염색체 Banding Pattern에 관한 연구

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적 요

한국내 4개 지역에서 채집된 17마리의 등줄쥐 (*Apodemus agrarius coreae*)의 G-와 C-banding pattern을 분석한 결과 첫번째 염색체쌍의 다형현상 (차단부 및 단부)은 동원체 주위의 이질염색질이 어떤 때는 short arm의 형태로 나타나기 때문이라는 것이 밝혀졌다.

한국내 2개 지역에서 채집된 곰쥐 (*R. rattus rufescens*)의 G-와 C-banding pattern을 분석한 결과 첫번째 염색체쌍의 다형현상 (차단부및 단부)은 pericentric inversion에 기인한다는 것이 밝혀짔다.

INTRODUCTION

The role of chromosomal rearrangements in speciation has been intensively studied and discussed (Mayr, 1969; Jackson, 1971; Wilson *et al.*, 1975; Bush *et al.*, 1977; White, 1978). After the development of G- and C-banding techniques which make possible the precise identification of individual chromosomes and the comparison of chromosome complements between species (Lubs *et al.*, 1973; Schnedl, 1974), more reliable evolutionary relationships have been suggested for rodents, e.g., in *Mus* (Markvong *et al.*, 1975), *Neotoma* (Mascarello *et al.*, 1974), and *Peromyscus* (Greenbaum *et al.*, 1978). However, the nature of chromosome banding is still unknown (Comings, 1978).

In the genus *Apodemus*, supernumerary chromosomes were found in *A. specious* from

USSR (Kràl, 1971). Centric fusion was suggested to explain the chromosomal variation of *A. specious* from Japan (Shimba and Kobayashi, 1969). In *A. agrarius* No. 1 chromosome pair of Korean specimens were found to differ from that of European mice (Kang and Koh, 1976).

In the genus *Rattus*, Yosida and Sagai (1973) suggested that pericentric inversion results in the chromosomal variation among several species of this genus. In *R. rattus*, three chromosomal types were reported (Yosida *et al.*, 1974); the variations of C-bands were found (Yosida and Sagai, 1975); and supernumerary chromosomes were reported (Yosida, 1977). Furthermore, the polymorphism of No. 1 chromosome pair was noted in black rats (*R. rattus*) from Korea and Japan (Yosida, 1967) and pericentric inversion was suggested for the variation in Japanese black rats by the comparison of bandig patterns (Yosida *et al.*, 1974).

In this paper, G- and C-banding patterns of striped field mice (*Apodemus agrarius coreae*) and black rats (*R. rattus rufescens*) from Korea were analyzed to determine in No. 1 pair the types of chromosome polymorphism—due to pericentric inversion or addition of heterochromatin.

MATERIALS AND METHODS

Materials

Seventeen specimens of striped field mice (*Apodemus agrarius coreae*) from four populations and four specimens of black rats (*R. rattus rufescens*) from two localities in Korea were studied for conventional, G-banded, and C-banded karyotypes (for details, see Table 1).

Table 1. Localities and number of specimens studied.

Locality	*A. agrarius coreae*	*R. rattus rufescens*
Mt. Weolak	3[♀2(K-29, K-34); ♂1(K-37)]	
Cheongju	5[♀3(K-23, K-24, K-82); ♂2(K-42, K-44)]	3[♀1(K-28); ♂2(K-27, K-39)]
Mt. Taebaek	5[♀3(K-47, K-48, K-51); ♂2(K-46, K-56)]	
Mt. Palgong	4[♀1(K-71); ♂3(K-68, K-69, K-72)]	1[♀1(K-62)]

Samples were trapped with live traps in 1981 and were kept alive in cages before experiments were conducted. Skins and skulls of all specimens are in the collection of the author (Department of Biology, College of Natural Sciences, Chungbuk National University).

Methods for chromosomal preparation

The bone-marrow *in vivo* method of Ford and Hamerton (1956) were used with modification. Before bone-marrow cells from femora were washed with 7 ml of isotonic NaCl solution, 0.01 ml of 0.03% colchicine solution was injected and kept for 1 hour. The cell suspension was centrifuged at 700 rpm for 8 minutes and resuspended in 3 ml of hypertonic solution (0.075 M KCl) for 22 minutes. Two ml of fixative (3 methanol : 1 acetic acid) were added and cells were spun down at 1,000 rpm for 10 minutes. The fixation-centrifuge sequence was repeated at least twice. The air-drying method by Rothfels and Siminovitch (1957) was used for chromosome preparation.

Methods for conventional, G-banded, and C-banded chromosomes.

For conventionally stained chromosomes, slides were stained with 4% Giemsa solution (GIBCO) for 7 minutes, rinsed with distilled water and air-dried.

To obtain G-banded chromosomes Seabright's (1971) trypsin G-banding method was combined with Sumner *et al.*'s method (1971). Slides were dipped in 0.1% trypsin solution for 7 to 20 seconds at 5°C, rinsed with distilled water, dipped in 2X SSC (0.3 M sodium chloride and 0.03 M sodium citrate) solution at 60°C for 1 minute, rinsed again with distilled water, and stained with 4% Giemsa for 5 minutes.

Sumner's method (1972) was modified to obtain C-bands. Slides prepared by the air-drying method were treated in 0.2 N HCl for 20 minutes, rinsed briefly with distilled water, treated in 5% $Ba(OH)_2$ for 5 minutes, rinsed again with distilled water, incubated in 2X SSC solution at 60°C for 30 minutes, and finally rinsed with distilled water. Treated slides were stained for 10 minutes.

For sequential G-banded and C-banded chromosomes, G-banded slides were destained by a treatment consisting of xylol-absolute alcohol-50% acetic acid sequence.

Slides were dipped in each solution for 10 to 30 seconds. Destained G-banded slides were subjected to a reduced C-banding schedule as follows: 10 minutes in 0.2 N HCl; 5 minutes in 5% $Ba(OH)_2$, and 10 minutes in 2X SSC solution. Metaphases photographed for G-banded chromosomes were then rephotographed.

Methods for printing and idiograming

Metaphases were printed so that the largest chromosomes were similar in size (about 2 cm). First of all telo- and subtelocentric chromosomes were idiogrammed in order of decreasing length, and then submeta- and metacentric chromosomes were done. In addition, G-banded karyotypes of *R. rattus* (Yosida and Sagai, 1973) were compared with G-banded karyotypes of *R. rattus rufescens* in this study.

The chromosome nomenclature of Levan *et al.* (1964) was used as follows: metacentric with an arm ratio (long arm to short arm) of less than 1.9; submetacentric with an arm ratio between 1.9 and 3.0; subtelocentric with an arm ratio between 3.0 and 7.0; and telocentric with an arm ratio of more than 7.0.

RESULTS

Fig. 1 shows metaphase chromosomes of striped field mice, *Apodemus agrarius coreae*, and enlarged No. 1 chromosome pair (arrows indicate No. 1 chromosomes). Conventionally stained metaphases of a male (K-68) from Mt. Palgong area are shown in Fig. 1-a and 1-b; G-banded metaphase chromosomes of the same specimen as above are in Fig. 1-c and C-banded counterpart is in Fig. 1-d; C-banded metaphase of a male (K-46) from Mt. Taebaek area is in Fig. 1-e; and C-banded one of a female (K-23) from Cheongju is in Fig. 1-f. As shown in Fig. 1, No. 1 chromosomes were varied from telocentric to subtelocentric ones even in the same specimen; their G-banded patterns are similar with each other; C-positive region of telocentric No. 1 chromosome is near the centromere; and the short arm of the subtelocentric No. 1 chromosome is also C-positive (totally heterochromatic), indicating that centromeric heterochromatin of No. 1 chromosome pair sometimes appears to be recognized as short arm.

Fig. 2 indicates the haploid complement of black rats, *R. rattus rufescens*. Conventionally stained chromosomes of a male (K-27) from Cheongju are shown in Fig. 2-a; G-banded chromosomes of K-27 are in Fig. 2-b; diagrams of G-banded chromosomes shown in Fig. 2-b are in Fig. 2-c; G- and C-banded chromosomes of K-27 are in Fig. 2-d; and C-banded chromosomes of a female (K-62) from Mt. Palgong area are in Fig. 2-e. Conventionally stained, G-banded, and C-banded metaphases idiogrammed in Fig. 2 are shown in Fig. 3-a to 3-e (Fig. 2-a vs. Fig. 3-a; Fig. 2-b vs. Fig. 3-b; Fig. 2-d vs. Fig. 3-c and 3-d; and Fig. 2-e vs. Fig. 3-e). G- and C-banded No. 1 chromosome pair of K-27 and K-62 are shown in Fig. 3-f.

As shown in Fig. 2, black rats have the chromosome complements of 24 telocentric (No. 1 to 12), 2 subtelocentric (No. 13), and 14 meta- and submetacentric autosomes. X chromosome was telocentric with the same size as that of No. 3 and Y was telocentric with the similar size as No. 13 chromosome. C-banding pattern analyses revealed clear positive regions near the centromeric region in 4 telocentric pairs (No. 1, 3, 9, and 12), 7 meta- and submetacentric pairs (No. 14 to 20), and X chromosome.

As shown in Fig. 3-f, G- and C-banding pattern analyses revealed that No. 1 chromosome polymorphism (subtelocentric/telocentric) in black rate appears to be due to pericentric inversion. G-banded patterns of No. 1 pair (subtelocentric/telocentric) of K-62 are similar with each other; telocentric chromosome of No. 1 pair of K-62 has the same G- and C-banding patterns as those of No. 1 pair of K-27; and subtelocentric No. 1 chromosome of K-62 has no clear C-band region.

DISCUSSION

Kral (1970) reported that No. 1 chromosome of *A. agrarius* from USSR was telocentric. Kang and Koh (1976) noted that No. 1 chromosome of the same species mentioned above from Korea was subtelocentric. Subtelocentric No. 1 chromosome was also revealed in this paper, although telocentric No. 1 chromosome was also found in the same and different cells of the same specimen of K-68 (see Fig. 1). G- and C-banding pattern analyses showed that No. 1 chromosome polymorphism of this species is not due to pericentric inversion but due to the centromeric heterochromatin, i.e., heterochromatin sometimes appears as short arm.

Intercellular and individual karyotype variation has been notes in *Peromyscus* populations (Ohno *et al.*, 1966). Thomas (1973) reported that subtelocentric chromosomes appear in *Peromyscus* as telocentric types, depending upon the time which the DNA replication cycle was arrested, and named these chromosomes as "rabbit-eared" ones, although he had not studied C-banding patterns. Polymorphism due to centromeric heterochromatin was reported in deer mice, *Peromyscus maniculatus* (Koh, 1980). C-banding polymorphism was also noted in other rodents, i.e., *R. rattus* (Yosida, 1979) and *Neotoma microps* (Mascarello *et al.*, 1974). Considering that heterochromatin differs from euchromatin in heteropycnosis (White, 1948) and later replication (Barigozzi *et al.*, 1966) and that C-banded regions correspond to the regions of heterochromatin (Hsu, 1971), it needs further analyses that No. 1 chromosome variation of *A. agrarius* due to centromeric heterochromatin is resulted from the different amount of heterochromatin or from the different degree of constriction of heterochromatin.

Yosida and Sagai (1973) reported that No. 9 and 13 chromosome pairs in Japanese black rats were polymorphic in respect to the telo- and subtelocentrics. In this paper, it is found that No. 9 chromosome in four black rats was telocentric and that No. 13 chromosome was subtelocentric (see Fig. 2), although it needs further studies. In addition, Yosida and Sagai (1975) stated that chromosome pair No. 3, 4, 7, 9, 11, and 13 were polymorphic in regard to presence and absence of C-bands in Japanese black rats. In the present paper, No. 3 and 9 showed C-band and polymorphism was not recognized (see Fig. 2), although it also needs further analyses.

Yosida (1967) reported No. 1 chromosome polymorphism in black rats from Korea and Japan. Yosida *et al.* (1974) suggested in the analyses of G-banding pattern that pericentric inversion resulted in this polymorphism mentioned above in Japanese black rats. Yosida and Sagai (1975) reported C-banding patterns of No. 1 chromosome pair in Japanese black rats, i.e., telocentric chromosome with C-band near the centromere and subtelocentric one without C-band. In the present paper, it is found that No. 1 chromosome polymorphism in black rats from Korea is due to pericentric inversion and C-banding pattern of

No. 1 chromosome pair was the same with that of No. 1 chromosome pair in Japanese black rats (see Figs. 2 and 3). Chromosome polymorphism due to pericentric inversion was noted in other rodents, e.g., in *Peromyscus maniculatus* (Sparkes and Arakaki, 1971; Koh, 1980) and *Mastomys natalensis* (Matthey, 1966).

Considering the importance of chromosomal rearrangements such as pericentric inversion in speciation, as noted by White (1978), further studies with more specimens are needed to decide whether No. 1 chromosome polymorphism of *R. rattus* from Korea is fixed in some areas or not.

SUMMARY

G- and C-banding pattern analyses of striped field mice (*Apodemus agrarius coreae*) using 17 specimens from four localities in Korea revealed that centromeric heterochromatin results in the variation of No. 1 chromosome pair (telocentric/subtelocentric), i.e., centromeric heterochromatin sometimes appeared to be recognized as short arm.

G- and C-banding patterns of four black rats (*R. rattus rufescens*) from two localities in Korea showed that No. 1 chromosome polymorphism (telocentric/subtelocentric) is due to pericentric inversion.

In addition, G- and C-banding patterns of black rats mentioned above are idiogrammed.

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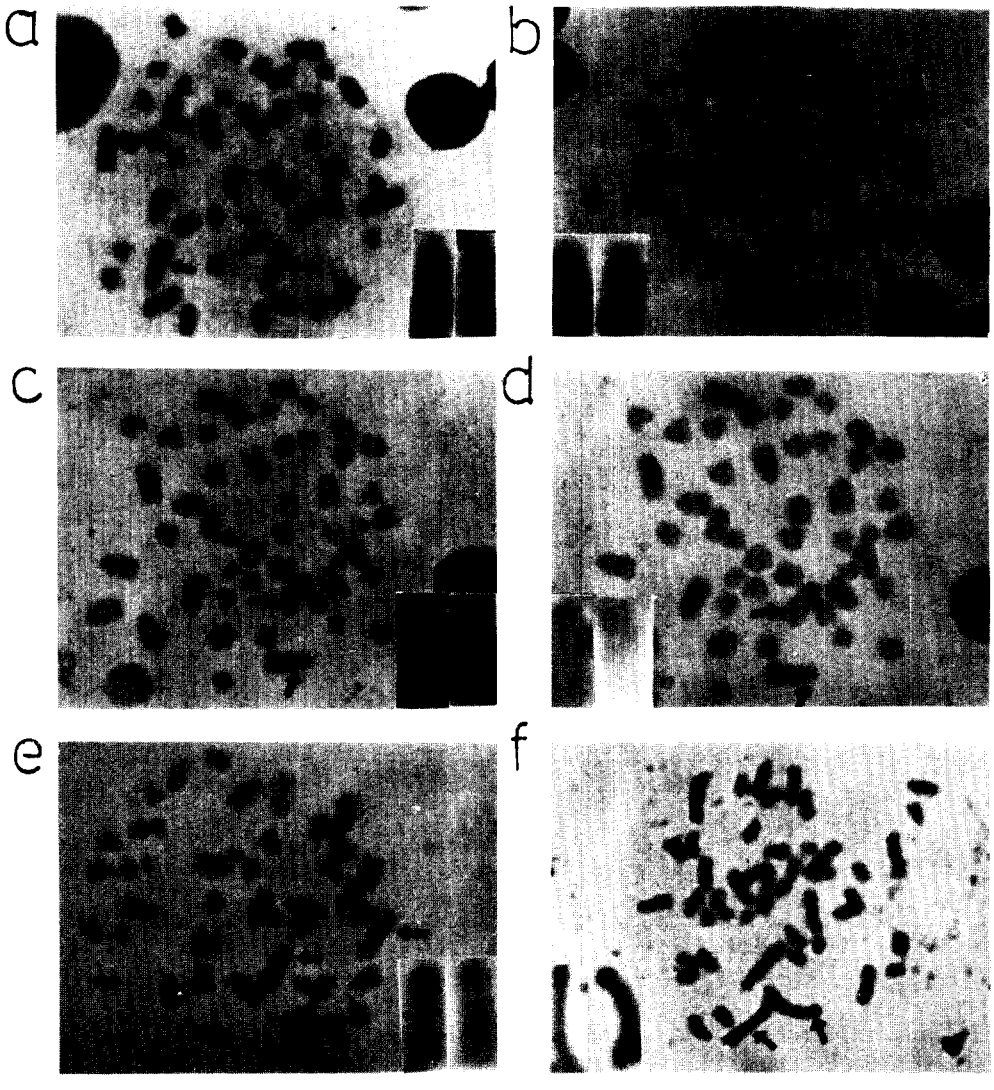
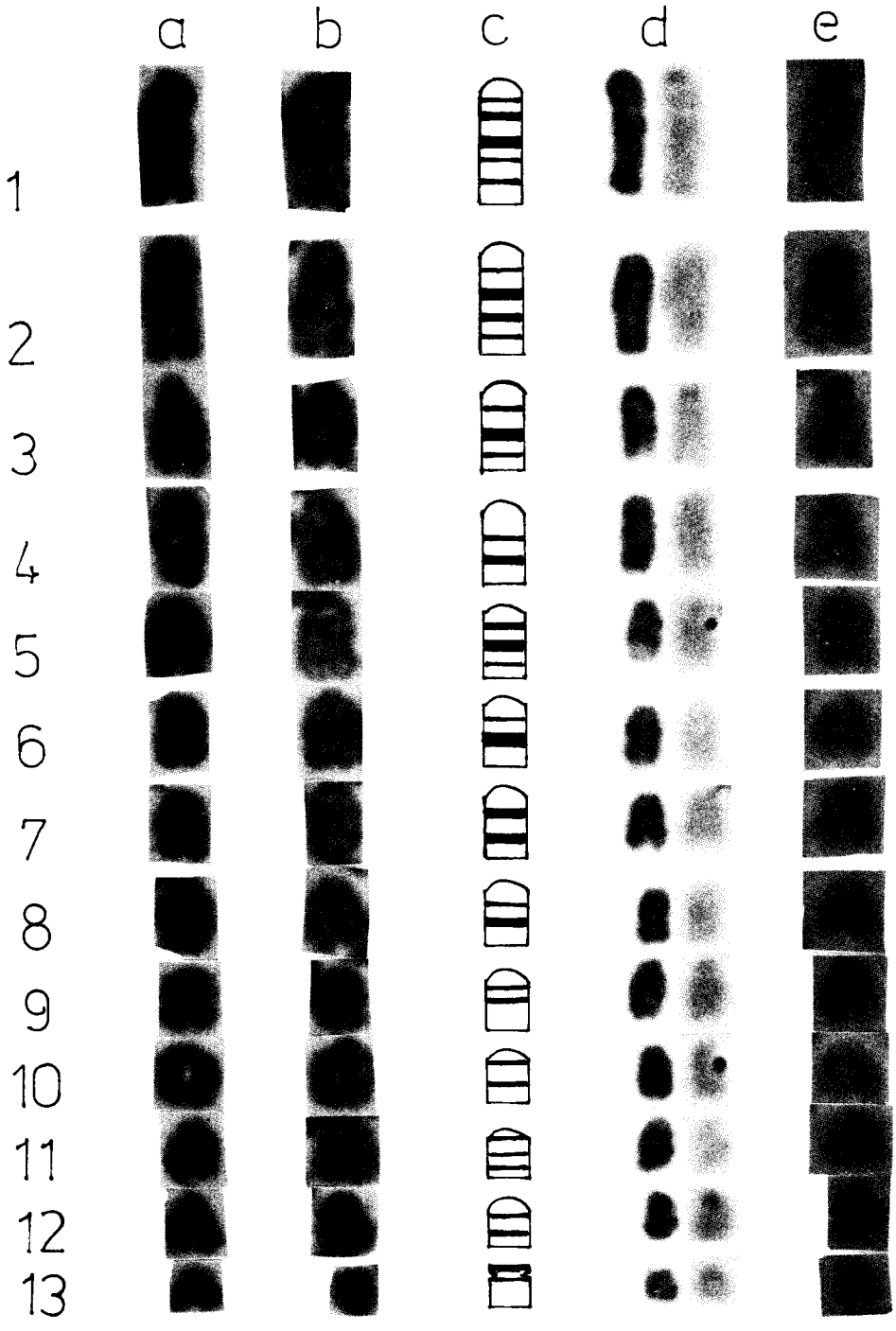


Fig. 1. Metaphase chromosomes of striped field mice, *Apodemus agrarius coreae*, and enlarged No. 1 pair. The arrows indicate No. 1 chromosomes. a) and b) Conventionally stained chromosomes of a male (K-68) from Mt. Palgong area. c) G-banded chromosomes of a male (K-68). d) C-banded counterpart of c). e) C-banded chromosomes of a male (K-46) from Mt. Taebaek area. f) C-banded metaphase of a female (K-23) from Cheongju.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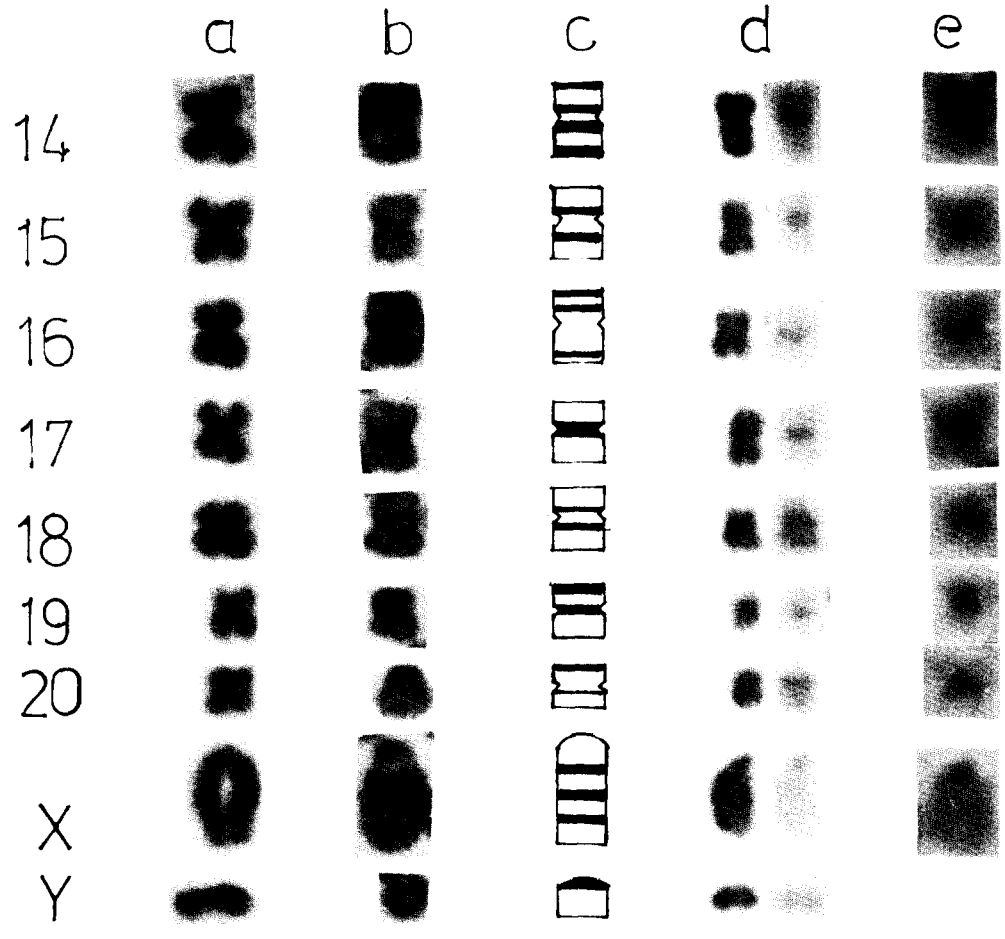

Fig. 2. The haploid complements of black rats, *R. rattus rufescens*. Idiogrammed metaphases are shown in Fig. 3. a) Conventional karyotype of a male (K-27) from Cheongju. b) G-banded karyotype of K-27. c) Diagram of G-banded karyotype in b). d) G-and C-banded karyotypes of K-27. e) C-banded karyotype of a female (K-62) from Mt. Palgong area.

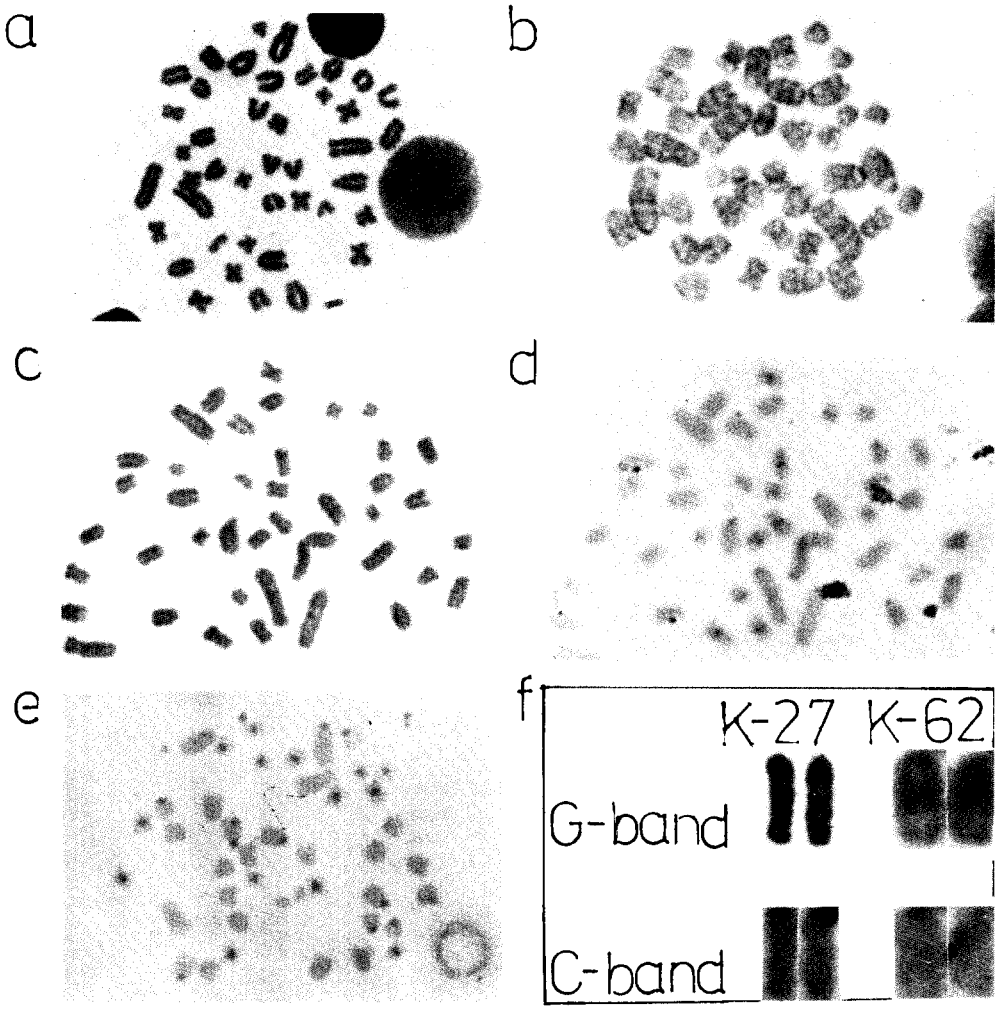

Fig. 3. Metaphase chromosomes of black rats, *R. rattus rufescens*, idiogrammed in Fig. 2 and enlarged No. 1 chromosome pair. a) Conventionally stained chromosomes idiogrammed in Fig. 2-a. b) G-banded chromosomes idiogrammed in Fig. 2-b. c) and d) G-and C-banded chromosomes idiogrammed in Fig. 2-d. e) C-banded chromosomes idiogrammed in Fig. 2-e. f) G-and C-banded No. 1 chromosome pair of K-27 from Cheongju and K-62 from Mt. Palgong area.