

Systematic Studies of Korean Rodents: IV. Morphometric and Chromosomal Analyses of two Species of the Genus *Apodemus* (Muridae).

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한국산 설치류의 계통분류학적 연구 : 4. 붉은쥐 속 2종의 염색체 및 형태적 형질의 분석

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적 요

한국에 서식하고 있는 붉은쥐속 2종, 등줄쥐 (*Apodemus agrarius coreae*, *A. agrarius chejuensis*) 와 흰넓적다리 붉은쥐 (*A. peninsulae peninsulae*)의 염색체 및 형태적 형질의 분석을 하였다.

*A. agrarius coreae*는 작은 형이고, *A. agrarius chejuensis*와 *A. peninsulae peninsulae*는 큰 형이었다. 또한 *A. agrarius chejuensis*는 *A. peninsulae peninsulae* 보다도 큰 편이었다.

*A. peninsulae peninsulae*에 있는 B chromosomes은 C-negative 즉 진정염색질임이 밝혀졌다.

Key words: *Apodemus*, systematic, chromosome, morphometric

INTRODUCTION

Most taxonomic works on Korean mammals were based on the description of pelage colour and external measurements of type specimens (Woon, 1967 and Corbet, 1978). However, age variation,

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secondary sexual variation and geographic variation of morphometric characters should be analyzed before species status is considered (Mayr, 1969). Chromosomal and electrophoretic analyses are also necessary to confirm species status (Koh, 1981).

The genus *Apodemus* is a fairly distinct genus of about 12 species and confined to the Palearctic and northern part of the Oriental regions: two species of it inhabit in Korea, i.e. Korean field mice, *Apodemus peninsulae* Thomas, and striped field mice, *A. agrarius* Pallas (Corbet, op. cit.).

Korean field mice, *Apodemus peninsulae* distribute from Altai to Ussuri through Manchuria and the type locality of this species is Mungyong, 110 miles of S.E. of Seoul, Korea (Jones, 1956). Vorontsov *et al.* (1977) described on the basis of morphometric and karyological data *A. peninsulae* as field mice of middle size with diploid chromosome number of 48 to 61 due to the presence of supernumerary chromosomes. Koh (1986a) analyzed chromosomes and morphometric characters of 5 samples of Korean field mice from Mungyong and confirmed that these samples are *A. peninsulae peninsulae*, as noted by Jones (op. cit.), rather than *A. speciosus peninsulae*, as suggested by Woon (1967).

Striped field mice, *Apodemus agrarius* is a distinct species in the genus *Apodemus*. It distributes from West Germany to Korea and most of subspecies were designated based on slight differences in pelage colour and/or mean body size (Corbet, op. cit.). Kral (1970) reported that the karyotype of *A. agrarius* from various localities of Eurasia is consisted of 40 acrocentric and eight metacentric chromosomes with diploid number of 48.

Jones and Jonson (1965) noted that four subspecies of *Apodemus agrarius* were recognized in Korea: *A. agrarius manchuria* in the extreme northern part, *A. agrarius coreae* throughout the major portion of the peninsula, *A. agrarius pallescens* in the coastal lowlands of southern and southwestern Korea, and *A. agrarius chejuensis* in Jeju-Do (Jeju island). Evident age variation and no significant secondary sexual variation were found from uni- and multivariate analyses with 31 morphometric characters of *A. agrarius coreae* from Cheongju (Koh, 1983). In morphometric analyses with samples of three subspecies of *A. agrarius* from ten localities of Korea it was found that *A. agrarius pallescens* is a synonym of *A. agrarius coreae* and that *A. agrarius chejuensis* differ from *A. agrarius coreae* (Koh, 1985; Koh, 1986 b; Koh, 1987). In chromosomal analyses with specimens of *A. agrarius* from ten localities of Korea the diploid number is 48 with the chromosomal complement of 40 acrocentric and eight metacentrics (Kang and Koh, 1976; Koh, 1982, and Koh, 1986b; Koh, 1987).

The objective of this paper is to analyze 31 morphometric characters and chromosomal karyotypes with samples of two species (*Apodemus agrarius coreae*, *A. agrarius chejuensis* and *A. peninsulae peninsulae*) from various localities of Korea in order to determine the range of variation within each species and difference between the two species. The degree of variation of morphometric characters among samples of different chromosomal types in *A. peninsulae peninsulae* is also analyzed by multivariate method.

MATERIALS AND METHODS

Materials

For morphometric analyses 279 samples of striped field mice from nine localities (247 specimens of *Apodemus agrarius coreae* from seven localities and 32 of *A. agrarius chejuensis* from two localities) were used. Thirty seven samples of Korean field mice (*A. peninsulae peninsulae*) from four localities were also used (for details see Table 1).

Table 1. Specimens examined. SA, YA, MA and OA indicate subadult, young adult, middle-aged adult and old adult respectively.

Species	Locality	Age classes				Total	OTU
		SA	YA	MA	OA		
<i>Apodemus agrarius coreae</i>	Chongju	37	43	42	9	131	1
	Mungyong	1	7	12	2	22	2
	Mt. Weolak	9	8	15	3	35	3
	Mt. Taebaek	4	9	9	3	25	4
	Mt. Seolak		4	2		6	5
	Mt. Palgong	3	4	9	4	20	6
	Kunsan			1	6	1	8
<i>Apodemus agrarius chejuensis</i>	Mosulpo		3	9	1	13	8
	Sanchondan		8	10	1	19	9
<i>Apodemus peninsulae peninsulae</i>	Mungyong		2	3		5	10
	Mt. Weolak		13	13	3	29	11
	Mt. Taebaek			1		1	12
	Mt. Seolak		1	1		2	13
Total		54	103	132	27	316	

For chromosomal analyses three samples of striped field mice from Mt. Weolak (*A. agrarius coreae*; K-317, K-321, K-331) and 15 specimens of Korean field mice from three localities (one from Mt. Taebaek, K-223; two from Mt. Seolak, K-291, K-292; 12 from Mt. Weolak, K-309, K-315, K-316, K-319, K-322, K-325, K-326, K-327, K-328, K-329, K-336, K-337) were utilized.

Samples were collected with live traps and specimens for chromosomal analyses were kept alive for a few days before chromosomal analyses were conducted. Skins and skulls of all specimens are in the collection of the author, Department of Biology, Chungbuk University, Chongju, Korea.

Chromosomal analyses

The bone-marrow *in vivo* method by Ford and Hamerton (1966) was used with modification. Before bone-marrow cells from femora were washed with 7 ml of isotonic NaCl solution, 0.03 ml of 0.03% colchicin solution was injected and kept for 8 minutes and kept for one hour. The cell suspension was centrifuged at 700 rpm for 8 minutes and resuspended in 7 ml of hypotonic solution (0.075 M KCl) for 22 minutes. Five ml of fixative (3 methanol: 1 acetic acid) were added and cells were spun down at 1,000 rpm for 1 minutes. The fixation-centrifuge sequences were repeated at least twice. The air-drying method by Rothfels and Siminovitch (1957) was used for chromosomal preparation.

For conventionally stained chromosomes, slides were stained with 4% Giemsa solution (GIBCO) for 7 minutes, and rinsed with distilled water and air-dried. Sumner's method (1972) was modified to obtain C-bands. Slides prepared by the air-drying method was 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. Good metaphases were photographed and printed so that the largest chromosomes were similar in size (about 1cm).

Morphometric analyses

Analyses were based on four external and 27 cranial characters as follows (for details see Koh, 1983): 1, greatest length of the skull; 2, condylobasal length; 3, length between incisor and incisive foramen; 4, length of the nasal bone; 5, zygomatic width; 6, mastoid width; 7, width of brain case; 8, height of brain case; 9, width between infraorbital canals; 10, length of rostrum; 11, length of hard palate; 12, interorbital constriction; 13, width across upper third molars; 14, incisor-upper-first-molar length; 15, width across upper first molars; 16, length of incisive foramen; 17, width of the interparietal bone; 18, length of the interparietal bone; 19, postpalatine length; 20, height of rostrum; 21, bullae-brain case height; 22, greatest length of mandible; 23, length of mandibular tooth row; 24, height of mandibles; 25, length of ramus; 26, length of upper third molars; 27, length of upper first molar; 28, length of tail vertebrae; 29, length of hind foot; 30, body length; 31, length of ear.

In striped field mice sexual variation was not significant in morphometric characters, whereas age variation was reported to be significant (Koh, 1983). Therefore, each specimen was assigned to one of five age classes (juvenile, subadult, young adult, middle-aged adult, and old adult) based on the eruption of upper third molar, degree of tooth-wear, and pelage colour (for details see Koh, 1983). All 279 samples of striped field mice were classified into 54 subadults, 87 young adults, 114 middle-aged adults, and 24 old adults and all 37 specimens of Korean field mice into 16 young adults, 18 middle-aged adults, and 3 old adults.

In morphometric analyses with two species, samples of middle-aged adults, which show the largest number of specimen among three adult age classes, were used. However, to analyze morphological variation among karyological morphs in *A. peninsulae peninsulae*, young adults were also used together with middle-aged adults.

Although sufficiently large samples were not available for each locality to be used as a basic unit, samples of the same species collected from each locality were grouped as Operational Taxonomic Units, OTU's (see Table 1).

All computations were made using the Chungbuk University HP-3000/48 computer. In each species sample statistics such as standard deviation, skewness, and kurtosis were carried out by ELEMSTAT program of Interactive Statistical Packages, ISP, with morphometric characters of samples in each age class.

For the analysis of morphometric variation between two species (13 OTU's), principal component analysis, PCA (Seal, 1964), was performed with the individual measurements of 31 characters using subprogram PCAS of ISP: the data were singular. Therefore, 13 characters (1, 3, 4, 6, 9, 13, 16, 19, 20, 22, 25, 28, and 30), showing significant difference among means in the analyses with the four OTU's of *A. agrarius coreae* (see Koh, 1983), were selected and used for PCA (the means of 13 selected characters were also used and the measurements from the OTU's with one sample were considered as the means of each OTU). Discriminant analysis with the measurements of 31 characters of two species was also carried out by DISCRIM of Statistical Packages for the Social Sciences, SPSS (Nie *et al.*, 1975).

For a cluster analysis, raw data were standardized using Sokal's equation (1961) and phenogram was constructed with the average taxonomic distances based on standardized means of 13 OTU's by Unweighted Pair Group Method using Arithmetic Averages, UPGMA (Sneath and Sokal, 1973).

For the analysis of morphometric variation among karyological morphs in *A. peninsulae peninsulae*, PCA was carried out.

RESULTS

Diploid chromosome number and chromosome frequency of striped field mice from Mt. Weolak are shown in Table 2. The representative karyotype of K-331 is shown in Fig. 1. The diploid number of samples from Mt. Weolak was 48 (40 acrocentric and 8 metacentric chromosomes).

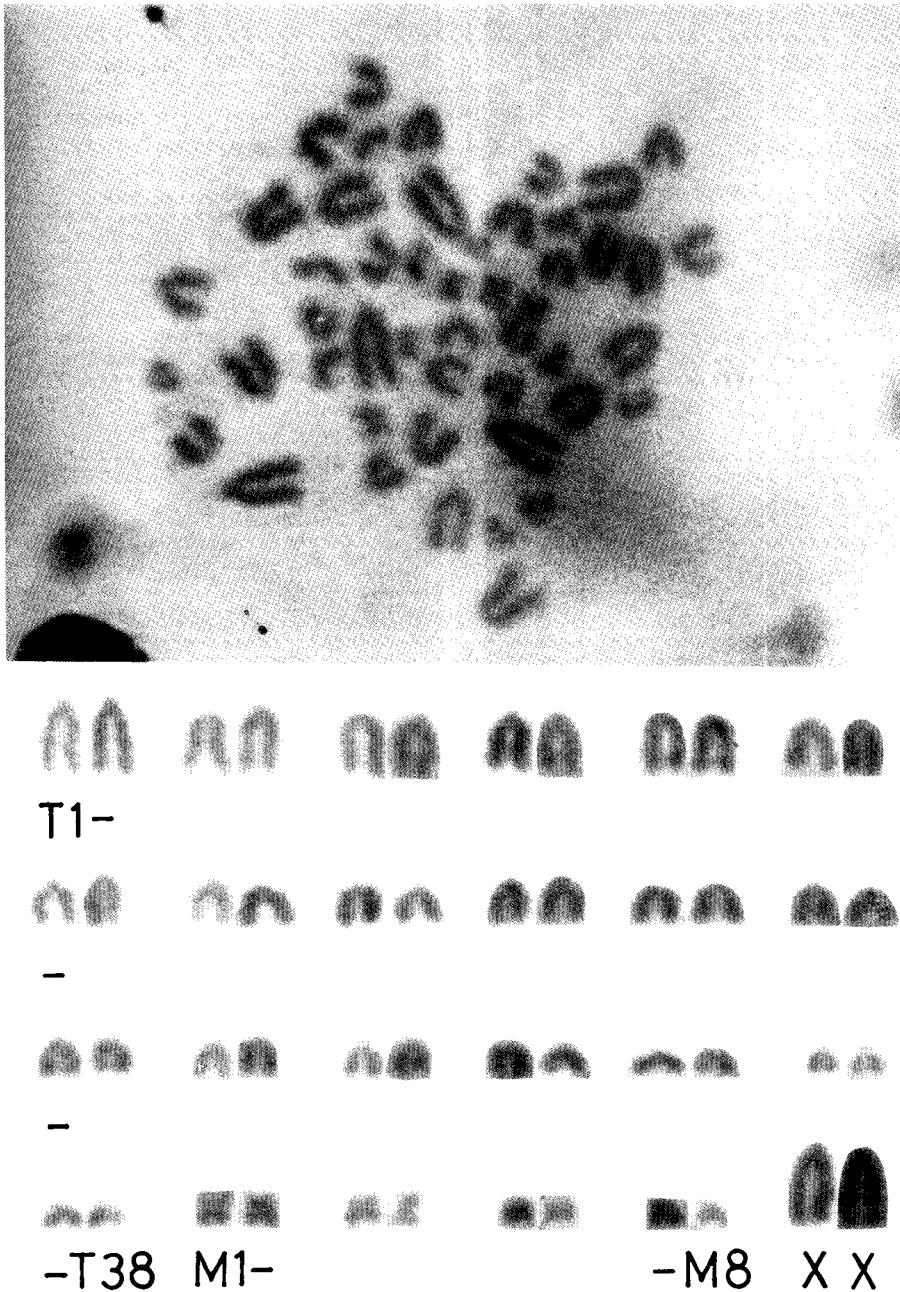


Fig. 1. Karyotype of a striped field mouse (K-331, ♀), *Apodemus agrarius coreae*, from Mt. Weolak.
T an M indicate acrocentric and metacentric chromosomes, respectively.

Table 2. Summary of chromosomes counted in three samples of *Apodemus agrarius coreae* from Mt. Weolak.

Specimen no.	Frequency of chromosome counted					2n
	46	47	48	49	50	
K-317		1	17	1		48
K-321		2	15	1	1	48
K-331	1		21			48

Table 3. Summary of chromosomes counted in 15 samples of *Apodemus peninsulae peninsulae* from three localities.

Locality	Specimen no.	Chromosome frequency					2n
		48	49	50	51	52	
Mt. Taebaek	K-223		13	1			49
Mt. Seolak	K-291		1	9	1		50
	K-292			12	1		50
Mt. Weolak	K-309	1	16				49
	K-315		18	1			49
	K-316			1	13		51
	K-319	1	23	1			49
	K-322			1	8		51
	K-325			14			50
	K-326		1	15			50
	K-327	1	12	1			49
	K-328			1	21		51
	K-329		15				49
K-336		2	21			50	
K-337		5				49	

Diploid chromosome number and chromosome frequency of 15 Korean field mice from three localities are shown in Table 3. The representative standard and C-banded karyotype of K-315 and K-328 are shown in Figs. 2 and 3, respectively. Diploid chromosome number varied from 49 to 51, indicating that supernumerary chromosomes varied from 1 to 3. Moreover, clear positive bands were appeared near the centromeric regions of all acrocentrics. X chromosomes show strong positive bands near the centromeric region, and Y chromosomes are heterochromatic. Supernumerary metacentric chromosomes appeared to be euchromatic (C-negative).

Two dimensional configurations from PCA with the means of 13 selected characters of 13 OTU's are shown in Fig. 4 (numerals indicate OTU numbers). The correlations between original characters and the principal components are given in Table 4 (factors I, II, and III represented 73, 9, and 8 per cent of the variance, respectively). Two dimensional configurations from PCA with the measurements of 13 selected characters of 132 specimens (13 OTU's) are shown in Fig. 5 (numerals indicate OTU numbers and factors I, II, and III represented 58, 7, and 6 per cent of the variance, respectively).

Two dimensional plotting from discriminant analysis with the measurements of 31 characters are

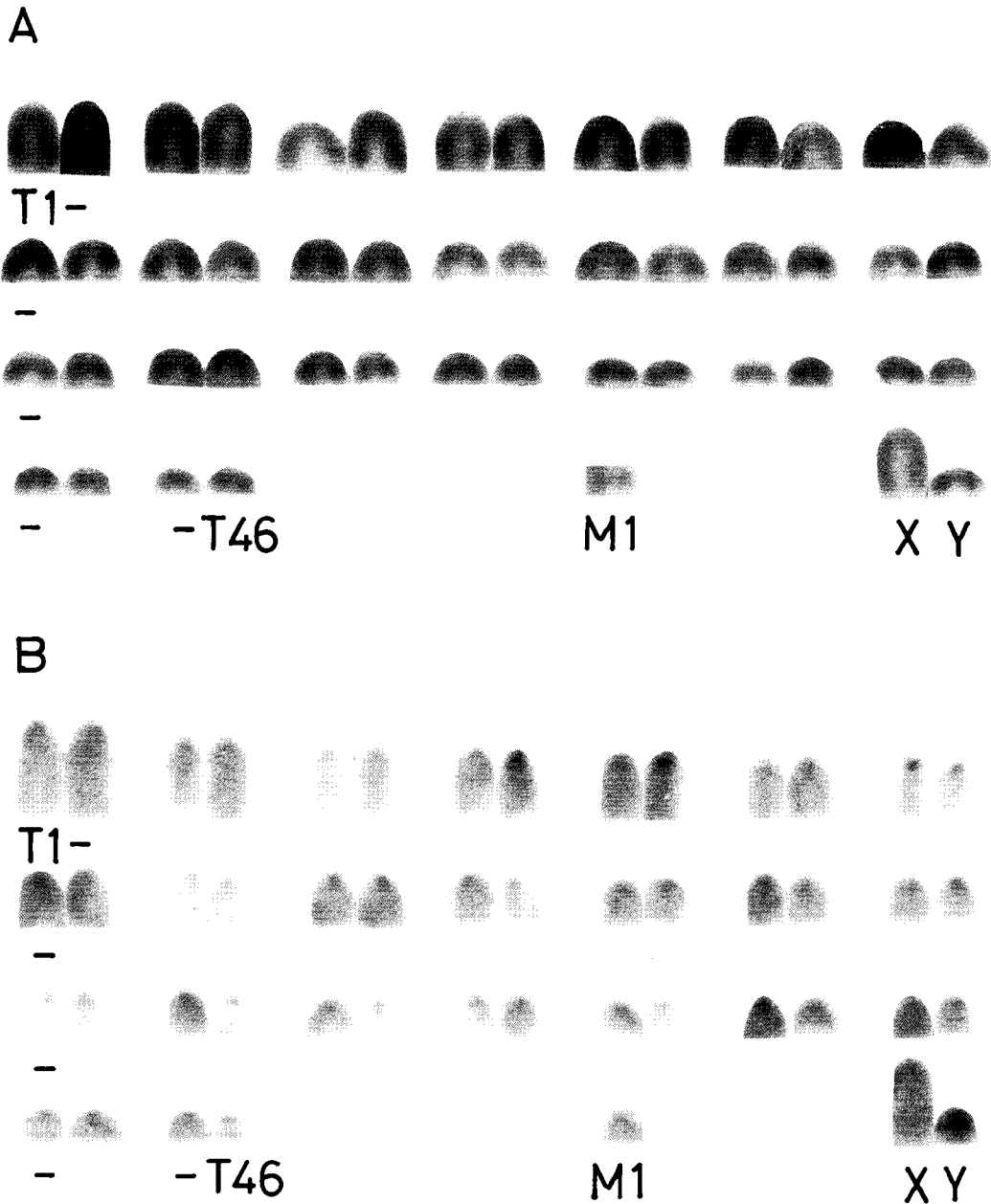


Fig. 2. Standard idiogram (A) and C-banded idiogram (B) of a Korean field mouse (K-315, ♂, 2n 49), *Apodemus peninsulae peninsulae*, from Mt. Weolak. T indicate acrocentric chromosomes: B, metacentric, supernumerary chromosomes: and X and Y, sex chromosomes.

shown in Fig. 6 (numerals indicate OTU numbers). The correlations between original characters and canonical discriminant functions are given in Table 5 (functions I and II represented 49 and 23 per cent of the variance, respectively). The 13 OTU's of two species of the genus *Apodemus* were also grouped by UPGMA cluster analyses as shown in Fig. 7.

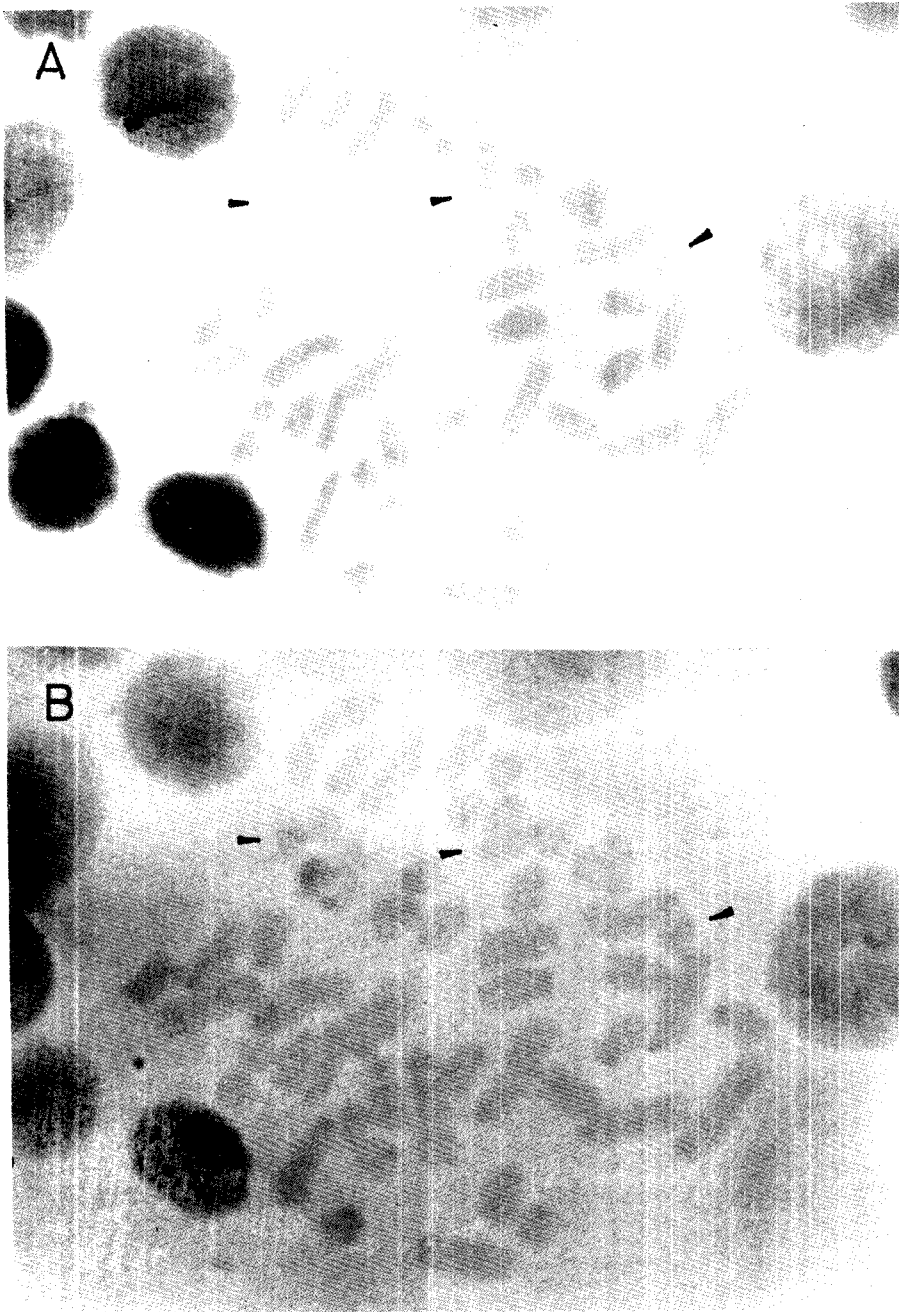


Fig. 3. Standard metaphase (A) and C-banded metaphase (B) from a Korean field mouse (K-328, ♀, $2n=51$), *Apodemus peninsulae peninsulae*, from Mt. Weolak. Arrows indicate B chromosomes. The heterochromatin is limited to small centric blocks of the chromosomes.

In conclusion, two groups appeared to be evident: a small-size group of seven OTU's (OTU's 1, 2, 3, 4, 5, 6, and 7; *A. agrarius coreae*) and a large-size group of six OTU's (OTU's 8 to 13; *A. agrarius chejuensis* and *A. peninsulae peninsulae*). Within the large-size group two subgroups recognized:

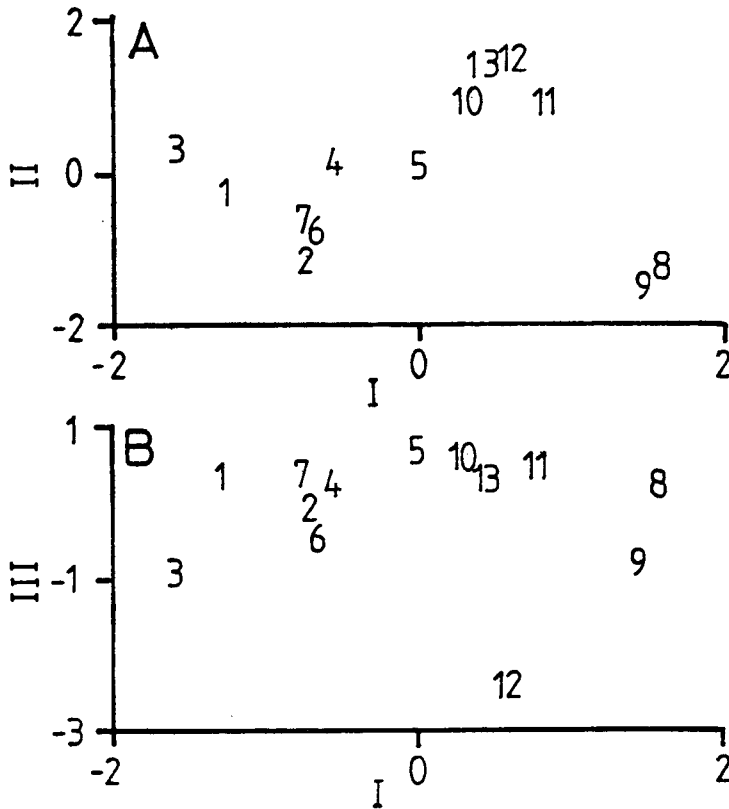


Fig. 4. Projections of 13 OTU's of two species of *Apodemus* based on principal component analysis in three dimensions. The means of 13 selected characters were used. (Factors I, II and III represented 73, 9 and 8 per cent of the variance, respectively). Numerals indicate OTU number. For species name and locality of each OTU see Table 1. A, OTU's ordinated with factor I vs. factor II. B, OTU's ordinated with factor I vs. factor III.

Table 4. Principal components I, II and III expressed as correlations between characters and individual components from an analysis of two species of *Apodemus*. The means of 13 selected characters from 13 OTU's were used.

Character	Factor I	Factor II	Factor III
1	0.97	-0.09	0.10
3	0.95	0.20	0.17
4	0.89	-0.89	0.28
6	0.75	0.48	-0.14
9	0.86	0.21	-0.04
13	0.67	0.10	-0.62
16	0.79	-0.30	-0.48
19	0.65	-0.73	-0.13
20	0.94	0.03	0.16
22	0.94	0.30	-0.00
25	0.90	0.01	-0.23
28	0.84	0.02	0.29
30	0.85	-0.28	0.39
% of trace	73	9	8

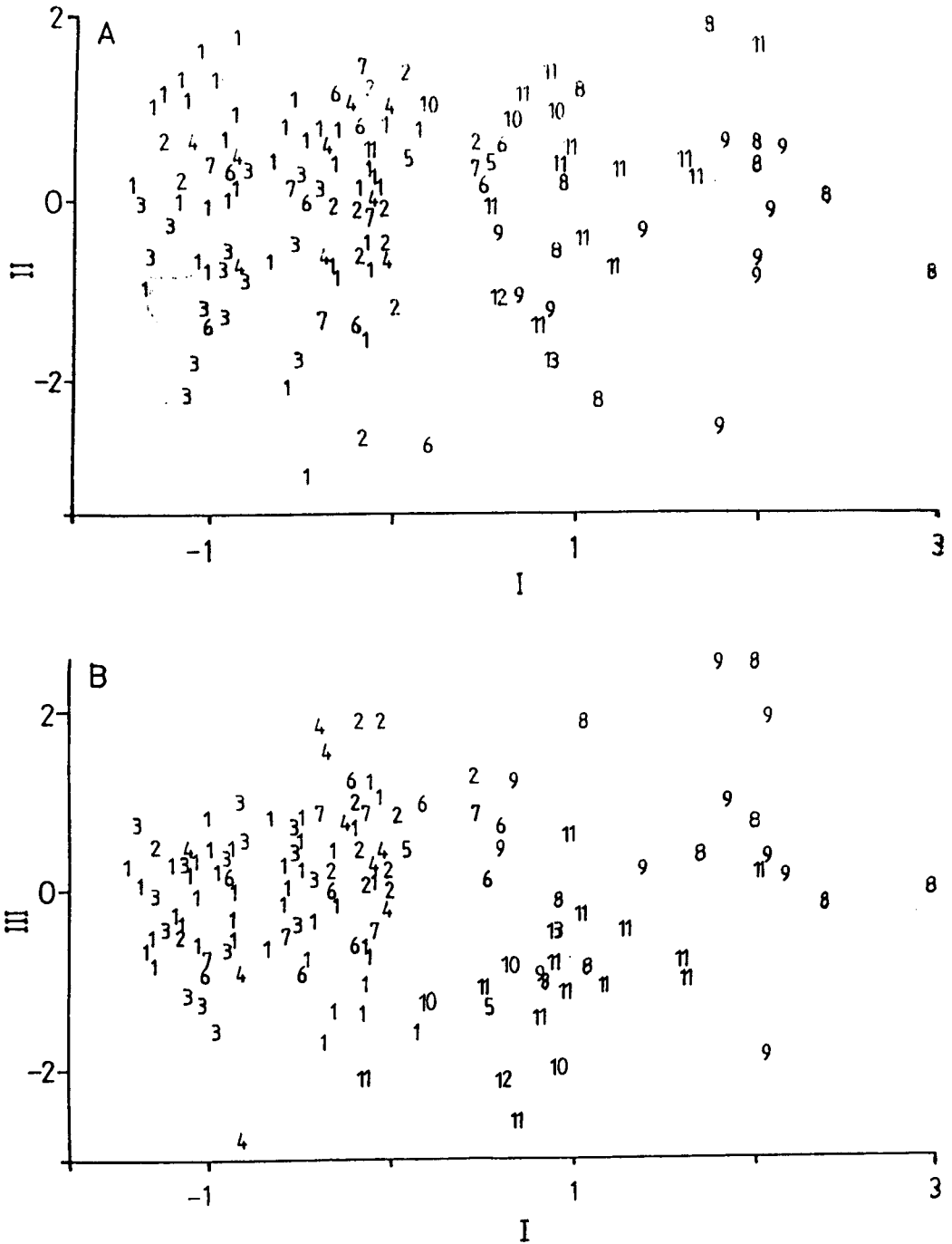


Fig. 5. Projections of 132 samples of two species of *Apodemus* based on principal component analysis in three dimensions. The individual measurements of 13 selected characters were used. Factors I, II and III represented 59, 7 and 6 per cent of the variance, respectively. Numerals indicate OTU number. For species name and locality of each OTU see Table 1. A, Samples ordinated with factor I vs. factor II. B, samples ordinated with factor I vs. factor III.

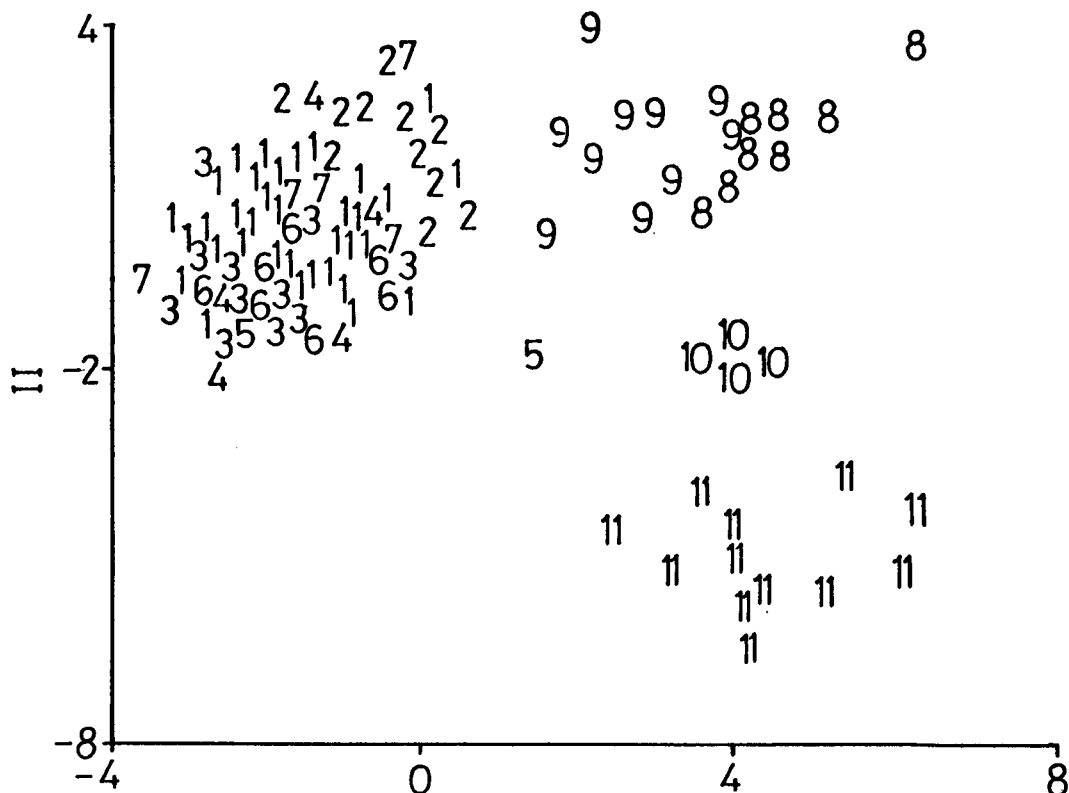


Fig. 6. Projections of samples of two species of *Apodemus* on the basis of discriminant function analysis in two dimensions. The individual measurements of 31 characters were used. Functions I and II represented 49 and 23 per cent of the variance, respectively. Numerals indicate OTU number. For species name and locality of each OTU see Table 1.

subgroup I of two OTU's (OTU's 8 and 9; *A. agrarius chejuensis*) is larger than subgroup II of four OTU's (OTU's 10 to 13; *A. peninsulae peninsulae*).

Two dimensional plottings from PCA with individual measurements of 13 selected characters in 34 young and middle-aged adults of *A. peninsulae peninsulae* are shown in Fig. 8 (large circle, small circle, large rectangle, and small rectangle indicate samples from OTU's 10, 11, 12, and 13, respectively: young adults are shown with unshaded circle and rectangle, whereas middle-aged adults are shown with shaded circle and rectangle: numerals indicate chromosome number of specimen studied). The correlation between original characters and the principal components are given in Table 6 (factors I, II, and III represented 43, 12, and 8 per cent of the variance, respectively). A discrete pattern of variation was not revealed, indicating that the presence of supernumerary chromosomes seemed not to effect on the difference of morphometric characters.

DISCUSSION

Sneath (1966) and Boyce (1969) found that average linkage or UPGMA represents a distance matrix of random points better than either complete or single linkage. The relationships between close

Table 5. Discriminant functions I and II expressed as correlations between characters and individual functions from an analysis with two species of *Apodemus*. The 31 characters of middle-aged adults were used.

Character	Function		Character	Function	
	I	II		I	II
1	0.44	0.56	17	-0.34	0.51
2	0.32	0.28	18	0.29	-0.01
3	-0.26	0.03	19	-0.72	0.32
4	0.01	-0.31	20	0.06	-0.14
5	-0.12	0.16	21	-0.10	0.18
6	0.31	-0.40	22	0.46	-0.15
7	0.23	-0.11	23	-0.04	0.31
8	-0.12	0.22	24	-0.14	-0.17
9	-0.11	-0.29	25	0.21	0.16
10	-0.24	-0.40	26	0.27	-0.08
11	-0.50	-0.04	27	-0.00	0.17
12	0.11	0.14	28	-0.06	-0.11
13	0.41	-0.05	29	-0.17	-0.02
14	0.87	0.12	30	0.33	-0.14
15	-0.33	0.15	31	0.22	-0.19
16	-0.34	0.00			
% of trace				49	23

Table 6. Principal components I, II and III expressed as correlations between characters and individual components from an analysis of *Apodemus peninsulae peninsulae*. The individual measurements of 13 selected characters of 34 samples were used.

Character	Factor I	Factor II	Factor III			
1	0.91	-0.14	0.10			
3	0.56	-0.25	-0.30			
4	0.68	-0.17	-0.19			
6	0.68	0.35	0.01			
9	0.48	0.67	-0.29			
13	0.27	0.53	0.21			
16	0.53	0.51	0.32			
19	0.89	0.07	0.04			
20	0.65	-0.43	0.20			
22	0.83	-0.21	-0.03			
25	0.79	-0.21	0.05			
28	0.15	0.11	-0.84			
30	0.66	-0.44	0.09			
% of trace				43	12	8

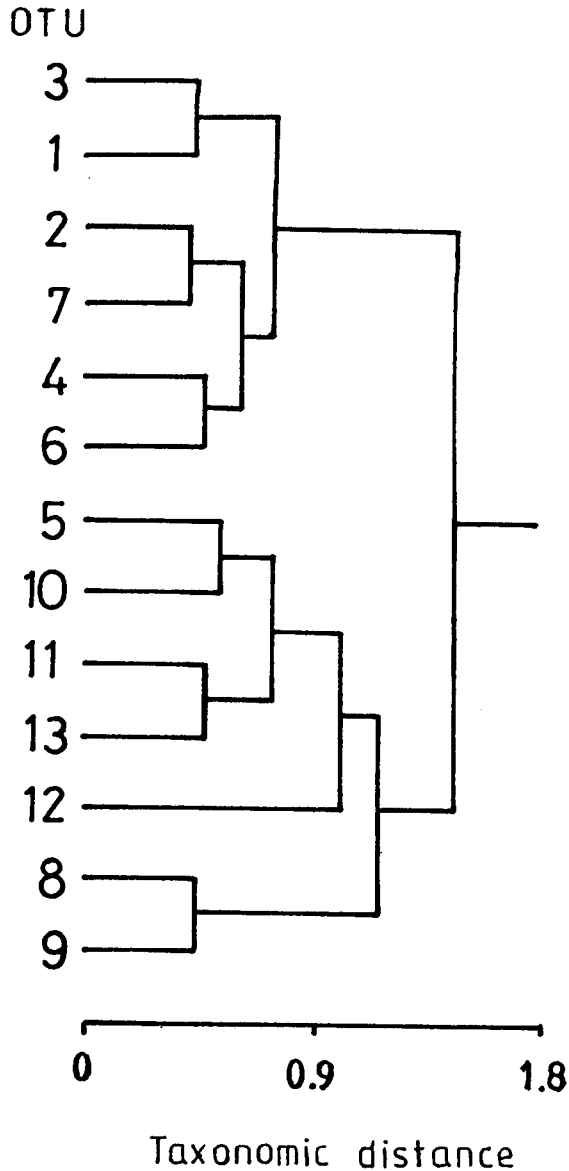


Fig. 7. Groupings of 13 OTU's of two species of *Apodemus* based on UPGMA analysis using average taxonomic distance from standardized means of 13 selected characters. For species name and locality of each OTU see Table 1.

neighbors are frequently distorted in an ordination, especially one based on principal component analysis (Rohlf, 1970). There are as yet no satisfactory methods for telling whether clustering or ordination is most appropriate (Sneath and Soakl, 1973). In this paper, a cluster analysis by UPGMA, principal component analysis, and discriminant function analysis were used with the same data and similar results were obtained, as shown in Figs. 4, 5, 6 and 7.

Apodemus agrarius, striped field mice, is a distinct species and a sole member of subgenus *Apodemus* with narrow black mid-dorsal stripe: the insular form, *A. agrarius chejuensis*, from Jeju island, is somewhat larger than other subspecies, but is not very distinctive (Corbet, op. cit.). No differences

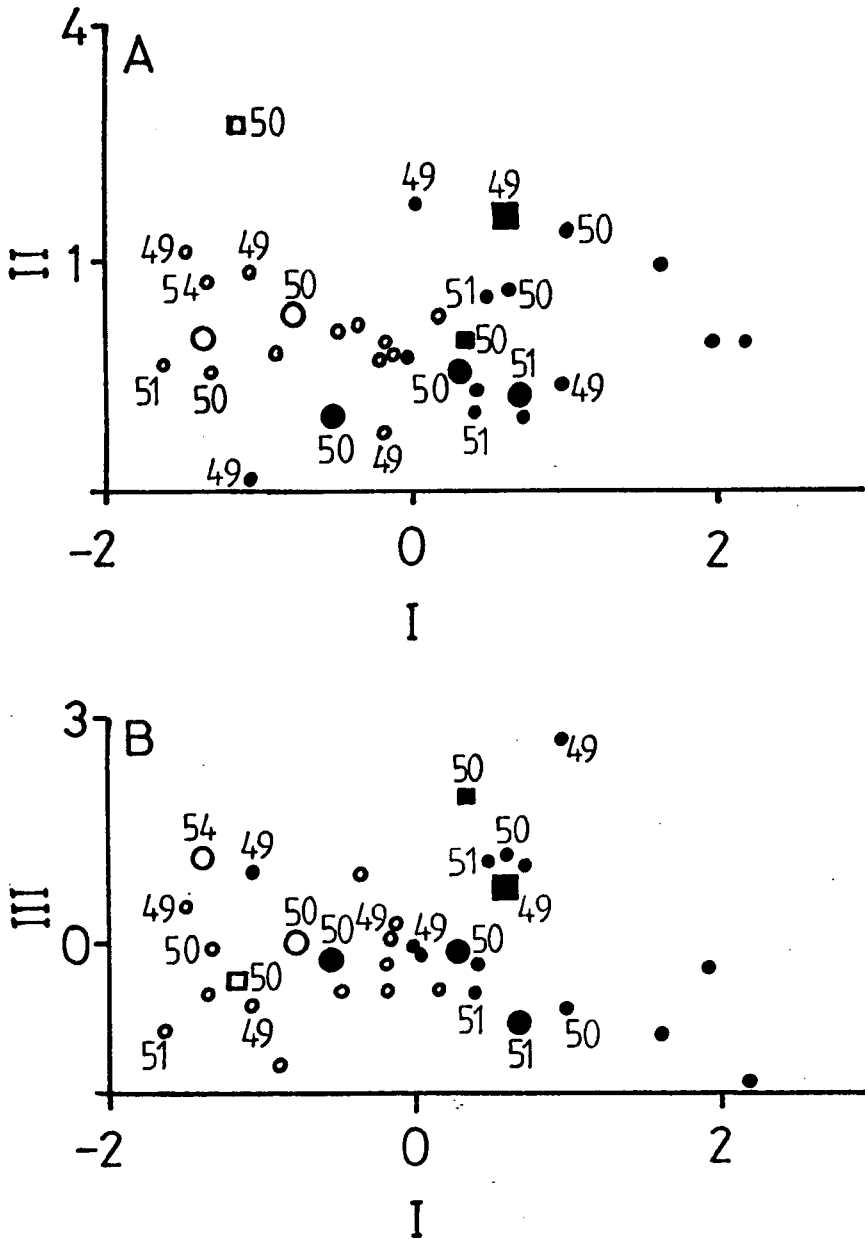


Fig. 8. Projections of 34 samples of *Apodemus peninsulae peninsulae* based on principal component analysis in three dimensions. The individual measurements of 13 selected characters of young and middle-aged adults were used. Large circle, small circle, large rectangle, and small rectangle indicate samples of OTU's 10, 11, 12 and 13 respectively. Young adults are shown with unshaded circle and rectangle, and middle-aged adults with shaded circle and rectangle. Numerals indicate chromosome number. For locality of each OTU see Table 1. For the chromosome number of samples from OTU 10, Mungyong, see Koh (1986a). A, samples ordinated with factor I vs. factor II. B, samples ordinated with factor I vs. factor III.

were found in the number and morphology of chromosomes among various populations of *A. agrarius* (Bekasova *et al.*, 1980). Vorontsova *et al.* (op. cit.) reported *Apodemus peninsulæ*, Korean field mice, as field mice both of middle-size among species in subgenus *Alsomys* and of diploid number of 48 to 61.

In the present morphometric analyses with the samples of two species (*A. agrarius coreae*, *A. agrarius chejuensis*, and *A. peninsulæ peninsulæ*), *A. agrarius chejuensis* (OTU's 8 and 9) and *A. peninsulæ peninsulæ* (OTU's 10 to 13) formed a large-size group and *A. agrarius coreae* (OTU's 1 to 7) a small-size group (see Figs. 4, 5, 6, and 7). And *A. agrarius chejuensis* was larger than *A. peninsulæ peninsulæ*, indicating that a decided gap appeared to be evident between *A. agrarius coreae* and *A. agrarius chejuensis*.

Geographically isolated populations may be either species or subspecies (Wiley, 1982) and it is preferable for various reasons to treat allopatric populations of doubtful rank as subspecies (Mayr, 1969). Ross (1974), however, stated that when there is no intermediate between two parapatric groups they are different species. Therefore, samples of striped field mice from southwestern coastal islands of the Korean peninsula are needed for further analyses to clarify the status of *A. agrarius chejuensis*.

In chromosomal analyses with striped field mice from Korea, karyotype contains 48 chromosomes, including 40 acrocentrics and 8 metacentrics (see Table 2 and Fig. 1; Koh, 1982; Koh, 1987). It was also found that diploid chromosome number of *A. peninsulæ peninsulæ* from Korea varied from 49 to 51 with one sample of 54 due to the presence of supernumerary chromosomes (see Table 3 and Figs. 2 and 3; Koh, 1986a). Bekasova *et al.* (op. cit.) reported that in continental populations of *A. peninsulæ* in Asia a relatively small number of B chromosomes (0 to 5) was observed and that animals with 6 to 13 B chromosomes, B's, were found in Hokkaido populations.

It was also found that metacentric, supernumerary chromosomes of *A. peninsulæ peninsulæ* from Korea were C-negative, euchromatic, and that Y chromosome is totally heterochromatic (see Figs. 2 and 3). Bekasova *et al.* (1980) reported that supernumerary chromosomes of samples from Siberia and continental part of the Far East were heterochromatic. Jones and Rees (1982) noted that the majority of B's in animals are described as heterochromatic. Hsu and Arrighi (1971) reported that the Y chromosome of deer mice, *Peromyscus maniculatus*, and white mice, *Mus musculus*, are C-positive.

Morphological divergence among chromosomal morphs due to the presence of B chromosomes in *A. peninsulæ peninsulæ* from Korea was not evident, as shown in Fig. 8. Jones and Rees (op. cit.) noted that while there is less information about the effects of B's on growth and development in animals there is enough to confirm that the effects of B's in high frequency, at any rate, are generally deleterious. Thorpe *et al.* (1982) noted that the Robertsonian populations in *Mus musculus* ($2n = 26$, $2n = 24$, and $2n = 22$) are clearly, morphometrically distinct.

ABSTRACT

Chromosomal and morphometric analyses were performed with the samples of two species of the genus *Apodemus* (*A. agrarius coreae*, *A. agrarius chejuensis* and *A. peninsulæ peninsulæ*) from Korea. *A. agrarius coreae* formed a small-size group and *A. agrarius chejuensis* and *A. peninsulæ peninsulæ* formed a large-size group; *A. agrarius chejuensis* was somewhat larger than *A. peninsulæ peninsulæ*. B chromosomes in *A. peninsulæ peninsulæ* were C-negative and euchromatic.

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