

Sperm Morphology of Two Species of the Genus *Apodemus* (Rodentia, Mammalia) in Korea

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Sperm morphology of two species (*Apodemus agrarius coreae* and *Apodemus peninsulae peninsulae*) of the genus *Apodemus* in Korea was investigated. Two species were similar in six qualitative characters, i. e., head shape, presence of apical ridge, process, cone-like process, and mitochondrial sheath, and the degree of development in outer dense fiber. Lengths of head and midpiece were not different with each other, but length of principal plus end piece was significantly larger ($P \ll 0.001$) in *Apodemus agrarius coreae* than that in *Apodemus peninsulae peninsulae*.

KEY WORDS: Sperm morphology, Genus *Apodemus*

The genus *Apodemus*, confined to the Palaearctic and northern part of the Oriental region, is a fairly distinctive genus classified into 11 species (Corbet and Hill, 1986). In Korea two species of this genus inhabit, i. e., striped field mice, *Apodemus agrarius*, and Korean field mice, *A. peninsulae* (Koh, 1988): the former with black mid-dorsal stripe is a sole member of the subgenus *Apodemus* and other ten species including the latter are the members of the other subgenus *Sylvaemus*, which has no mid-dorsal stripe (Corbet, 1978).

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of mammalian spermatozoa are useful tools for the study of mammalian evolution (Fawcett, 1975; Matano *et al.*, 1976; Breed and Inns, 1985) and systematically oriented comparisons have been made of the morphology of the spermatozoa in a wide range of mammalian taxa (Matano *et al.*, 1976; Visser and Robinson, 1987).

Here we thus present results on sperm morphology of two species of the genus *Apodemus* in Korea.

Materials and Methods

Three adult males of *Apodemus agrarius coreae* from Chongju, two of *A. peninsulae peninsulae*

from Mt. Weolak, and one of *A. peninsulae peninsulae* from Chongju were used. From the epididymides of each sample, spermatozoa were extruded into physiological saline, spun, and resuspended twice. The sperms were then fixed with 2.5% glutaraldehyde for 15 minutes (Hafez and Kanagawa, 1973). For inspection under light microscope one drop of sperm suspension was placed on a slide glass and air-dried. The slides were stained with 50% silver nitrate (Bloom and Goodpasture, 1976) or 4% Giemsa solution. The lengths of head, midpiece, and principal plus end piece were measured. The numbers of spermatozoa measured from each sample were 20, and t-test between two species was undertaken with the measurements of three continuous characters mentioned above.

For SEM the spermatozoa placed on the coverslips were coated with gold (Dott, 1969) and observed under Hitachi S-570 scanning electron microscope at 20 kv accelerating voltage. For TEM small pieces of epididymides were immersed in the fixative for 1 hour, washed in buffer, osmicated, dehydrated in a series of alcohols, and embedded in Spurr's resin (Kim, 1986). Ultrathin sections were cut and stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined under Zeiss EM 109 transmission electron microscope at 50kv accelerating voltage.

Results

The sperm morphology of *Apodemus agrarius coreae* is represented in Fig. 1. With the SEM apical ridge (Ar), process (Pr), and cone-like process (C) are visible at the ventral region of falciform head. With TEM mitochondrial sheath (MS) is revealed, and outer dense fiber (ODF) number 1, 4, 5 and 9 are larger than others. As given in Table 1, lengths of head, midpiece and principal plus end piece in the spermatozoa of *Apodemus agrarius coreae* are $8.0 \pm 0.07 \mu\text{m}$, $24.8 \pm 0.14 \mu\text{m}$, and $101.8 \pm 0.56 \mu\text{m}$, respectively.

Fig. 2 shows the morphology of spermatozoa of *Apodemus peninsulae peninsulae*. Head is falciform in outline and Ar, Pr, and C are also visible, as shown in Fig. 2-A. MS are observed and ODF number 1, 4, 5, and 9 are larger than the rest (see Fig. 2-B). As given in Table 1, length of head, midpiece length, and length of principal plus end piece in the spermatozoa of *Apodemus peninsulae peninsulae* are $7.9 \pm 0.07 \mu\text{m}$, $24.9 \pm 0.01 \mu\text{m}$, and $94.8 \pm 0.39 \mu\text{m}$, respectively.

The two species studied are similar in six qualitative characters (falciform head shape, the presence of Ar, Pr, C, and MS, and the degree of development in ODF). They are not different in two of three continuous variables, i. e., length of head and midpiece length, whereas *Apodemus agrarius coreae* seems to be significantly larger in length of principal plus end piece than *A. peninsulae peninsulae* (see Table 1).

Discussion

Rodents have been divided into three suborders (Sciuromorpha, Caviomorpha, and Myomorpha). Matano *et al.* (1976) reported that the sperm head of myomorphs is falciform in outline, and that apical ridge (Ar) is visible. In this study with two species of the genus *Apodemus* (suborder Myomorpha), falciform sperm head and Ar were observed, as shown in Figs. 1 and 2.

Matano *et al.* (1976) also noted that a process (Pr) is present in two species of the family Muridae, *Rattus rattus* and *Mus musculus*, whereas cone-like process (C) is observed only in *Rattus rattus*. In the present study with two species of the genus *Apodemus* (family Muridae), both Pr and C were visible.

Fawcett (1970) stated that species differences are great in thickness of outer dense fiber and midpiece length. Baccetti (1985) also noted that animals evolved toward elongation and sophistication of the sperm flagellum. Visser and Robinson (1987) found that length of principal piece was different between two cytotypes of *Aethomys crysophilus*. In this study, the sperms of two species of the genus *Apodemus* were similar with each other in the degree of development of outer dense fiber, length of head, and length of midpiece, while *Apodemus agrarius coreae* was significantly larger in length of principal plus end piece than *A. peninsulae peninsulae*, as shown in Figs. 1 and 2 and Table 1.

Table 1. Comparison of three continuous characters in spermatozoa of two species of the genus *Apodemus*. Three characters are length of head, midpiece length, and length of principal plus end piece. Three samples in each species were used and 20 sperms were measured in each sample.

Region of sperm	Lengths		Significance
	<i>A. agrarius coreae</i>	<i>A. peninsulae peninsulae</i>	
Head	$8.0 \pm 0.07^*$ [7.5-8.8]**	7.9 ± 0.07 [7.5-8.5]	n. s.
Midpiece	24.8 ± 0.14 [23.3-26.3]	24.9 ± 0.11 [23.8-26.3]	n. s.
Principal plus end piece	101.8 ± 0.56 [95.0-106.3]	94.8 ± 0.39 [87.5-98.0]	$P \ll 0.001$

*Mean \pm S. E (μm), **[range]

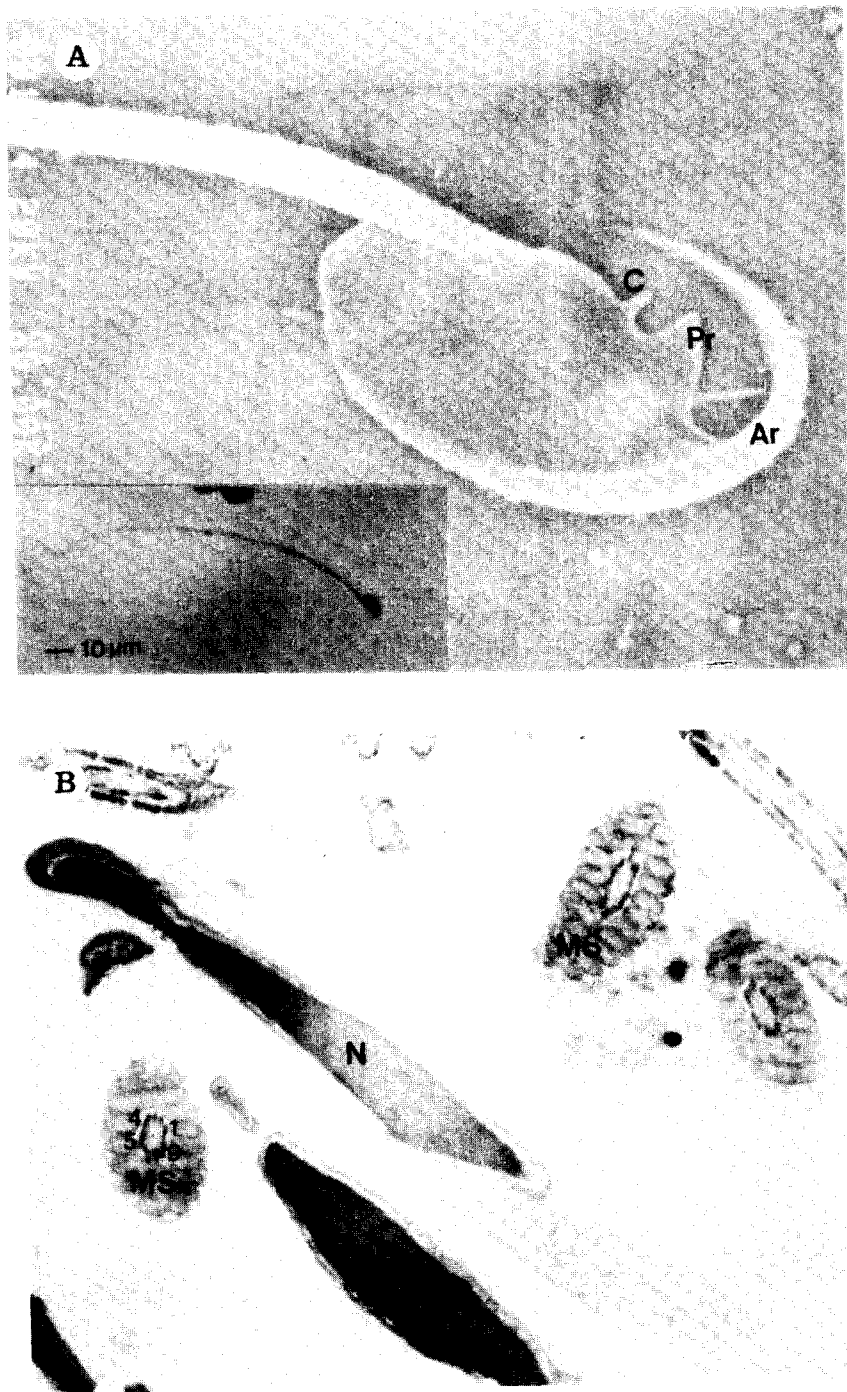


Fig. 1. Spermatozoa of *Apodemus agrarius coreae* from Chongju. A, SEM of falciform head showing apical ridge (Ar), process (Pr), and cone-like process (C), $\times 8,000$. Inset: silver stained spermatozoon, $\times 400$. B, TEM section of head showing nucleus (N) and midpiece showing well developed mitochondrial sheath (MS) and outer dense fiber (ODF), $\times 24,000$. Numerals indicate larger ODF numbers.

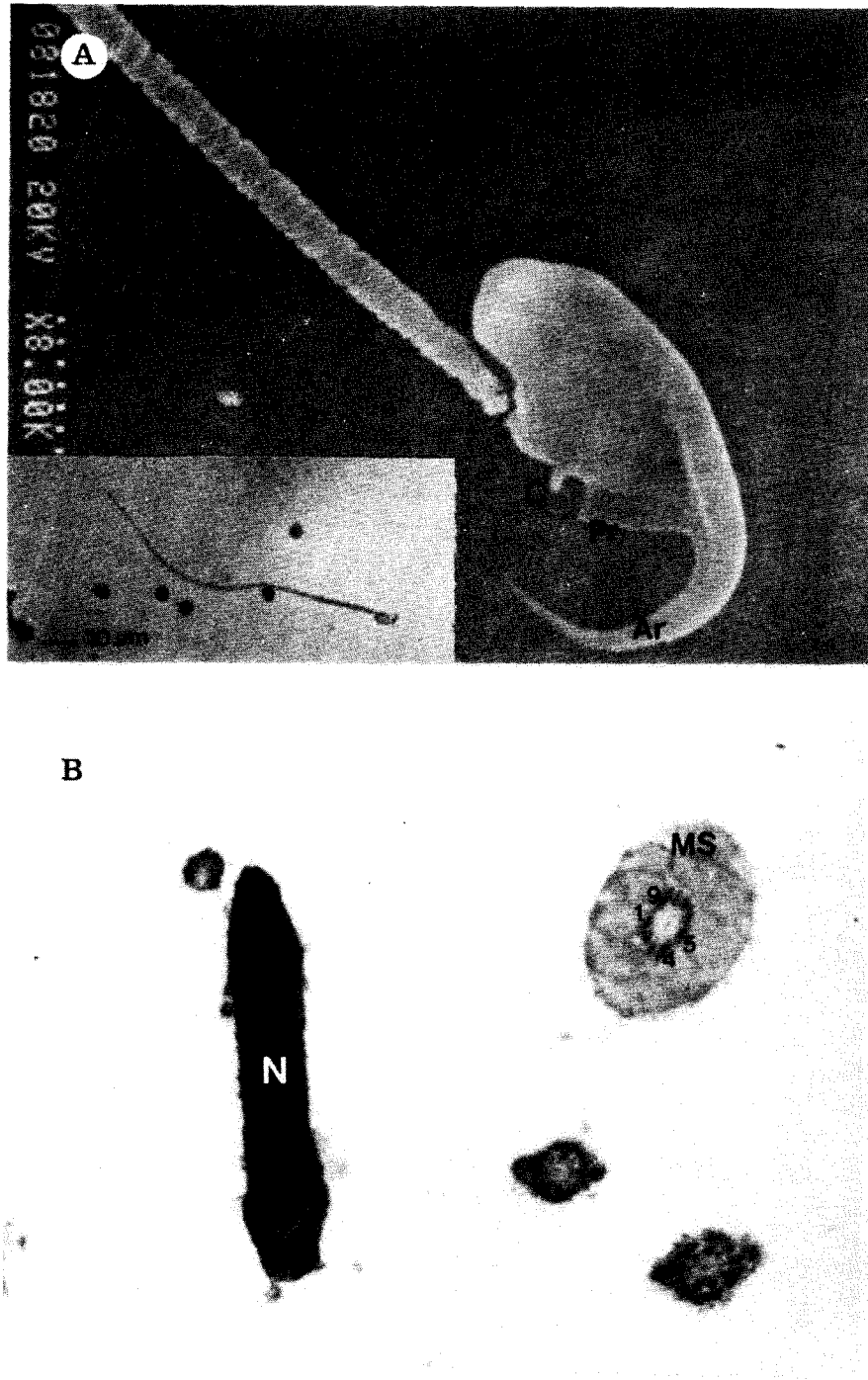


Fig. 2. Spermatozoa of *Apodemus peninsulae peninsulae* from Mt. Weolak. A, SEM of falciform head showing Ar, Pr, and C, $\times 8,000$. Inset: silver stained spermatozoon, $\times 400$. B, TEM section of head showing N and midpiece showing well developed MS and ODF, $\times 24,000$. Numerals indicate larger ODF numbers. For abbreviations see Fig. 1.

In the genus *Apodemus*, one subgenus *Apodemus* differ morphologically in the possession of the upper third molar with 2 internal lobes and distinct mid-dorsal stripe from the other subgenus *Sylvaemus* (Corbet, 1978). Moreover, *Apodemus agrarius* (a sole member of the subgenus *Apodemus*) has larger fundamental number of 52 in karyotypes than the species of the subgenus *Sylvaemus* (Gagia et al., 1985). In this study, it was found that length of principal plus end piece of the spermatozoa in *Apodemus agrarius coreae* was significantly larger than that in *A. peninsulae peninsulae*, and it is necessary to analyze sperm morphology of the other subspecies of two speceis and other nine species of the subgenus *Sylvaemus* in order to determine whether or not length of principal plus end piece of *Apodemus agrarius* is the largest among the members of the genus *Apodemus*.

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한국산 붉은쥐속 2종의 정자의 형태

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한국산 설치류 2종 (*Apodemus agrarius coreae*와 *Apodemus peninsulae peninsulae*)의 정자형태를 관찰하여 비교하였다. 두종의 정자는 6가지의 질적 형질 (두편의 형태, apical ridge, process, cone-like process, 및 mitochondrial sheath의 존재, 그리고 outer dense fiber의 발달정도)에 있어서 같았다. 두편의 길이와 중편의 길이는 두 종간에 차이가 없었으나, 주편과 단편의 합한 길이에 있어서는 *Apodemus agrarius coreae*가 *Apodemus peninsulae peninsulae* 보다 크다는 것이 밝혀졌다($P \ll 0.001$).