

Cytological Studies of Two Species of Genus *Cipangopaludina* (Mesogastropoda: Viviparidae) in Korea

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= 국문 요약 =

한국산 논우렁이 2종의 세포학적 연구

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한국에 분포되어 있는 논우렁이과(Viviparidae) 권패류는 논우렁이(*Cipangopaludina chinensis malleata*)와 큰논우렁이(*C. japonica*), 두 종이 보고되어 있으나 형태학적 유사성 때문에 감별이 용이치 않다. 우선, 본 연구에서는 두 종간에 염색체 수와 핵형분석 결과를 비교해 보고자 하였다.

두 종 모두 염색체 수는 $2n=18$ 이었으며 성염색체는 구별할 수 없었고, 두쌍의 metacentric과 7쌍의 submetacentric 염색체를 갖고 있었다. 두 종간 평균 염색체 크기에서 큰논우렁이가 논우렁이보다 약 $10 \mu\text{m}$ 정도 크게 나타났다.

Key words : Viviparidae, Chromosome, *Cipangopaludina chinensis malleata*, *C. japonica*

INTRODUCTION

Comparative chromosome studies in related species have been of great value for the establishment of systematic relations in many animals

and plants. In mollusca, however, the literatures on karyotype analysis are not abundant due to difficulties of obtaining mitotic fields with enough quality to carry out chromosome studies.

The family Viviparidae contains three subfamilies, Bellamyinae, Viviparinae and Lioplacinae, and their chromosome numbers were ranged from $n=7$ to $n=14$ by the literature survey. The haploid chromosome numbers of *C. chinensis malleata* have been reported by the former investigators (Pollister

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and Pollister, 1940; Pollister, 1943; Inaba and Tanaka, 1953). Also, the chromosome numbers of 19 species in the family Viviparidae have been studied by Patterson (1969). In Korean viviparid snails, Park *et al* (1988) has reported the karyologic analysis of a population of *C. chinensis malleata*. Two viviparid snail species in Korea, *C. chinensis malleata* (Reeve), 1912 and *C. japonica* Martens, 1860 are morphologically similar and closely related on the taxonomic basis (Kwon *et al.*, 1993). Especially, *C. japonica* occur in a restricted area in the southern part of Korea, while *C. chinensis malleata* distribute widely in all of the freshwater systems.

The purpose of this study was to compare the karyotypes of two viviparid snail species occurring in Korea.

MATERIALS AND METHODS

The snails used in this study were collected from August 1995 to April 1996 in Korea and examined shortly after being collected. Six males and five females of *C. chinensis malleata* were collected from Chunchon, Kangweon Do, and five males and five females of *C. chinensis malleata* were collected from Kimhae, Kyungsangnam-Do. Four males and six female specimens of *C. japonica* were collected from Kimhae. Chromosome preparations were made from gonadal tissues by the air-drying method of Park (1994). Morphological features of the chromosomes used to compare karyotypes were the relative and total lengths of the mitotic metaphase chromosomes. Nomenclature of morphological types of chromosomes was adopted from the paper of Leven *et al.* (1964). Voucher specimens of the *C. chinensis malleata* and *C. japonica* have been in the Inha University, Korea.

RESULTS

1. *Cipangopaludina chinensis malleata* (Reeve), 1912

Meiotic chromosomes in males were observed (Fig. 1-a, b). Pachytene bivalents were randomly scattered on the plane and appeared like long threads and homologous pairs could not be distinguished from each other (Fig. 1-a). At late diakinesis phase, five of bivalents were ring-shaped with two chiasmata and one of them appeared as rod-shaped with one terminal chiasma. The others were multiple loop-shaped bivalents with three chiasmata (Fig. 1 b). The haploid chromosome numbers in the snail specimens (11 males and 10 females) collected from two localities were all nine ($n=9$). Spermatogonial divisions in metaphase were observed in 32 cells.

From ten female specimens, ovarian mitotic divisions were observed in 22 cells. In these cells, 18 chromosomes ($2n$) were also counted (Fig. 1-c, d).

The results of chromosome measurement are shown in Table 1. Total lengths of the chromosomes in males and females ranged from 1.8 to 3.9 μm and 1.8 to 4.1 μm , respectively. There were no remarkable differences from the total chromosome lengths in male and female (Fig. 3). Mean total lengths of the diploid complements in male and female were 25.9 ± 2.21 (S.D) μm and 26.0 ± 3.22 μm , respectively. Karyotype of this species consists of seven pairs of submetacentrics and two pairs of metacentric chromosomes (Fig. 2-a, b).

2. *Cipangopaludina japonica* Martens, 1860

This is the first report on the chromosomes of *C. japonica* in Korea. Chromosomes were observed in four males and six females. The mitotic metaphase chromosomes were observed in both sexes and the diploid chromosome numbers were 18 ($2n=18$) (Fig. 1-e, f). The karyotypes of this species were arranged by shape and size (Figs. 2-c, d, 3) and Table 2 shows the results of chromosome measurement from this species. Total lengths of chromosomes in males and females ranged from 3.0 to 5.5 μm and 2.6 to 5.6 μm , respectively. Mean

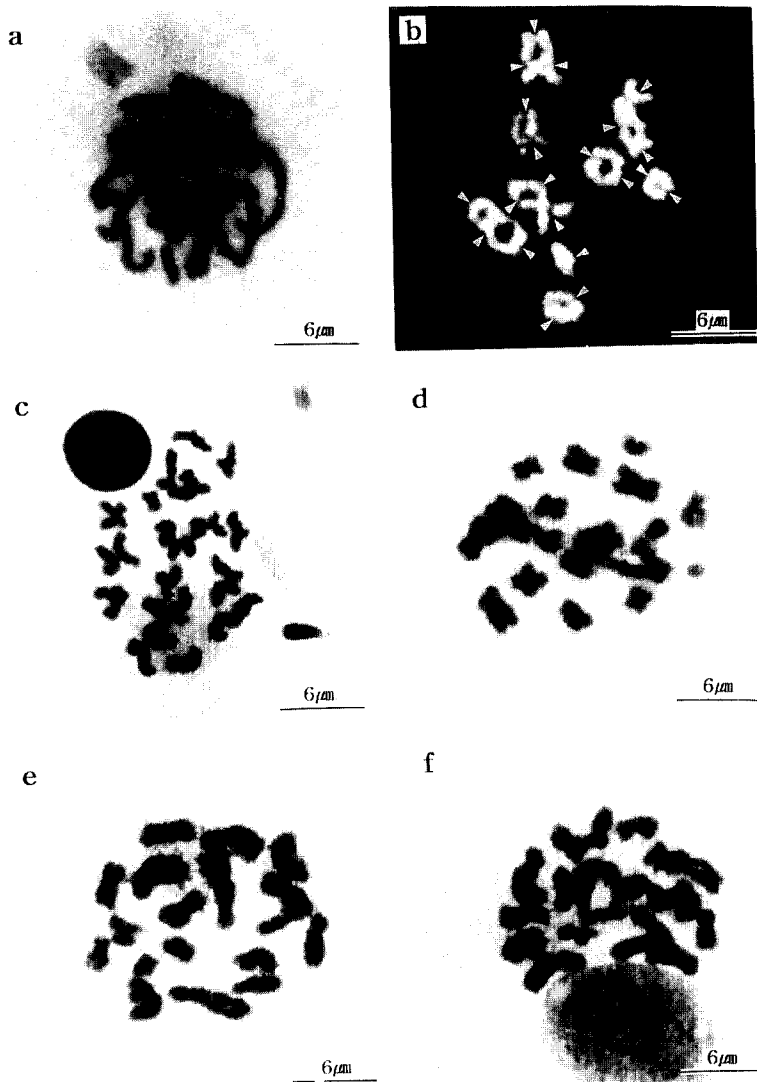


Fig. 1. Meiotic and mitotic chromosomes of *C. chinensis malleata* and *C. japonica*.

- a. Chromosomes in prophase I (pachytene) (*C. chinensis malleata*, male)
- b. Chromosomes in diakinesis phase (*C. chinensis malleata*, male). Ring-shaped bivalents, each with two chiasmata; rod-shaped bivalent with one terminal chiasma; multiple loop-shaped bivalents with three chiasmata are noticeable. Arrow heads indicate positions of chiasmata.
- c. Spermatogonial metaphase chromosomes of *C. chinensis malleata*
- d. Chromosomes in metaphase I (*C. chinensis malleata*, female)
- e. Spermatogonial metaphase chromosomes of *C. japonica*
- f. Chromosomes in metaphase I (*C. japonica*, female)

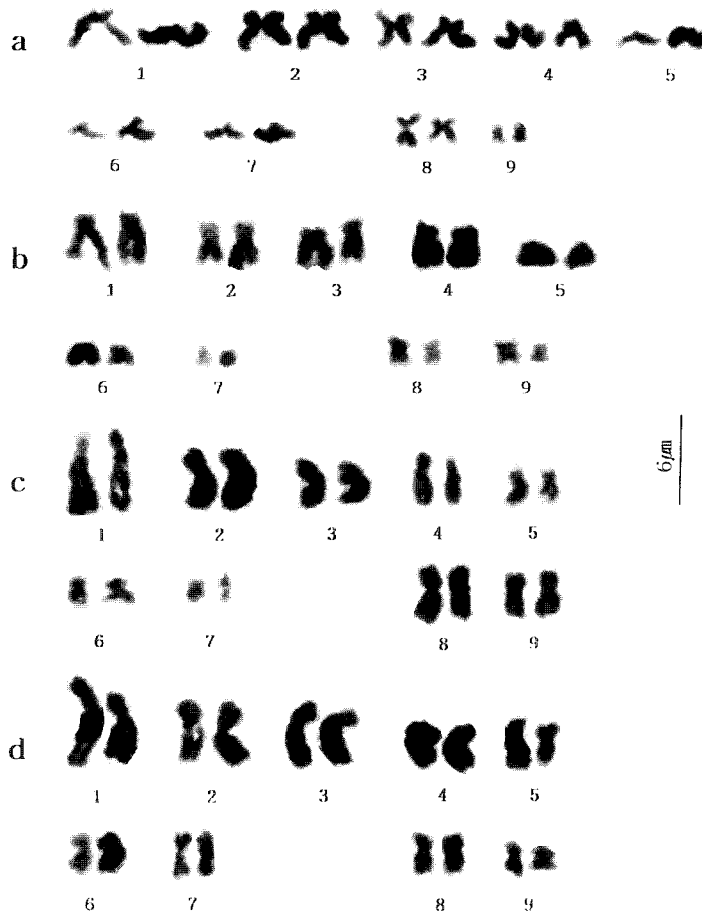


Fig. 2. Karyotypes in male and female of *C. chinensis malleata* and *C. japonica*

- a. Karyotype of male *C. chinensis malleata*, constructed from Fig. 1-c
- b. Karyotype of female *C. chinensis malleata*, constructed from Fig. 1-d
- c. Karyotype of male *C. japonica*, constructed from Fig. 1-e
- d. Karyotype of female *C. japonica*, constructed from Fig. 1-f

total lengths of the diploid complements in male and female were $36.9 \pm 3.21 \mu\text{m}$ and $35.1 \pm 3.06 \mu\text{m}$, respectively. Karyotype of this species consists of seven pairs of submetacentrics and two pairs of metacentric chromosomes (Figs. 2-c, d, 3). Total lengths of mitotic metaphase chromosomes in both sexes were of no difference in the karyotype analysis (Fig. 3).

DISCUSSION

The general reviews on the chromosomal attributes of molluscs have been presented mostly by Burch and Patterson since the late 1960's (Burch, 1967; Patterson, 1969). Patterson (1969) made a general review of all molluscan chromosome

Table 1. Measurements of the mitotic metaphase chromosomes of *C. chinensis malleata*.

No. chromosome pair	Male*			Female*		
	Total length	Relative length	Type	Total length	Relative length	Type
1	3.9±0.12	15.06±0.19	SM	4.1±0.03	15.76±0.23	SM
2	3.6±0.23	13.90±0.23	SM	3.6±0.17	13.85±0.15	SM
3	3.5±0.08	13.50±0.13	SM	3.5±0.05	13.46±0.13	SM
4	3.1±0.05	11.96±0.11	SM	3.3±0.16	12.69±0.23	SM
5	2.6±0.16	10.04±0.16	SM	2.8±0.23	10.76±0.32	SM
6	2.4±0.07	9.27±0.37	SM	1.9±0.34	7.30±0.07	SM
7	2.3±0.11	8.88±0.03	SM	1.8±0.35	6.92±0.30	SM
8	2.7±0.02	10.41±0.17	M	2.7±0.05	10.38±0.16	M
9	1.8±0.24	6.94±0.24	M	2.3±0.01	8.84±0.15	M

* Measurements were done with three sets of karyotyped male cells and two sets of female cells.

$$\text{Relative length} = \frac{\text{Total length of a pair}}{\text{Sum of total length}} \times 100$$

M = metacentric chromosome ; SM = submetacentric chromosome

studies reported after 1930's. Park *et al.* (1988) previously studied a Korean viviparid species, *Cipangopaludina chinensis malleata*, which had a haploid chromosome number of $n=9$ in the male. In this study, both sexes of *C. chinensis malleata* were employed to compare the sexual differences of chromosomes. The chromosome number and centromere positions of this species were same as the results of Park *et al.* (1988) in spite of applying different methods. We concluded that there was no appreciable morphological difference of chromosomes, even with populations from different localities. However, total lengths of chromosome bivalents were a little different from each other. These results may be due to technical differences.

The chromosome numbers and karyotypes of *C. japonica*, were same as those of the other *Cipangopaludina* species with minor differences in total chromosome lengths.

Sex chromosomes in both male and female individuals of a viviparid species, *Tulotoma angulata*, have been determined by Patterson (1965). She concluded that this species has two relatively large sex chromosomes in spermatogonial metaphase cells. In this study, we have not observed heterochromatic or heteromorphic chromosomes in mitosis phase at both sexes. The identification of the homologous chromosomes is, of course, very important in order to establish the karyotype. However, the detection of sex chromosomes is very difficult in cases that the chromosomes are closely similar both in size and in shape. The future application of chromosome banding techniques will be helpful to identify homologous chromosomes and sex chromosomes.

SUMMARY

Table 2. Measurements of the mitotic metaphase chromosomes of *C. japonica*.

No. chromosome pair	Male*			Female*		
	Total length	Relative length	Type	Total length	Relative length	Type
1	5.5±0.02	14.91±0.04	SM	5.6±0.01	16.67±0.16	SM
2	4.9±0.11	13.28±0.33	SM	4.8±0.06	14.29±0.11	SM
3	4.7±0.03	12.74±0.09	SM	4.6±0.02	13.69±0.47	SM
4	4.6±0.05	12.47±0.47	SM	4.2±0.07	12.50±0.12	SM
5	3.3±0.06	8.94±0.08	SM	3.8±0.20	11.30±0.25	SM
6	3.2±0.31	8.67±0.28	SM	3.3±0.02	9.82±0.24	SM
7	3.0±0.28	8.13±0.20	SM	3.2±0.05	9.52±0.18	SM
8	4.1±0.24	11.11±0.23	M	3.0±0.24	8.92±0.09	M
9	3.6±0.33	9.76±0.14	M	2.6±0.08	7.74±0.05	M

* Measurements were done with three sets of karyotyped male cells and three sets of female cells.

The mitotic and meiotic chromosomes of two

viviparid snail species, *Cipangopaludina chinensis malleata* (Reeve), 1912 and *C. japonica* Martens, 1860 were investigated by means of air-dry method. The diploid chromosome numbers were 2n=18 (n=9) in both species, and the sex chromosomes could not be distinguished. Chromosome complements of both species consist of two pairs of metacentrics and seven pairs of submetacentric chromosomes.

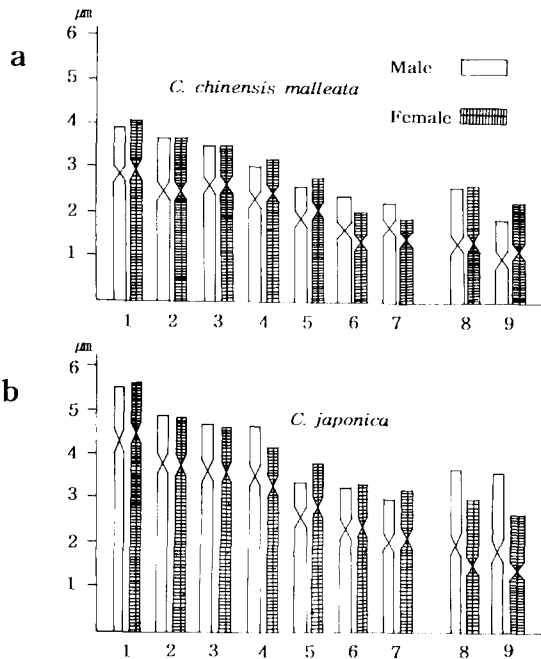


Fig. 3. Idiograms of chromosome pairs detected in two viviparid species of Korea, *C. chinensis malleata* (a) and *C. japonica* (b); drawn from the data in Tables 1 and 2.

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