

Karyotypes of Korean Endemic Land Snail, *Koreanohadra koreana* (Gastropoda: Bradybaenidae)

Gab-Man PARK

Department of Environmental Medical Biology, Kwandong University College of Medicine, Gangneung 210-701, Korea

ABSTRACT

The karyotypes of Korean endemic land snail, *Koreanohadra koreana*, using air-drying method were investigated. Somatic cells of this species had $2n = 58$. Karyotypes were also analysed with 16 metacentric, 12 submetacentric and one subtelocentric chromosome pairs. Observed chromosomes ranged from 2.6 to 8.9 μm and the total length was 122.3 μm . This is the second report on the chromosome numbers and the karyotype of *K. koreana*.

Keywords : Karyotype, *Koreanohadra koreana*, Land snail

Introduction

Bradybaenidae is a taxonomic family of medium-sized to small land snails, terrestrial pulmonate gastropod mollusks in the superfamily Helicoidea. These snails are found mainly in Asia, with only one species occurring in Northwestern Europe: *Fruticicola fruticum*. Korean Bradybaenidae snails have been classified into 24 species by Kwon *et al.*, (1993).

In recent years, through a considerable number of works, a large amount of information has been accumulated on the chromosomes of the mollusks. Cytogenetic studies of mollusks have been important in aspects of phylogenetic and cytogenetic relationships among the species (Thiriot-Quiévreux, 2003). Detailed studies of chromosome morphology and population cytology of the Bradybaenidae are very little. In fact modern cytogenetic techniques have only recently been adopted for studies of Gastropoda. The family Bradybaenidae is conservative in regard to chromosome numbers that 36 species have 28, 29 and 30 pairs of chromosomes (Table 1). The chromosome numbers of these animals in Korea were studied by Lee & Kwon

(1993), based on the air-drying technique with gonadal tissues. In this study, the karyotype of *Koreanohadra koreana* was studied in order to analyse their genetic relationships.

Materials and Methods

The eight specimens used in this study were collected in Hongdo, Sinan-gun, Jeollanam-do, Korea, June 2010, and examined shortly after collection. The chromosome preparations were made on gonad of the specimens by the usual air-drying method as follows. Live specimens were set aside for one day after injection with 0.3 ml of 0.05% colchicine solution. The treated tissues were dissected and minced with needles in a hypotonic 0.01% NaCl solution. Separated cells were collected by centrifugation at 1500 rpm for 10 minutes. These cells were then fixed in freshly mixed modified Carnoy's fixative (three parts methyl alcohol and one part glacial acetic acid). Supernatants were replaced with fresh fixative and centrifugation (1500 rpm, 10 minutes) was repeated three or more times. A single drop of each cell suspension obtained was then pipetted using a microhematocrit capillary tube onto a clean slideglass pre-cooled to 4°C. Cells remaining on slides were air-dried and then stained for 7 minutes with 4% Giemsa (Gurr R66) solution made up in 0.1 M phosphate buffer, pH 7.0. The prepared slides were observed under an Olympus (BX51) microscope. Voucher specimens of the

Table 1. Chromosome numbers and karyotypes of Bradybaenidae

Species	Haploid No.	Karyotype*	Source	References
<i>Acusta despecta sieboldiana</i>	29	21M + 7SM + 1ST	Japan	Tatewaki & Kitada, 1987
"	29	12M + 17SM	Korea	Lee & Kwon, 1993
<i>A. despecta</i>	29	-	Japan	Burch (pers. comm.)
<i>A. d. sieboldiana</i>	29	12M + 17SM	Korea	Lee & Kwon, 1993
<i>Aegista vatheleti</i>	29	-	Japan	Inaba, 1959
<i>A. chosenica</i>	28	10M + 18SM	Korea	Lee & Kwon, 1993
<i>A. (Plectotropis) diversa</i>	28	14M + 14SM	"	"
<i>A. (P) quelpartensis</i>	28	15M + 10SM + 3T	"	"
<i>Bradybaena ravida</i>	29	19M + 3SM + 7T	China	Sun, 1995
<i>B. similaris</i>	28	-	Japan	Inaba, 1959
<i>B. gainesi</i>	29	-	"	Kawabe, 1947
<i>Cathaica fasciola</i>	30	7M + 1SM + 22T	China	Sun, 1995
<i>Euhadra amaliae</i>	28	19M + 9SM	Japan	Tatewaki & Kitada, 1987
<i>E. callizona</i>	28	-	"	Inaba, 1959
"	28	19M + 9SM	"	Tatewaki <i>et al.</i> 1987
<i>E. congenital</i>	28	-	"	Inaba, 1959
<i>E. dixoni</i>	28	19M + 9SM	"	Tatewaki & Kitada, 1984, 1987
<i>E. eoa</i>	28	-	"	Inaba, 1959
"	28	19M + 9SM	"	Tatewaki & Kitada, 1996
<i>E. grata</i>	28	-	"	Inaba, 1959
<i>E. peliomphala</i>	28	-	"	"
<i>E. peliomphala simodae</i>	28	18M + 10SM	"	Tatewaki <i>et al.</i> 1987
<i>E. sadoensis</i>	28	-	"	Inaba, 1959
<i>E. sandai</i>	28	-	"	"
<i>E. sandai oki</i>	28	21M + 7SM	"	Tatewaki & Kitada, 1994
<i>E. senckenbergiana</i>	28	-	"	Inaba, 1959
<i>E. subnimbosa</i>	28	21M + 9SM	"	Tatewaki & Kitada, 1984
<i>E. awaensis</i>	29	-	"	Inaba, 1959
<i>E. idzumonis</i>	29	-	"	"
<i>E. quaesita</i>	29	-	"	"
<i>E. scaevola</i>	29	-	"	"
<i>E. subnimbosa</i>	28	21M + 7SM	"	Tatewaki & Kitada, 1984
<i>Eulota fruticum</i>	29	-	Europe	Perrot, 1938
<i>Fruticicola despecta</i>	29	-	Japan	Inaba, 1959
<i>Koreanohadra kurodana</i>	29	11M + 17SM + 1T	Korea	Lee & Kwon, 1993
<i>K. koreana</i>	29	10M + 13SM + 1ST + 5T	"	"
"	29	16M + 12SM + 1ST	"	Present study
<i>Karatohelix adamsi</i>	29	10M + 18SM + 1ST	"	Lee & Kwon, 1993
<i>Nesiohelix samarangae</i>	29	12M + 9SM + 2ST + 6T	"	"

*M, metacentric; SM, submetacentric; ST, subtelocentric; T, Telocentric chromosomes

samples studied have been deposited at the Department of Environmental Medical Biology, Kwandong University College of Medicine, Korea.

Results

A microphotograph of somatic metaphase chromosomes

and the karyogram are shown in Fig. 1, and measurements of the chromosomes in Table 2. The present cytological preparations showed 12 well-spread mitotic cells on a slide. This species showed a diploid chromosome number of $2n = 58$, with 16 metacentric, 12 submetacentric and one subtelocentric chromosome

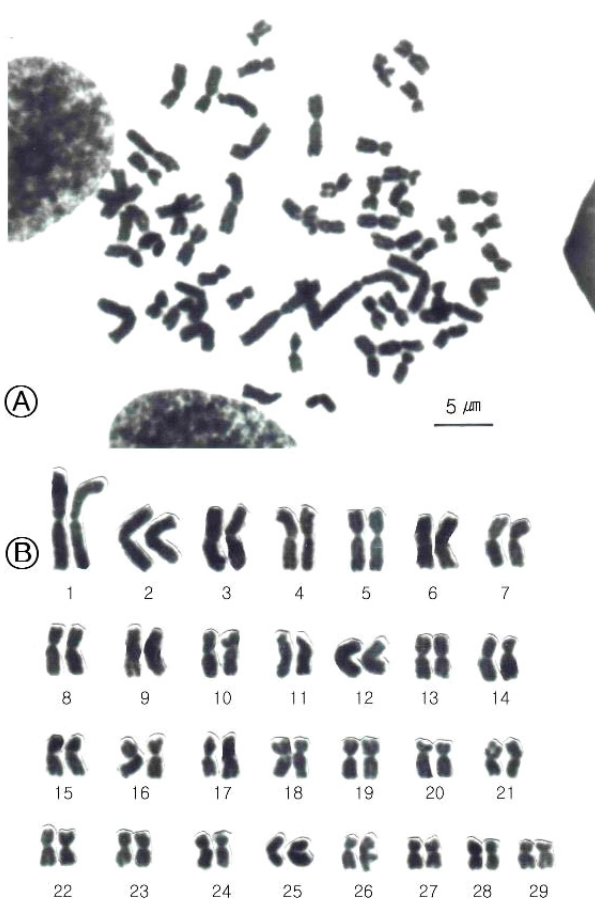


Fig. 1. Metaphase chromosomes of *Koreanohadra koreana* (A) and karyotype constructed from A (B).

pairs. Observed chromosomes ranged from 2.6 to 8.9 μm in length. This species was no inter-specimen variability in chromosome counts. Also, sexual dimorphism of chromosomes in this study did not find.

Discussion

The chromosome numbers of 36 species in Helicoidea until now studied by some workers and including the present study were $n = 28$, $n = 29$ and $n = 30$ (Table 1). Most of the Bradybaenidae chromosomes in the karyotype were metacentric or submetacentric. In this study, though the chromosome number is coincided from Lee and Kwon (1993) findings, but the karyotype is differs. The present preparations for the karyotype analysis get the good spread mitotic cells than the air-drying technique by them. With regard to karyotype

Table 2. Relative lengths and total lengths (μm) of chromosomes of *Koreanohadra koreana*

Chromosome	RL \pm SE	TL \pm SE	Type
1	7.3 \pm 0.25	8.9 \pm 0.21	M
2	6.4 \pm 0.89	7.8 \pm 0.75	M
3	4.8 \pm 0.39	5.9 \pm 0.33	M
4	4.7 \pm 0.78	5.7 \pm 0.66	M
5	4.6 \pm 0.30	5.6 \pm 0.25	SM
6	4.3 \pm 0.19	5.2 \pm 0.16	M
7	4.1 \pm 0.74	5.0 \pm 0.62	M
8	3.9 \pm 0.24	4.8 \pm 0.20	SM
9	3.8 \pm 0.64	4.6 \pm 0.54	SM
10	3.7 \pm 0.18	4.5 \pm 0.15	SM
11	3.5 \pm 0.53	4.3 \pm 0.45	ST
12	3.4 \pm 0.39	4.2 \pm 0.33	M
13	3.4 \pm 0.19	4.1 \pm 0.16	M
14	3.3 \pm 0.78	4.0 \pm 0.66	SM
15	3.2 \pm 0.44	3.9 \pm 0.37	SM
16	3.1 \pm 0.23	3.8 \pm 0.19	SM
17	3.0 \pm 0.09	3.7 \pm 0.08	M
18	2.9 \pm 0.26	3.5 \pm 0.22	SM
19	2.8 \pm 0.49	3.4 \pm 0.41	M
20	2.7 \pm 0.20	3.3 \pm 0.17	SM
21	2.6 \pm 0.24	3.2 \pm 0.20	M
22	2.6 \pm 0.18	3.2 \pm 0.15	M
23	2.5 \pm 0.08	3.1 \pm 0.07	M
24	2.5 \pm 0.21	3.0 \pm 0.18	SM
25	2.4 \pm 0.30	2.9 \pm 0.25	M
26	2.3 \pm 0.14	2.8 \pm 0.12	SM
27	2.2 \pm 0.08	2.7 \pm 0.07	M
28	2.1 \pm 0.39	2.6 \pm 0.33	M
29	2.1 \pm 0.18	2.6 \pm 0.15	SM

* Based on measurements from three sets from *Koreanohadra koreana* of karyotyped cells. **M**, metacentric; **RL**, relative length; **SE**, standard error; **SM**, submetacentric; **ST**, subtelocentric; **TL**, total length

analysis in the genus *Koreanohadra*, only one paper about *K. kurodana* and *K. koreana* has been published (Lee & Kwon, 1993). *K. kurodana* has 29 pairs of 11 metacentric chromosomes and 17 submetacentric chromosomes and one telocentric chromosome, on the other hand, *K. koreana* consisted of 10 metacentric chromosomes, 13 submetacentric chromosomes, one subtelocentric chromosomes and five telocentric chromosomes by Lee & Kwon (1993), and 16 metacentric chromosomes, 12 submetacentric chromosomes and one

subtelocentric chromosomes by this study. They result was based on the contracted at metaphase I in meiosis. It has often been pointed out that such an some researchers often leads to mis-karyotyping of the chromosome morphology analysis (Thiriot-Quévieux, 2003), and this may be the reason for the difference. Submetacentrics and subtelocentrics chromosomes with satellite in the Bradybaenidae were rarely found. In the present study, a secondary constriction was not detected in the chromosome pair.

Further study will need various analyses for the investigation of the karyo-systematical evolution, and accumulation of karyological information is very much required in the family Bradybaenidae.

REFERENCES

- Lee, J.S. and Kwon, O.K. (1993) Chromosomal studies of eight species of Bradybaenidae in Korea. *Korean Journal of Malacology*, **9**: 30-43.
- Inaba, A. (1959) Cytological studies in mollusks. II. A chromosome survey in the stylommatophoric Pulmonata. *Journal of Science of the Hiroshima University, Series B. Division 1*, **18**: 71-93.
- Kawabe, B. (1947) Notes on the chromosomes of Bradybaena (*Ezohelix gainesi*). *La Kromosomo*, **3-4**: 133-134.
- Sun, T. (1995) Chromosomal studies in three land snails. *Sinozoologia*, **12**: 154-162.
- Tatewaki, R. and Kitada, J. (1984) Comparative karyotypes of two land snails, *Euhadra subnimbosa* and *Euhadra dixonii* (Bradybaenidae, Gastropoda). *Proceeding of Japan Acadmy*, **60**: 77-80.
- Tatewaki, R. and Kitada, J. (1987) Karyological studies of five species of land snails (Helicoidea: Mollusca). *Genetica*, **74**: 73-80.
- Tatewaki, R. and Kitada, J. (1994) Karyotype of *Euhadra sandai oki* (Helicoidea: Mollusca). *Chromosome Information Service*, **57**: 17-18.
- Tatewaki, R. and Kitada, J. (1996) Karyotype of a Japanese land snail, *Euhadra eoa* (Helicoidea: Mollusca). *Chromosome Information Service*. **60**: 3-4.
- Tatewaki, R., Kitada, J. and Masuda, O. (1987) Karyotypes of two species of genus *Euhadra*, *Euhadra callizona* and *Euhadra peliomphala simodae* (Bradybaenidae, Gastropoda). *Proceedings of the Japan Academy*, **63**: 293-296.
- Thiriot-Quévieux, C. (2003) Advances in chromosomal studies of gastropod mollusks. *Journal of Molluscan Study*, **69**: 187-201.