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Chromosome Study of Two Similar Lymnaeid Snail Species, Korean *Austropeplea ollula* and an Exotic Species in Australia (Pulmonata: Lymnaeidae)

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ABSTRACT

The chromosomes of two similar lymnaeid snail species, *Austropeplea ollula* from Korea and "*Lymnaea*" sp. introduced to Australia, were karyologically investigated by using an air-dry method. The diploid chromosome number found in *A. ollula* was 32, and that of "*Lymnaea*" sp. was 30. The mitotic chromosome complements of *A. ollula* were 5 metacentric, 9 submetacentric, and 2 subtelocentric pairs. "*Lymnaea*" sp. had 5 metacentric, 8 submetacentric, and 2 subtelocentric pairs. *Austropeplea ollula* is distinguishable from "*Lymnaea*" sp. by their chromosome numbers.

Keywords: *Austropeplea ollula; "Lymnaea viridis";* Chromosomes, Korea, Australia.

INTRODUCTION

Three lymnaeid snail species of the family Lymnaeidae have been reported from Korea, *Radix auricularia coreana, Austropeplea ollula* and *Fossaria truncatula* (Burch *et al.*, 1988). These Korean lymnaeid snail species play an important role as molluscan intermediate hosts for several human intestinal trematodes, e.g., *Fasciola hepatica, Echinostoma cinetorchis*, and *Neodiplostomum seoulense* (Jang *et al.*, 1987; Ahn *et al.*, 1989; Lee, 1993; Lee *et al.*, 19f95; Chung *et al.*, 2001). However, the taxonomic status of *A. ollula* remains as a problem, since *A. ollula* (Gould, 1859) has been placed in the synonymy of *Lymnaea viridis* (Quoy and Gaimard, 1833) from Guam (Hubendick, 1951). Therefore taxonomic distinction between *A. ollula* and *A. viridis* needs to be settled. In an attempt to do that, we obtained specimens of *A. "viridis"* from Australia from Dr. Joseph Boray because we did not have access to specimens of true *A. viridis* from Guam.

Comparative chromosome studies in related species have been of great value for the establishment of systematic relations in many animals and plants. In molluscs, however, the literature on karyotype analysis is not abundant due to difficulties of obtaining mitotic fields with enough quality to carry out chromosome studies. Nevertheless, some chromosome studies of lymnaeid snails have been reported previously by the earlier investigators (Inaba and Tanaka, 1953; Natarajan, 1960; Burch *et al.*, 1964; Burch, 1965; Inaba, 1969). Recently, the chromosome studies of the Korean lymnaeid snail species have been done for confirming their cyto-taxonomic positions (Park *et al.*, 1992; Park and Kim, 1996).

The present study was carried out to compare the karyotypes of Korean *A. ollula* with that of Australian *L. "viridis"*.

MATERIALS AND METHODS

The specimens of *A. ollula* were collected in

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Kangwha, Kyunggi-do, Korea in July 1999, and identified on the basis of taxonomic keys according to Burch *et al.* (1988) and Kwon (1990). The snail specimens of Australian "*L. viridis*" were supplied from the laboratory of Dr. Joseph C. Boray, Elizabeth Macarthur Institute, Camden, Australia, then reared in Department of Parasitology, Inha University College of Medicine, Inchon, Korea. These Australian specimens were originally from a creek in a suburb of Queensland.

Preparations were made from the gonadal tissues of snails by the air-drying method of Kligerman and Bloom (1977), with minor modification. A 0.05% colchicine fluid (0.1 ml) were injected to the gonadal tissues and set aside for 20-24 hours in a moist chamber. The tissues were then dissected and minced with needles in a 0.01% hypotonic NaCl solution. Separated cells were collected by centrifugation at 930 x g for 10 minutes. The cells were fixed in the fresh Carnoy's fixative (3 parts of methyl alcohol and 1 part of glacial acetic acid). The supernatant was replaced with the fresh fixative by the two or more times of centrifugations (930 x g, 10 minutes).

A drop of the cell suspension was then pipetted by a microhematocrit capillary tube and dropped onto a clean glass slide pre-cooled at 4℃. The cells left on the slide were air-dried, and then stained with 4% Giemsa (Gurr R66) solution in 0.1M phosphate buffer (pH 7.0) for 10 minutes. The chromosomes were observed with a Olympus (VANOX) microscope with a 100x (n.a. 1.25) oil immersion objective and a 10x ocular.

To observe the morphological features of the karyotypes, relative and total lengths of the mitotic metaphase chromosomes were measured. Nomenclature of morphological types of chromosomes was followed by the methods of Leven *et al.* (1964). Voucher specimens of the two snail species used in this investigation have been placed in the Museum of Zoology, University of Michigan, Ann Arbor, Michigan 48109, U. S. A.

RESULTS

The mitotic metaphase chromosomes were observed in two lymnaeid snail species, the Korean *A. ollula* and Australian "*L. viridis*" (Fig. 1). The diploid chromosome numbers were 32 (2n= 32) in *A. ollula* and 30 (2n = 30) in "*L. viridis*." The karyotypes were arranged according to their sizes and centromeric positions. The chromosome complements of *A. ollula* have 5 metacentric, 9 submetacentric, and 2 subtelocentric pairs, while "*L. viridis*" showed 5 metacentric, 8 submetacentric, and 2 subtelocentric pairs.

Chromosome measurements are shown in Table 1. In *A. ollula*, the lengths of chromosomes ranged from 2.6 μm. to 3.7 μm., and the mean total length of the diploid complements was 50.2 ± 1.56 μm.. In *L. viridis*, the lengths of chromosomes ranged from 1.8 μm. to 4.7 μm., and the mean total length of the diploid complements was 43.1 ± 1.60 μm.

DISCUSSION

The chromosomes and karyotypes of three Korean lymnaeid snail species have been reported by Park *et al.* (1992) and Park and Kim (1996). In their karyological studies, 17 pairs of chromosomes of *R. auricularia coreana* and 16 pairs of chromosomes of *A. ollula* were observed. Five pairs of metacentric and 12 pairs of submetacentric chromosomes in *R. auricularia coreana*, and 5 pairs of metacentric, 9 pairs of submetacentric and 2 pairs of telocentric chromosomes in *A. ollula* were karyotyped (Park *et al.*, 1992). Fifteen pairs of chromosomes and one non-paired submetacentric sex-determining chromosome in *F. truncatula* were also karyotyped (Park and Kim, 1996). Chromosome numbers and karyotypes of *A. ollula* from Kangwha, Kyunggi-do, Korea observed in this study were in accord with the results of Park *et al.* (1992), suggesting that there was no chromosomal difference between two populations from Kangwha, Kyunggi-do, Korea in this study and from Chunchon, Kangwon-do, Korea in the study of Park *et al.* (1992). In addition, the chromosome numbers of Korean *A. ollula* were the same as those obtained from the Japanese *A. ollula* specimens by Burch *et al.* (1964). However, the chromosome numbers of *A. ollula* (n = 16) were different from those of *A. "viridis"* (n = 15) in this study.

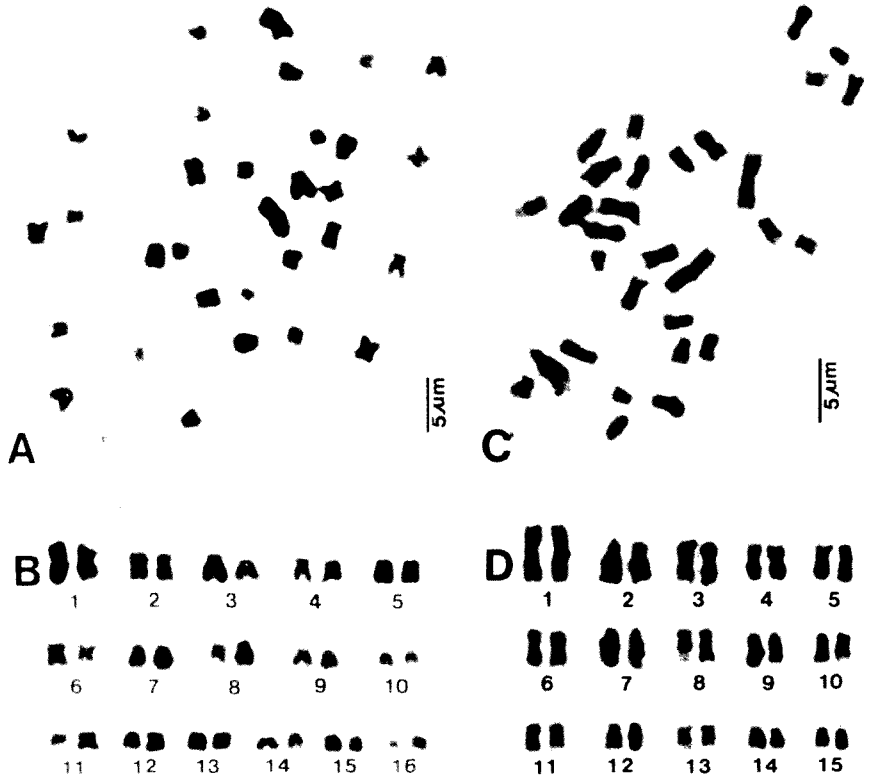

Fig. 1. Mitotic metaphase chromosomes (A) and karyotypes (B) of *Austropeplea ollula*, and mitotic metaphase chromosomes (C) and karyotypes (D) of *Lymnaea viridis*.

Pace (1973) reviewed *Lymnaea* [*Austropeplea*] *ollula* from Ito's monograph (1964) of trematode cercariae in Japan and adjacent territories. He reported that this snail species plays a role as the molluscan intermediate host for several trematodes such as *Echinostoma hortense, E. revolutum, Echinoparyphium recurvatum, Fasciola hepatica* and *Plagiorchis muris*. *Lymnaea* [*Austropeplea*] *ollula* was also reported to be an intermediate host of *Fasciola gigantica* in Hawaii (Alicata, 1938). The Korean *A. ollula* (= *L. ollula*) has been reported as the second intermediate host of *Fasciola* sp. (Jang *et al.*, 1987). On the other hand, some Korean parasitologists reported that *L. viridis* collected in Korea act as the first and second molluscan intermediate hosts of *F. hepatica* (Kim and Shin, 1978; Lee, 1993; Lee *et al.*, 1995). However, it should be mentioned that many Korean former researchers have used the scientific name of *A. ollula* together with *L. viridis* without any conchological and taxonomical comparison of the two nominal species.

This study mainly focused on a cytological approach to solve the taxonomic problem between these two morphologically similar lymnaeid snail species. Previously, Burch *et al.* (1964) and Burch (1965) considered that *L. ollula* (= *viridis*?) belongs to genus *Radix* rather than to genus *Fossaria*, because it seemed cytologically closer to Radix spp. Burch (1965) pointed out that *L. ollula* (= *viridis*?) perhaps should be placed in the genus *Radix* instead of genus *Fossaria*, because its chromosome number (n=16) is closer to *Radix* (n = 17) than to *Fossaria* (n = 18, 19). Pace (1973) reviewed the taxonomic remarks of *A. ollula*, mentioning that *L. ollula* and *L. pervia* are synonyms of *L. viridis* according to Hubendick (1951).

Table 1. Measurement of the mitotic metaphase chromosomes of *Austropeplea ollula* and *Lymnaea viridis*.

Austropeplea ollula†				*Lymnaea viridis*‡		
No. of chromesome	Total length (μm.)	Relative length (μm.)	Type	Total length (μm.)	Relative length (μm.)	Type
1	3.7 ± 0.17	7.4 ± 0.31	M§	4.7 ± 0.16	10.8 ± 0.33	M
2	3.6 ± 0.16	7.2 ± 0.08	M	3.6 ±0.14	8.2 ± 0.28	SM
3	3.4 ± 0.15	6.7 ± 0.40	SM¶	3.5 ± 0.19	8.0 ± 0.32	M
4	3.4 ± 0.07	6.7 ± 0.14	SM	3.3 ± 0.20	7.6 ± 0.41	M
5	3.3 ± 0.12	6.5 ± 0.18	SM	3.2 ± 0.16	7.5 ± 0.26	SM
6	3.3 ± 0.10	6.5 ± 0.18	M	3.2 ± 0.16	7.4 ± 0.23	M
7	3.2 ± 0.09	6.3 ± 0.14	SM	3.0 ± 0.04	7.0 ± 0.08	ST
8	3.2 ± 0.08	6.3 ± 0.15	SM	2.9 ± 0.08	6.7 ± 0.09	SM
9	3.1 ± 0.08	6.2 ± 0.15	SM	2.7 ± 0.12	6.3 ± 0.14	ST
10	3.0 ± 0.02	6.0 ± 0.16	SM	2.5 ± 0.05	5.8 ± 0.09	SM
11	3.0 ± 0.20	6.0 ± 0.20	M	2.4 ± 0.03	5.6 ± 0.07	M
12	3.0 ± 0.06	6.0 ± 0.42	M	2.2 ± 0.07	5.1 ± 0.12	SM
13	2.9 ± 0.06	5.8 ± 0.40	SM	2.1 ± 0.07	4.9 ± 0.12	SM
14	2.8 ± 0.05	5.6 ± 0.35	ST‖	2.0 ± 0.08	4.6 ± 0.13	SM
15	2.7 ± 0.05	5.4 ± 0.37	SM	1.8 ± 0.05	4.2 ± 0.11	SM
16	2.6 ± 0.09	5.2 ± 0.10	ST			

†Measurements were done with seven sets of karyotyped mitotic cells.
‡Measurements were done with four sets of karyotyped mitotic cells.
§M, metacentric chromosome.
¶SM, submetacentric chromosome.
‖ST, subtelocentric chromosome.

Dr. J.P.E. Morrison of the U. S. National Museum concluded that *L. pervia* Martens is a junior synonym of *L.* [*A.*] *ollula* Gould (Burch *et al.*, 1964). The shells of *A. ollula* have quite vertical shape of the whorls and evenly rounded outer apertural lip (Itagaki and Itagaki, 1955; Pace, 1973). However, previous studies have not discriminated clearly between *A. ollula* and *L. viridis*.

Although the identification of these two species is difficult due to the great amount of variability in the morphology of their shells, it is noted that the diploid chromosome number of *A. ollula* is 32, and that of *A. "viridis"* was 30 in this study.

Pace (1973) has reported distribution ranges of *A. ollula*: Japan, Hong Kong (type locality of *Limnaea ollula* Gould 1859), China (Tshi-fu, Shantung, type locality of *Limnaeus pervius* Martens 1867), Philippines, eastern Siberia, India and Burma. For the concrete classification of these vector snails of *Fasciola* spp. infecting man, it is essential to study these lymnaeids compared with specimens of true *A. viridis* from Guam.

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