

Chromosomes of the liver fluke, *Clonorchis sinensis*

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Abstract: A karyological study was carried out in order to compared the chromosome numbers, chromosome morphologies and karyotypes of the oriental liver fluke, *Clonorchis sinensis* (Trematoda: Opisthorchiidae), collected from Korea and China. Chromosome preparations were made by means of air-drying method. The chromosome number was $2n=56$ in both Korean and Chinese flukes, and chromosomes were divided into two groups based on this size; consisting of 8 pairs of large and 20 pairs of small chromosomes. However, the karyotypes showed some differences between Korean and Chinese flukes. The karyotype of liver flukes from Korea consisted of three metacentric pairs, one meta-/submetacentric pair, 16 submetacentric pairs and eight subtelocentric pairs of chromosomes. On the other hand, liver flukes from China consisted of two metacentric pairs, two meta-/submetacentric pairs, 16 submetacentric pairs and eight subtelocentric pairs of chromosomes.

Key words: Chromosome, Karyotype, *Clonorchis sinensis*, Korea, China

INTRODUCTION

Clonorchiasis is an important food-borne parasitic zoonosis in East Asia, being endemic in Korea, Japan, and all parts of China except the northwest, Taiwan and Vietnam. Although reports of this infection are infrequent in western countries, infection can be acquired by eating frozen, dried, or pickled freshwater fish imported from endemic areas (Rim, 1986, 1990). People in endemic areas acquire infection by eating raw or uncooked freshwater fish.

Cytotaxonomic studies of the parasites are important not only for the parasites of systematic analyses, but also for the basic information of the parasitic agents. From this

point of view, chromosomal studies on trematode parasites have been noticeably useful in systematic studies (Sakakuchi and Nakagawa, 1975; Sakaguchi and Tada, 1975; Hirai et al., 1985; Mutafova and Kanev, 1996; Terasaki et al., 1996; Park et al., 1998); but, until recently there have been relatively a few studies on the chromosomes of trematode parasites. Barsiene (1993) reviewed chromosome numbers and karyological data for 230 species of trematodes. Diploid chromosome numbers of the trematodes ranged from 16-22 (Short et al., 1989; Terasaki et al., 1995). Until now, no studies on the chromosome of *Clonorchis sinensis* from Korea and China have been attempted.

The purpose of this study was to analyze the chromosome numbers and karyotypes of the liver fluke, *C. sinensis*, collected from Korea and China.

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Table 1. Total lengths (μm) and relative lengths of chromosomes of *Clonorchis sinensis* from Korea and China

Chromosome No.	Korea ^{a)}			China ^{b)}		
	TL \pm SD	RL \pm SD	Type ^{c)}	TL \pm SD	RL \pm SD	Type
1	12.01 \pm 0.10	8.79 \pm 0.19	M	11.08 \pm 0.16	7.67 \pm 0.08	M/SM
2	10.50 \pm 0.08	7.68 \pm 0.26	SM	10.90 \pm 0.30	7.55 \pm 0.43	SM
3	10.13 \pm 0.33	7.41 \pm 0.13	SM	10.71 \pm 0.14	7.41 \pm 0.14	SM
4	9.76 \pm 0.25	7.14 \pm 0.28	SM	10.16 \pm 0.23	7.03 \pm 0.06	SM
5	8.63 \pm 0.18	6.32 \pm 0.11	M	8.31 \pm 0.02	5.75 \pm 0.25	M
6	8.27 \pm 0.18	6.05 \pm 0.24	M	8.13 \pm 0.21	5.63 \pm 0.27	M
7	8.27 \pm 0.11	6.05 \pm 0.16	M/SM	7.85 \pm 0.33	5.43 \pm 0.09	SM
8	6.76 \pm 0.25	4.95 \pm 0.06	SM	7.66 \pm 0.17	5.30 \pm 0.01	SM
9	4.65 \pm 0.38	3.40 \pm 0.10	SM	5.08 \pm 0.07	3.52 \pm 0.23	SM
10	4.50 \pm 0.30	3.29 \pm 0.33	SM	4.80 \pm 0.13	3.32 \pm 0.17	M/SM
11	4.13 \pm 0.12	3.02 \pm 0.25	SM	4.43 \pm 0.02	3.07 \pm 0.14	SM
12	3.99 \pm 0.15	2.92 \pm 0.08	SM	4.25 \pm 0.24	2.94 \pm 0.23	SM
13	3.90 \pm 0.32	2.85 \pm 0.42	ST	4.16 \pm 0.15	2.88 \pm 0.08	ST
14	3.75 \pm 0.21	2.74 \pm 0.31	ST	3.88 \pm 0.26	2.69 \pm 0.25	ST
15	3.60 \pm 0.03	2.63 \pm 0.04	SM	3.79 \pm 0.06	2.62 \pm 0.11	SM
16	3.53 \pm 0.07	2.58 \pm 0.09	SM	3.69 \pm 0.33	2.55 \pm 0.31	SM
17	3.38 \pm 0.12	2.47 \pm 0.33	SM	3.42 \pm 0.16	2.37 \pm 0.16	SM
18	3.08 \pm 0.07	2.25 \pm 0.40	SM	3.23 \pm 0.10	2.24 \pm 0.22	SM
19	3.00 \pm 0.25	2.20 \pm 0.03	ST	3.14 \pm 0.18	2.17 \pm 0.04	ST
20	2.85 \pm 0.10	2.09 \pm 0.28	ST	2.95 \pm 0.46	2.04 \pm 0.23	ST
21	2.78 \pm 0.12	2.03 \pm 0.15	SM	2.77 \pm 0.07	1.92 \pm 0.15	SM
22	2.63 \pm 0.18	1.92 \pm 0.47	SM	2.75 \pm 0.04	1.90 \pm 0.02	SM
23	2.33 \pm 0.26	1.70 \pm 0.05	SM	2.68 \pm 0.33	1.86 \pm 0.17	SM
24	2.25 \pm 0.08	1.65 \pm 0.27	SM	2.59 \pm 0.15	1.79 \pm 0.13	SM
25	2.18 \pm 0.17	1.60 \pm 0.43	ST	2.54 \pm 0.06	1.76 \pm 0.10	ST
26	2.10 \pm 0.10	1.54 \pm 0.24	ST	2.53 \pm 0.05	1.75 \pm 0.09	ST
27	1.95 \pm 0.16	1.43 \pm 0.28	ST	2.40 \pm 0.12	1.66 \pm 0.14	ST
28	1.73 \pm 0.43	1.27 \pm 0.10	ST	2.03 \pm 0.15	1.41 \pm 0.05	ST

Measurements were done with three sets^{a)} and five sets^{b)} of karyotyped mitotic cells.

TL, total length; SD, standard deviation; RL, relative length

^{c)}M, metacentric; SM, submetacentric; ST, subtelocentric chromosomes

MATERIALS AND METHODS

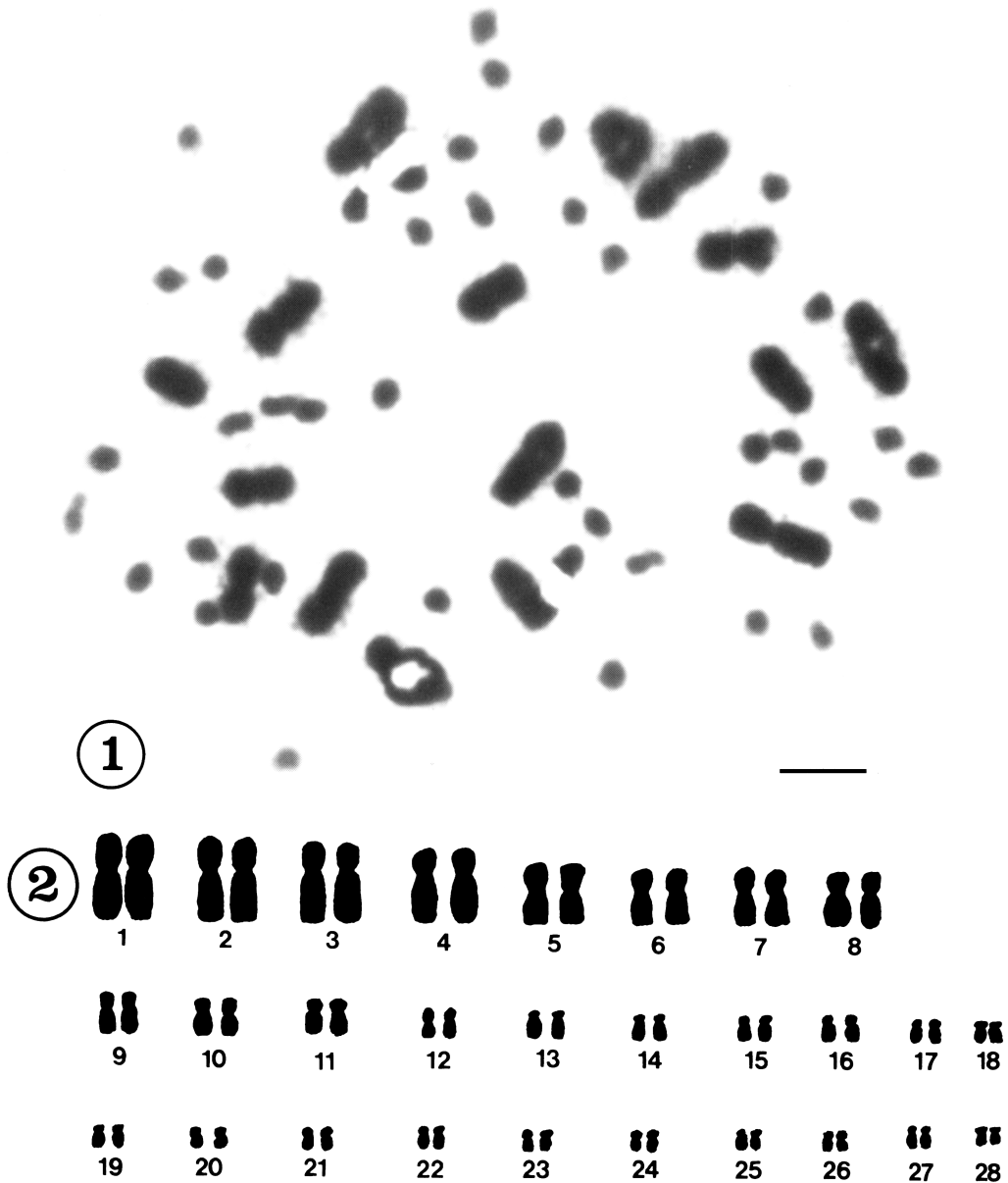
Metacercariae were removed from a fresh-water fish, *Pseudorasbora parva*, collected at Kimhae, Kyungsangnam-Do, Korea and at Shenyang, Liaoning Province, China. Metacercariae were orally inoculated into rabbits. Rabbits were sacrificed 3-5 months after infection and the adult flukes obtained were subjected to cytological examination. The chromosomes in the gonadal tissues of the fluke were studied by the simple cell cultivation method of Ando and Uchida (1973) with minor modification and by Park's (1994)

air-drying method. Morphological features of the chromosomes used to compare karyotypes were the relative length and total lengths of the mitotic metaphase chromosomes. Relative length was expressed as the ratio of absolute chromosome pair length over the total length of the haploid complement $\times 100$. The prepared slides were observed under an Olympus BX50 microscope with a 100X oil immersion objective and a 10X ocular. The nomenclature of the centromeric position for the classification of chromosomes was followed as proposed by Levan et al. (1964).

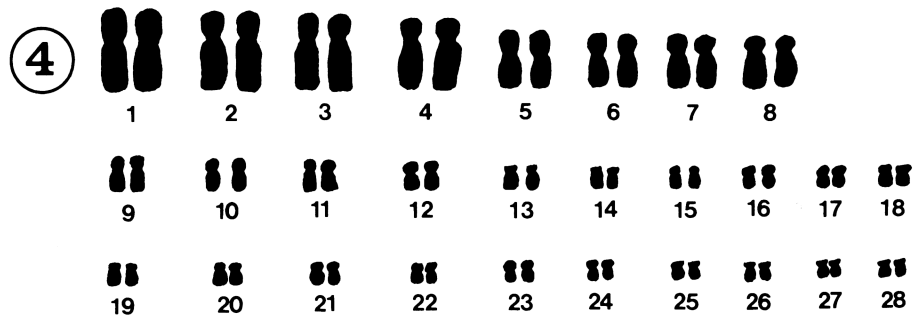
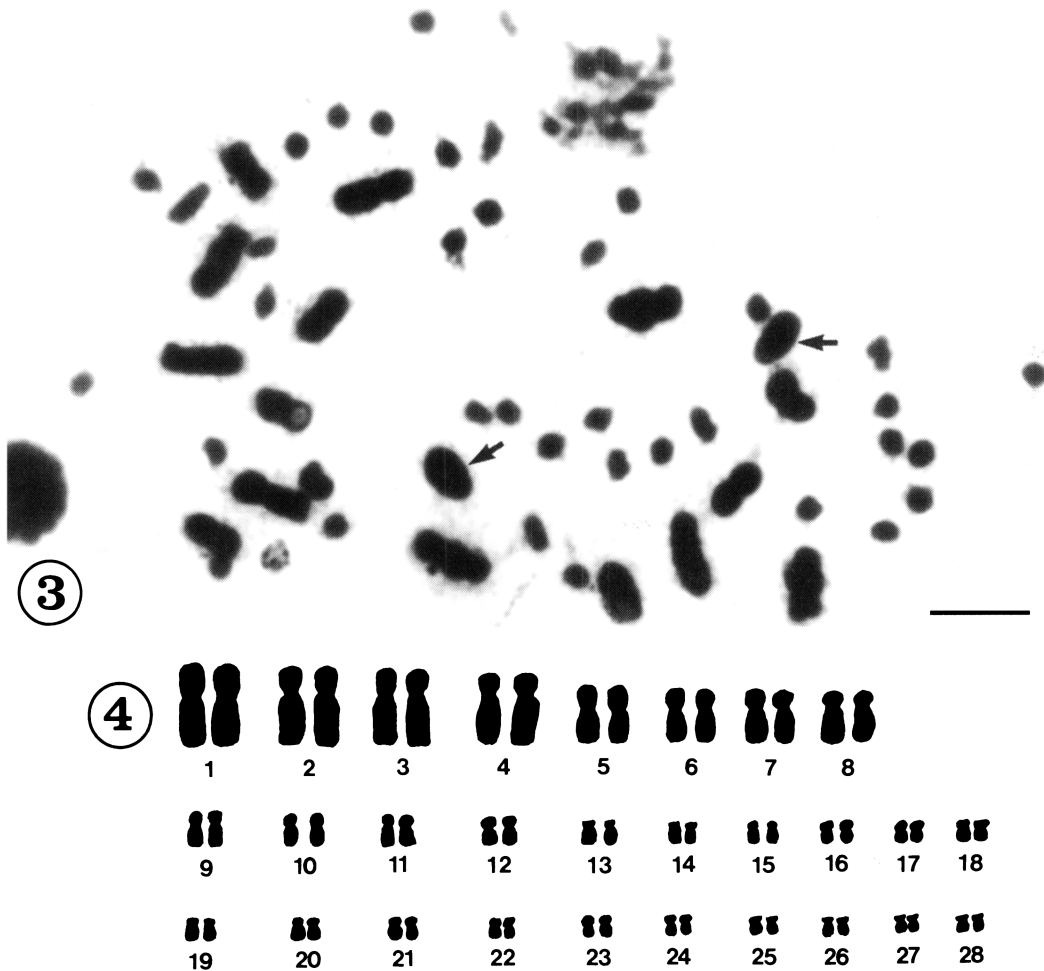
RESULTS

Mitotic chromosomes were observed in 118 cells from 12 individuals in Korea and in 121 cells from 18 individuals in China. All of the metaphase figures of mitosis in *C. sinensis*, 56(2n) chromosomes were recognized (Figs. 1-4). According to the size and shape of the

chromosomes, the karyotypes were established on plates for 28 pairs. Although there were size variations in the chromosomes, the complements were tentatively ordered into pairs according to size. They could be divided into two groups by size, 8 pairs of large and 20 pairs of small chromosomes. Table 1 shows the measurements of mitotic metaphase chromosomes in three sets of karyotyped cells



Figs. 1-2. Mitotic chromosomes of *Clonorchis sinensis* from Korea. **Fig. 1.** Diploid chromosomes in metaphase I. Scale bar=10 μ m. **Fig. 2.** Camera lucida drawings of Fig. 1.



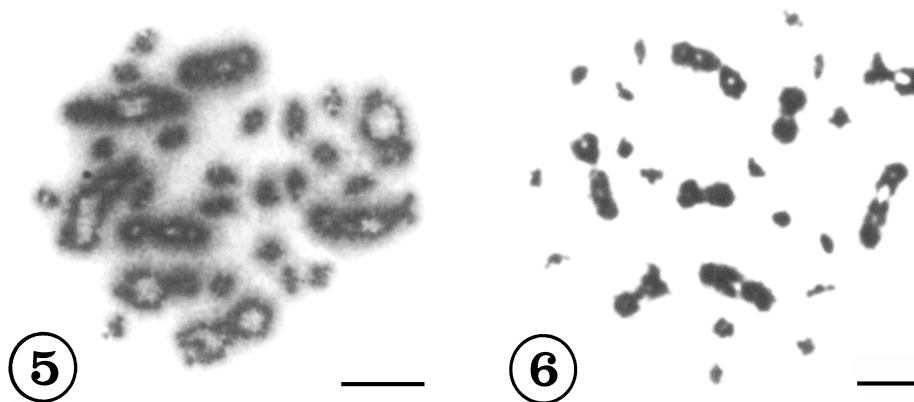
Figs. 3-4. Mitotic chromosomes of *Clonorchis sinensis* from China. **Fig. 3.** Diploid chromosomes in metaphase I. Arrows indicate sperm heads. Scale bar= 10 μ m. **Fig. 4.** Camera lucida drawings of Fig. 3.

from Korea and five sets of karyotyped cells from China population. The karyotypes of Korean population consisted of three metacentric pairs, one meta-/submetacentric pair, 16 submetacentric pairs and eight subtelocentric pairs of chromosomes (Fig. 2). The karyotypes of Chinese population consisted of two metacentric pairs of chromosomes, two meta-/submetacentric pairs, 16 submetacentric pairs and eight subtelocentric pairs of chromosomes (Fig. 4.). The mean lengths of the mitotic metaphase chromosomes ranged from $12.01 \pm 0.10 \mu\text{m}$ to $1.73 \pm 0.43 \mu\text{m}$ from Korea and $11.08 \pm 0.16 \mu\text{m}$ to $2.03 \pm 0.15 \mu\text{m}$ from China. The mean total length of diploid complement was $136.64 \pm 5.1 \mu\text{m}$ in Korea and $144.44 \pm 4.39 \mu\text{m}$ in China. The shapes of

the various diakinesis bivalents that have been observed in *C. sinensis* are shown in Figure 5 and 6.

DISCUSSION

The morphology of chromosomes among individuals of the same species is sufficiently constant that karyotypes are recognized as a definite species character (White, 1973). The study of chromosome numbers has been used as a valuable complement to biochemical methods for identifying species, hybrids and more rarely populations. It has also been of particular importance in the application of polyploidy. In fact, relationships within groups of species cannot be considered complete, in



Figs. 5-6. The meiosis diakinesis bivalents of *Clonorchis sinensis*. **Fig. 5.** *C. sinensis* from Korea. **Fig. 6.** *C. sinensis* from China. Scale bars= 10 μ m.

an evolutionary sense, without good cytotaxonomic data to reinforce conclusions drawn from morphological criteria.

Barsiene (1993) reviewed chromosome numbers and karyological data for 230 species of trematodes. Chromosome numbers of trematodes ranged from $2n = 16$ for *Schistosoma mansoni* (Short et al., 1989), $2n = 20$ for *Fasciola* spp. (Yin and Ye, 1990), *Neodiplostomum seoulense* (Park et al., 1998), *Pegosomum asperum* and *P. saginatum* (Aleksandrova and Podgornova, 1978) to $4n = 44$ for *Paragonimus westermani* (Terasaki et al., 1995). In this study, the chromosome number of *C. sinensis* from Korea and China was $2n = 56$. Until now, no *C. sinensis* have been investigated cytologically prior to this study. Karyotype studies prove of greatest value to systematic study when used with more closely related species. The mean total length of the diploid complements of liver flukes from China was a little longer than those from Korea. Sasaki (1961) stated that individual chromosomes in the metaphase stage usually exhibited considerable variation in length due to varying degrees of condensation on decomposing chromatids on mammalian somatic chromosomes. By his result, highly contracted chromosomes tend to have their centromeres more median than less contracted ones in different cells, while longer chromosomes tend to contract more strongly than shorter ones in the same cell by colchicine treatment. In this chromosomal

study, we made preparations in the same conditions. We expressed the lengths of individual chromosomes as relative lengths in terms of a percentage of the haploid autosomal complement. Actually, this air-drying technique produces a more uniform and complete flattening of cells than can be achieved by the usual squash method.

The karyotypes of *C. sinensis* from Korea and China were different. Differences in chromosome morphology could be due to inversions, additions, deletions, etc. which might still represent within species variability. Nonetheless, this speculation enhances the authors' conclusion that more study is warranted for these groups of flukes. This chromosomal data will provide basic information on the liver flukes, which can then be used for genome study.

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