CHROMOSOMES AND C-BANDING OF *OPISTHORCHIS VIVERRINI*

Chalit Komalamisra

Department of Helminthology, Faculty of Tropical Medicine, Mahidol University
420/6 Rajavithi Road, Bangkok 10400, Thailand

Abstract. Chromosome of *Opisthorchis viverrini* was observed by air-drying and C-banding techniques. The chromosome number was 2n=12 and n=6 consisting of one large-sized metacentric, one medium-sized metacentric, one small-sized metacentric, one small-sized submetacentric or subtelecentric, one small-sized subtelocentric or acrocentric and one small-sized acrocentric. The relative lengths of the chromosomes were 32.02 ± 2.52, 23.28 ± 1.98, 15.24 ± 3.40, 13.39 ± 3.11, 10.18 ± 1.56 and 5.82 ± 0.59 % respectively. After C-banding treatment, two of the small-sized chromosomes showed a remarkable constitutive heterochromatin.

INTRODUCTION

The liver fluke, *Opisthorchis viverrini* has been recognized as the only species of harmful parasite being associated definitively with human cancer (cholangiocarcinoma) in Thailand. Many aspects of this parasite have been studied, while its chromosomes have not yet been investigated. A small number of reports have been available in the literature and there have been a few studies on the chromosome of genus *Opisthorchis*. Jha (1975) reported that the chromosome of *O. felineus* was 2n= 16, n=8 and *O. germinus* was 2n=18, n= 9. Cho (1978) also reported that the chromosome number of *Clonorchis sinensis (Opisthorchis sinensis)* was 2n= 14, n=7. This paper aimed to check and demonstrate the chromosome of *O. viverrini* using conventional Giemsa staining as well as C-banding methods.

MATERIALS AND METHODS

Specimen preparation

Cyprinoid fishes, known to be the intermediate host of *O. viverrini* were collected from a large swamp in the Chonabot district, Khon Kaen Province, northeast Thailand. The metacercariae of *O. viverrini* were obtained from these fishes. After oral inoculation of metacercariae, 40-50 days later, adult specimens were recovered from the livers of golden hamsters.

Chromosome preparation

The Terasaki (1977) air-drying technique was modified and used for chromosome preparation. Live specimens were put into a colchicine-salt solution (0.005% w/v colchicine in 0.85% sodium chloride solution) for three hours at 37°C. One ovary and two testes per adult fluke were taken out with a micropin under a dissecting microscope. Then each of the ovaries and testes was put on a glass slide with a few drops of 0.6% sodium citrate solution, and was broken with a micropin under a dissecting microscope. Germ cells were spread out on the glass slide and were kept at room temperature for thirty minutes. Then these glass slides were put into a moisture box which contained Carnoy solution (methyl alcohol 1: acetic acid 1). After thirty minutes, Carnoy solution was put onto the glass slides with a pipette and five minutes later the solution was shed and blown dry. All slides were stained with 4% Giemsa solution pH 6.8 for thirty minutes at room temperature before conventional investigation.

C-banding technique

Chromosome banding of *O. viverrini* was performed by the Hirai (1985) method with a little modification. Dried chromosome preparations were left in a 37°C incubator for at least 1 week and then treated directly with 5% barium hydroxide solution for 10 minutes, followed by 2SSC (Standard saline-citrate) solution for 60 minutes. Both treatments were set at 60°C. Slides were stained with 4% Giemsa solution pH 6.8 for 90 minutes at room temperature. All preparations were then used for microscopic observation.

RESULTS

In the metaphase figures of germ cells of *O. viverrini*, the chromosome number was 2n=12, n=6 and divided into three groups (large, medium and small) according to their size. Based on chromo-

Table 1
Comparison of the chromosome number of the present study with other reported species.

Species	2n	n (chr nomenclature)	L	M	S	
Clonorchis sinensis	14	7 (2m+5st/a)	2	0	5	Cho, 1978
Opisthorchis felineus (India)	16	8	2	3	3	Jha, 1975
O. felineus (Siberia)	14	7 (2m + 3sm + 2a)				Ilyinskikh, 1982
O. germinus	18	9	2	6	1	Jha, 1975
O. viverrini	12	6 (3m+1sm/st+1st/a+1a)	1	1	4	Present study

Abbreviations: chr=chromosome, 2n=diploid number; n= haploid number; L= number of large-sized chromosome; M= number of medium-sized chromosome; S= number of small-sized chromosome, m=metacentric, sm= submetacentric, st= subtelocentric, a= acrocentric, sm/st=submetacentric or subtelocentric, st/a= subtelocentric or acrocentric

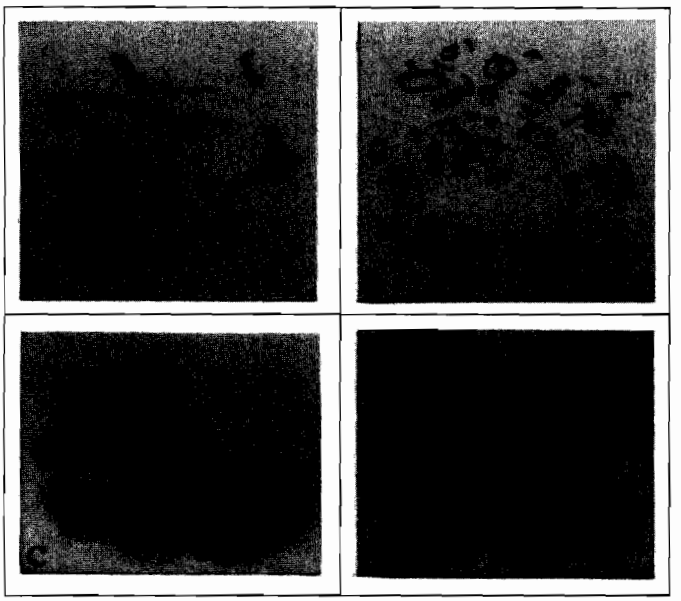

Fig 1–(A-D) Chromosomes of *Opisthorchis viverrini*, In D: Meiosis metaphase showing C-banded chromosomes.

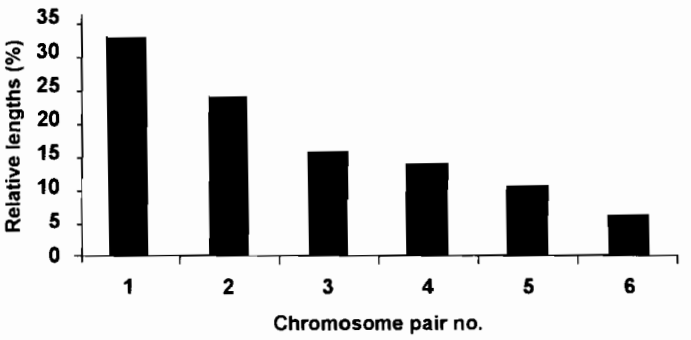

Fig 2–A bar chart showing relative lengths of chromosomes of *O. viverrini*.

some nomenclature recommended by Levan *et al* (1964), they consisted of one large-sized metacentric, one medium-sized metacentric, one small-sized metacentric, one small-sized submetacentric or subtelecentric, one small-sized subtelocentric or acrocentric and one small-sized acrocentric. On the basis of 27 metaphase figures, the relative length of each chromosome was calculated and the lengths are shown in Fig 2 and Table 2. After C-banding treatment, two of the small-sized chromosomes of *O. viverrini* showed a remarkable constitutive heterochromatin (Fig 1).

DISCUSSION

Jha (1975) studied the chromosome numbers of liver flukes from sectioned materials in India and found that the chromosome numbers of *O. felineus* and *O. germinus* were 2n = 16, n = 8 and 2n =18, n = 9 respectively. Cho *et al* (1978) also reported that the chromosome number of *C. sinensis* in Japan was 2n =14, n =7. Ilyinskikh (1982) found that the chromosome number of *O. felineus* in Siberia was 2n =14, n =7. The present study revealed that the chromosome number of *O. viverrini* in Thailand was 2n =12, n = 6, which was remarkably different from *O. felineus, O. germinus* and *C. sinensis* (Table 1).

Table 2
Measurement of the chromosome of *O. viverrini.*

Chromosome pair no.	Relative length* (mean ± SD)
1	32.02 ± 2.52
2	23.28 ± 1.98
3	15.24 ± 3.40
4	13.39 ± 3.11
5	10.18 ± 1.56
6	5.82 ± 0.59

*Relative length = (length of each chromosome / total length of whole chromosomes) x100.

C. sinensis, *O. felineus* and *O. viverrini* have been known to be the causative agents of liver fluke infection in a number of countries. *O. viverrini*, has been found to be the only species of liver fluke infection in Thailand (Maleewong *et al,* 1992; Radomyos *et al*, 1994; 1998) while *C. sinensis* is prevalent in China, Taiwan, Korea, Japan and North Vietnam (Choi, 1984; Cross, 1984; Chen *et al*, 1997). *O. felineus* used to be prevalent in Central and Eastern Europe and Siberia, especially in Germany and Poland (Beaver *et al*, 1984); currently this fluke is still endemic in Germany, Siberia and the Ukraine (Hinz, 1991; Hering-Hagenbeck *et al*, 1996; Loktieva,1997). *O. viverrini* and *O. felineus* are closely related species in terms of morphology of the adult flukes except that they are a little different in the shape of the testes and vitellaria, while both species are markedly different from *C. sinensis* (Beaver *et al*, 1984). The morphological characteristics of eggs and metacercariae of the three species are similar and difficult to differentiate from each other. Therefore, it is considered that the significant differences of chromosome numbers among *O. viverrini*, *O. felineus*, and *C. sinensis* may reflect genetic distinctions of the respective parasite groups, geographic distances and their biological differences. The finding may be used for identification and taxonomic purposes, especially in distinguishing between *O. felineus* and *O. viverrini.*

C-banding pattern has been used to study several aspects of closely related parasites and primates (Hirai *et al*, 1985; Zhao *et al*, 1989; Tan and Li 1990; Pieczarka *et al.*1998). The present study revealed that C-band formation in the chromosome of *O. viverrini* was only seen in two of the small-sized chromosomes (Fig 1). Up to the present time, there have been no reports concerning this aspect in other species of *Opisthorchis* and *Clonorchis*. This paper is the first report of demonstrated C-banding in the chromomsome of *O. viverrini*. Further study should emphasize phylogeny among the three species of liver flukes from different localities using biochemical and molecular analysis.

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