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A cytogenetic study on human intestinal trematodes of the genus *Metagonimus* (Digenea: Heterophyidae) in Korea

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Abstract: In order to analyze chromosome numbers and karyotypes of intestinal trematodes belonging to the genus, *Metagonimus*, the gonad tissues of *M. takahashii*, *M. miyatai*, and *M. yokogawai* were prepared and examined. The number of bivalents in the first meiotic division of *M. takahashii* was nine (n=9). The diploid number of *M. miyatai* was observed to be eighteen (2n=18) and their chromosomes consisted of one pair of metacentric, 7 pairs of submetacentric, and one pair of telocentric chromosomes. The diploid number of *M. yokogawai* was thirty-two (2n=32) and the chromosome complements were composed of two pairs of metacentric, 11 pairs of submetacentric, and three pairs of subtelocentric chromosomes. These results could be a supporting evidence for the validity of the new species, *M. miyatai*, distinct from *M. yokogawai*

Key words: karyotyping, *Metagonimus yokogawai*, *Metagonimus miyatai*, *Metagonimus takahashii*, taxonomy

INTRODUCTION

Metagonimiasis is an endemic intestinal trematode infection in Korea with 0.3% egg positives in the general population (i.e. 130,000 infected people) (Chai et al., 1993; Yu et al., 1994; Ministry of Health and Welfare and Korea Association of Health, 1997). Three species of the genus *Metagonimus* are found in Korea: *M. yokogawai* (Katsurada, 1912), *M. takahashii* (Suzuki, 1930), and *M. miyatai* (Saito et al., 1997). These species can be differentiated not only by their morphologies, geographical distributions, and their intermediate hosts which are mainly fish species, but also by their polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) patterns of the genes for 18 S rRNA, 5.8 S rRNA, and internal transcribed spacer 1 (ITS1) and by their random amplification of polymorphic DNA patterns (Saito et al., 1997; Yu et al., 1997a, 1997b; Chai et al., 1998). Chromosomal studies on trematode parasites have been significantly useful in taxonomic studies (Terasaki et al., 1982; Hirai et al., 1985; Park et al., 1998). In 1959, Walton phylogenetically studied the chromosome numbers of more than 50 species of helminthic parasites and showed definite chromosome numbers at the genus level. However, there have been few studies on karyotypes of human intestinal trematodes. In the present study, we

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compared the karyotypes of 3 species of *Metagonimus* in order to evaluate the taxonomical positions.

MATERIALS AND METHODS

Twenty adult specimens each of *M. takahashii, M. miyatai,* and *M. yokogawai* were obtained from rats (Sprague-Dawley, 4 to 6-week-old) at 7 days post-infection (PI). Metacercariae of each species were collected from *Carassius carassius, Zacco platypus,* and *Plecoglossus altivelis,* respectively (Saito et al., 1997; Yu et al., 1997b). After collecting flukes from the intestine of rats, they were cultivated in 1X liquid MEM-199 medium (GIBCO BRL Lab., Ontario, Canada, pH 7.4) containing 0.001% colchicine, at 37°C for 4 hr. Flukes were then fixed with modified Carnoy's fluid (1 part glacial acetic acid, 3 parts of 95% ethanol). The testis of each specimen was gently removed with a dissecting pin and transferred to the fixative using a fine forcep. Pieces of other tissues were removed as well. The testes were then minced gently in 45% acetic acid to prepare cell suspensions. The cells left on the slide were stained with acetic-orcein solution for 10 to 30 min. The over-stained cells were rinsed briefly with 45% acetic acid, covered with a cover glass, and then squashed. The slides were heated briefly with an alcohol lamp to remove air bubbles and mounted in Canada balsam. Observations were done under a light microscope. The absolute length of chromosomes, the total chromosome length of the haploid set, and the positions of centromeres were compared by each species. The classification of chromosomes was adopted according to the methods of Levan et al. (1964).

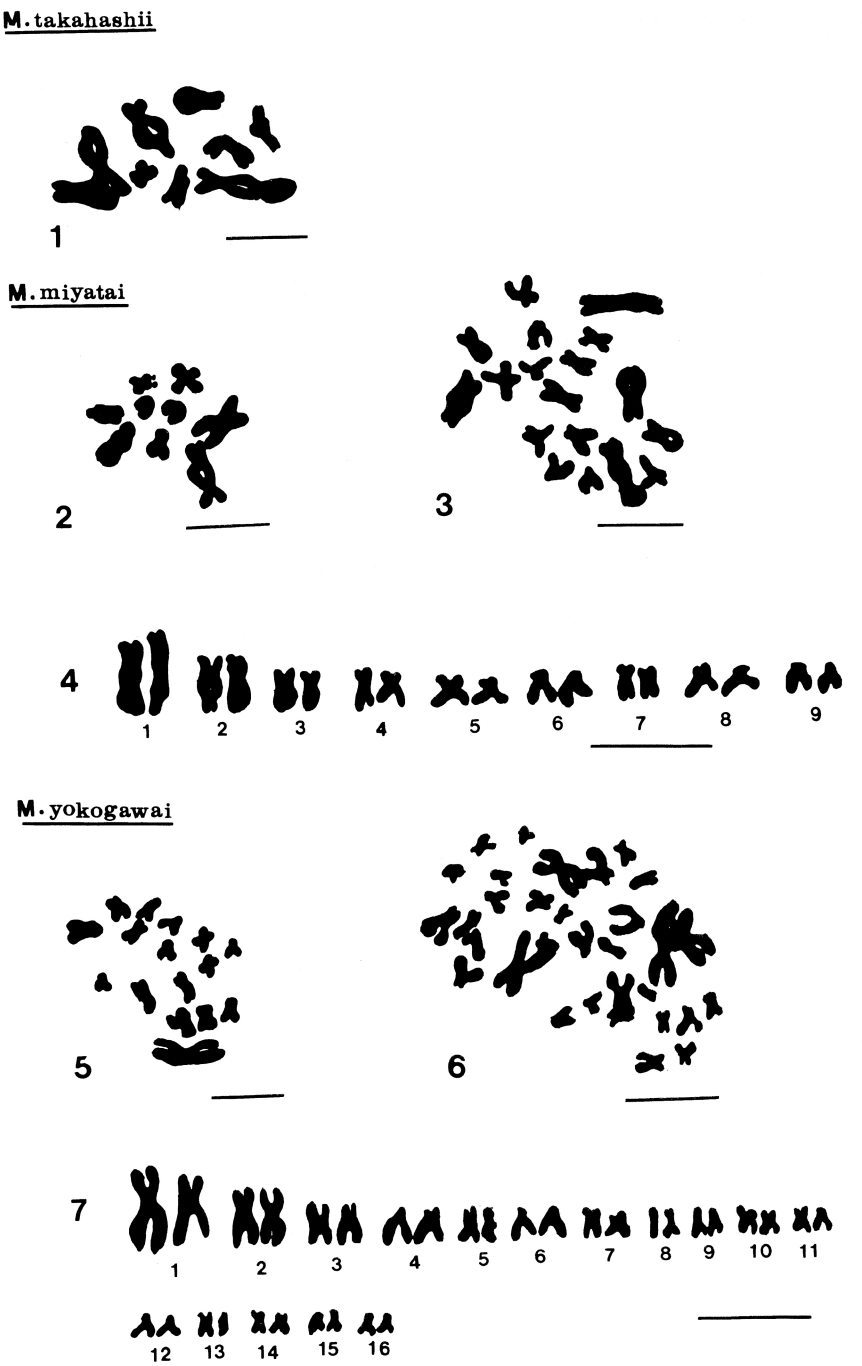
RESULTS

The number of bivalents of *M. takahashii* was nine at the meiotic metaphase of the primary spermatocytes (Fig. 1). However, diploid chromosomes of dividing spermatogonia were not detected. The spermatogonial metaphase of *M. miyatai* was observed and the diploid number was counted as eighteen (2n=18). Counted chromosomes were consisted of one pair of large metacentric (m) chromosomes (No. 1 of Fig. 4), seven pairs of submetacentric (sm) chromosomes (Nos. 2-8 of Fig. 4), and one pair of telocentric (t) chromosomes (No. 9 of Fig. 4) (2n=18: n=1m+7sm+1t). The length of chromosome pairs of *M. miyatai* was 3.19 -23.0 μm and the mean total length of the haploid components was 29.25±0.24 μm (Table 1; Fig. 4). The haploid state of *M. yokogawai* has a chromosome number of sixteen (n=16). Diploid number of *M. yokogawai* was thirty-two (2n=32) (Figs. 5, 6). The karyotype consisted of two pairs of metacentric (m) chromosomes (Nos. 1-2 of Fig. 7), 11 pairs of submetacentric (sm) chromosomes (Nos. 3, 5, 7-11, 13-16 of Fig. 7), and three pairs of subtelocentric (st)

Table 1. Measurements and classification of chromosomes of *Metagonimus miyatai* (2n=18)

Chromosome No.	Relative length[a)] (%)	Chromosome length (μm)	Type[b)]
1	23.93±0.01	7.00±0.01	m
2	17.09±0.00	5.00±0.03	sm
3	11.79±0.03	3.45±0.01	sm
4	11.11±0.02	3.25±0.04	sm
5	7.69±0.01	2.25±0.03	sm
6	7.35±0.00	2.15±0.02	sm
7	7.17±0.04	2.10±0.03	sm
8	7.00±0.03	2.05±0.05	sm
9	6.83±0.02	2.00±0.03	t
Total		29.25±0.24	

[a)]Relative length of each chromosome divided by the total length of the whole chromosome. [b)]Type of chromosomes: m, metacentric; sm, submetacentric; t, telocentric.



Figs. 1-7. Camera lucida drawings of meiotic and mitotic phase of chromosomes of *Metagonimus* spp. stained with aceto-orcein. Bar=10 μm. **1.** Meiotic phase of 9 metaphase I chromosomes of *Metagonimus takahashii* (n=9). **2.** Meiotic phase of 9 metaphase I chromosomes of *Metagonimus miyatai* (n=9). **3.** Mitotic metaphase of 18 chromosomes of *M. miyatai* (2n=18). **4.** Arrangement of mitotic metaphase chromosomes of *M. miyatai* from a drawing of Fig. 3. **5.** Meiotic phase of 16 metaphase I chromosomes of *Metagonimus yokogawai* (n=16). **6.** Mitotic metaphase of 32 choromosomes of *M. yokogawai* (n=32). **7.** Arrangement of mitotic metaphase chromosomes of *M. yokogawai* from Fig. 6.

Table 2. Measurements and classification of chromosomes of *Metagonimus yokogawai* (2n=32)

Chromosome No.	Relative length[a)] (%)	Chromosome length (μm)	Type[b)]
1	20.30±0.16	8.07±0.05	m
2	11.19±0.08	4.45±0.06	m
3	7.47±0.03	2.97±0.05	sm
4	7.47±0.01	2.97±0.02	st
5	6.39±0.11	2.54±0.10	sm
6	6.19±0.05	2.46±0.08	st
7	5.11±0.23	2.03±0.05	sm
8	5.10±0.09	2.09±0.04	sm
9	5.01±0.08	1.99±0.01	sm
10	4.58±0.03	1.82±0.04	sm
11	4.15±0.09	1.65±0.05	sm
12	3.72±0.04	1.48±0.03	st
13	3.62±0.02	1.44±0.01	sm
14	3.30±0.10	1.31±0.06	sm
15	3.19±0.01	1.27±0.05	sm
16	3.19±0.01	1.27±0.03	sm
Total		39.81±0.73	

[a)]Relative length of each chromosome divided by the total length of the whole chromosomes. [b)]Type of chromosomes: m, metacentric; sm, submetacentric; st, subtelocentric.

chromosomes (Nos. 4, 6, 12 of Fig. 7) (2n=32: n=2m+11sm+3st). The length of chromosomes of *M. yokogawai* ranged from 1.27 to 8.07 μm and the mean value of the total length of haploid components was 39.81±0.73 μm (Table 2; Fig. 7).

DISCUSSION

Karyotype data of human intestinal trematodes are rare (Walton, 1959). The diploid chromosome number of *Neodiplostomum seoulense* has been described as twenty (2n=20) (Park et al., 1998), and that of *Echinostoma hortense* and *Echinostoma cinetorchis* were twenty (2n=20) and twenty-two (2n=22), respectively (Terasaki et al., 1982). The present study revealed that the diploid chromosome number of *M. yokogawai* was thirty-two (2n=32) and that of *M. miyatai* was eighteen (2n=18). Therefore, these results could be an additional supporting evidence for the validity of new species, *M. miyatai*, distinct from *M. yokogawai* (Saito et al., 1997). Meanwhile, the presence of 9 bivalents in the first meiotic division of the primary spermatocytes of *M. takahashii* signified the existence of 18 chromosomes in the organism. Each of 9 bivalents consisted of two homologous chromosomes in synapsis. Therefore, we attempted to observe diploid chromosomes from *M. takahashii* several times, but we were not successful in obtaining the result.

The karyological results of our study showed the same haploid chromosome number of nine (n=9) from both *M. miyatai* and *M. takahashii*; however, the PCR-RFLP patterns of three *Metagonimus* spp. with a ITS1 site of rRNA and the mitochondrial cytochrome *c* oxidase genes showed different results with the estimation that the genetic distance between *M. yokogawai* and *M. takahashii* was closer than that of the distance between *M. miyatai* and *M. takahashii* (Yu et al., 1997b). Other related genes could probably give more information about the phylogenic distance.

Sasada (1978) reported 7 bivalents in the primary oocytes and the primary spermatocytes of *M. yokogawai* from Japan (2n=14), which did not coincide with the result obtained from the present studies. It is quite uncertain whether or not the difference is due to the intraspecific polymorphism or due to the intercellular junction of the gonocyte. It is necessary to simultaneously compare chromosomes of *M. yokogawai* from Korea

with those from Japan to further verify. Despite the fact that chromosome size and number will reflect the size of the whole genome, less attention has been paid to the length of the mitotic metaphase chromosomes or to the total length of the mitotic metaphase chromosomes. In this study, variations in the chromosome size occurred. The squash method has been reported to produce larger size of chromosome than the air-drying method. Sasaki (1961) reported that the process of chromatid condensation is not always uniform or synchronous in all complements at a given stage of the mitotic cycle. When using fresh gonadal tissue directly, as in our study, it is especially difficult to gain a large number of uniform chromosome complements. Also, there are still more problems to be solved before the karyotypes can be analyzed in detail. It is, however, possible to qualitatively analyze chromosome sets clearly.

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