

□ Brief Communication □

Karyotype analysis of *Neodiplostomum seoulense*

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Abstract: A karyotype analysis of the chromosome of *Neodiplostomum seoulense*, one of causative agents of human intestinal trematodiasis, was done from the gonad tissue by the squashing method. The chromosome number was $n=10$ and $2n=20$. Chromosome length was 1.30-4.0 μm . Chromosome pairs in the complement consisted of two pairs of metacentric, five pairs of submetacentrics/subtelocentric and three pairs of telocentric chromosomes. These data were comparable with those of other intestinal trematodes.

Key words: *Neodiplostomum seoulense*, karyotype, chromosome, trematoda

Neodiplostomum seoulense (Trematoda: Neodiplostomatidae) is one of causative agents of human intestinal trematodiasis (Hong and Shoop, 1995). A human infection is acquired by ingesting raw or half-done frogs or snakes. This 1 mm long trematode has been reported only in Korea (Hong *et al.*, 1986). We aimed to compare the karyotypes of *N. seoulense* with those of other intestinal trematodes as a basic taxonomic work and a basic study for the gene.

Metacercariae of *N. seoulense* were obtained after the artificial digestion of the mesentery and muscle of the European glass snake (*Rhabdophis tigrinus tigrinus*), and adult worms were recovered from rats (Sprague-Dawley, 4 to 6-week-old) seven days post-infection. The cytological preparations were made with tissue fixation followed by acetic-orcein squash procedures (Session, 1996). Adult worms were incubated in 0.05% colchicine for 2 hr at room temperature, and

fixed with modified Carnoy's fluid (1 part 100% glacial acetic acid, 3 parts of 95% ethanol). The testis of each specimen was gently removed with a dissecting pin and transferred with a fine forceps to the fixative, and the collected testis tissue was moved to fresh fixative. Tissues were then minced gently in 45% acetic acid to prepare a cell suspension. The cells left on the slide were stained with

Table 1. Chromosome lengths and karyotype of *Neodiplostomum seoulense*^{a)}

Chromosome No.	Chromosome length (μm)	Type ^{b)}
1	4.0 \pm 0.20	M
2	3.7 \pm 0.36	M
3	3.6 \pm 0.19	T
4	3.5 \pm 0.25	T
5	3.3 \pm 0.09	SM/ST
6	2.4 \pm 0.23	T
7	2.2 \pm 0.18	SM/ST
8	1.8 \pm 0.03	SM/ST
9	1.5 \pm 0.25	SM/ST
10	1.3 \pm 0.27	SM/ST

^{a)}Measurements were made from two sets of chromosome of *N. seoulense*.

^{b)}M, metacentric; SM/ST, submetacentric or subtelocentric; T, telocentric chromosome.

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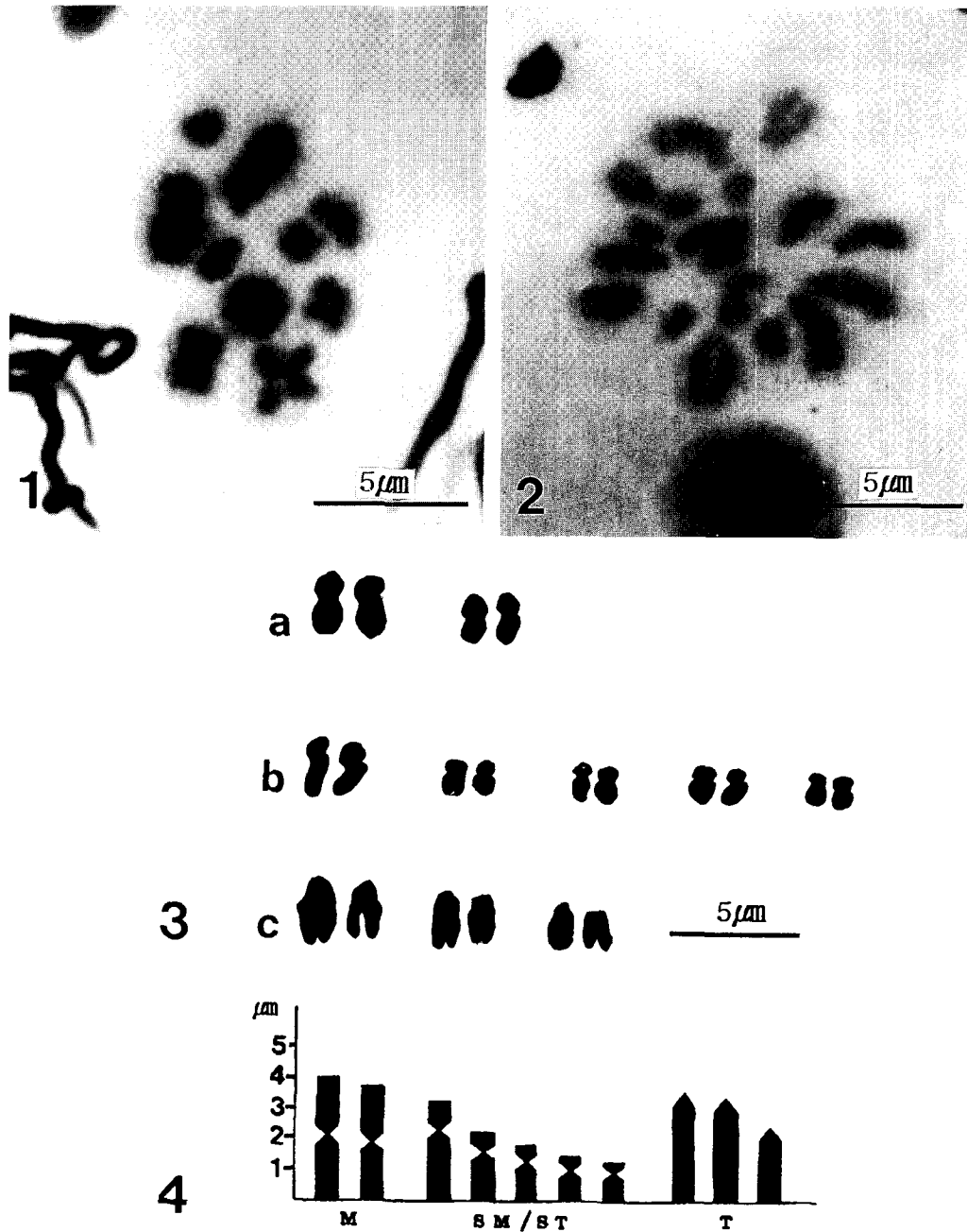


Fig. 1. Spermatogonial chromosomes of *Neodiplostomum seoulense* in meiotic phase ($n=10$). **Fig. 2.** Mitotic phase of *N. seoulense* ($2n=20$). **Fig. 3.** Karyotype constructed from Fig. 2 ($2n=20$). The homologous pairs have been arranged in type. a, metacentric chromosome; b, submetacentric chromosome; c, telocentric chromosome. **Fig. 4.** Schematic representation of the karyotype of *N. seoulense*. M, metacentric; SM/ST, submetacentric or subtelo-centric; T, telocentric chromosome.

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 then heated briefly on an alcohol lamp to remove air bubbles and mounted with Canada balsam. The chromosome length of the diploid set and chromosome types were compared. The nomenclature of chromosomes was adopted according to Levan *et al.* (1964).

A total of 10 well-spread metaphase plates was photographed and analyzed. Observed chromosomes ranged from 1.30 μm to 4.0 μm in their length (Table 1). The chromosome number was $n=10$ and $2n=20$. They consisted of two pairs of metacentric chromosomes, five pairs of submetacentric/subtelocentric chromosomes and three pairs of telocentric chromosomes (Figs. 1-4).

The karyological data of chromosomes of human intestinal trematodes are meager: *Metagonimus yokogawai*, $n=16$ and $2n=32$; *M. takahashii*, $n=9$; *M. miyatai* $n=9$, $2n=18$; *Echinostoma hortense* $2n=20$; *Echinostoma cinetorchis*, $2n=22$ (Terasaki *et al.*, 1992). Of the chromosomes of *N. seoulense*, the heteromorphic pair was observed. It is believed to be originated from the variation by the effect of colchicine (Grossman *et al.*, 1981). The above

data provide the basic information on the karyotype of *N. seoulense* in Korea, which can be used for the genome approach.

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=초록=

서울주걱흡충 염색체 핵형 분석

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우리 나라 인체 장흡충의 하나인 서울주걱흡충의 염색체 핵형 분석을 위하여 고환에서 압착법으로 염색체를 분리하여 관찰하였다. 분석 결과 $n=10$, $2n=20$ 이며, 2쌍의 중앙중심절 염색체 (metacentric chromosome), 5쌍의 아래중앙중심절/아래끝중심절 염색체 (submetacentric/subtelocentric chromosome), 3쌍의 끝중심절 염색체 (telocentric chromosomes)로 구성되어 있었다. 이 결과는 앞으로 계통 연구 등의 기본 자료로 쓰일 수 있을 것이다.

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1998년도 제36권 상호심의에 참여하신 분께 감사드립니다.

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