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Comparative Studies on the Karyotypes of *Echinostoma cinetorchis* and *E. hortense* (Echinostomatidae: Trematoda)

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Only a few reports have been made concerning the karyotypes of Echinostomatidae. First of all, Rees (1939) reported that the chromosome number of *Parorchis acanthus* (Nicoll, 1906) was n=11, 2n=22, after his observaiton of the germ cells in sectioned specimens. Churchill (1950, 1951) studied the germ cell cycle of *Echinostoma revolutum* (Froelich, 1802), and found that its diploid chromosome number was twenty-two. Both reports, however, cover little information on the karyotype of these species in detail.

Materials and Methods

Living loaches, *Misgurnus anguillicaudatus*, gained from a dealer in Akita and from another dealer in Yanagawa, Fukuoka, were fed to 16 and 35 rats, respectively. Several loaches were given to each rats. From 14 to 73 days after the feeding, the rats were anatomized and 99 *E. cinetorchis* Ando et Ozaki, 1923 and 255 *E. hortense* Asada, 1926 were collected from their small intestines and were used for the karyotypical analyses.

A simple cell cultivation method of Ando and Uchida (1973) was modified (Terasaki and Nakamura, 1978), and applied to the karyotypical analysis of germ cells of *E. cinetorchis* and *E. hortense*. The preparations were made and used for microscopic observation.

Good metaphase figures of mitosis and meiosis found in each of the preparations were photographed under magnification of ×2,500. Ten photographs of metaphase figures of mitosis in each species were used for karyotypic analyses. The chromosomes were arranged in order of their size as shown in Plate 1C and Plate 2C. The length of long and short arms of each mitotic chromosome was measured by a slide caliper. Relative arm lengths (the ratio of each chromosome length to the sum of all chromosome lengths) and arm ratios (the ratios of long arm length to short one of each chromosome) were calculated from the measurement.

Results

Plate 1 shows the chromosomes of *E. cinetorchis*. Plate 2 shows those of *E. hortense*. The averages and standard deviations of relative arm lengths and arm ratios of these chromosomes are figured in Table 1. Judging from the range of arm ratios calculated

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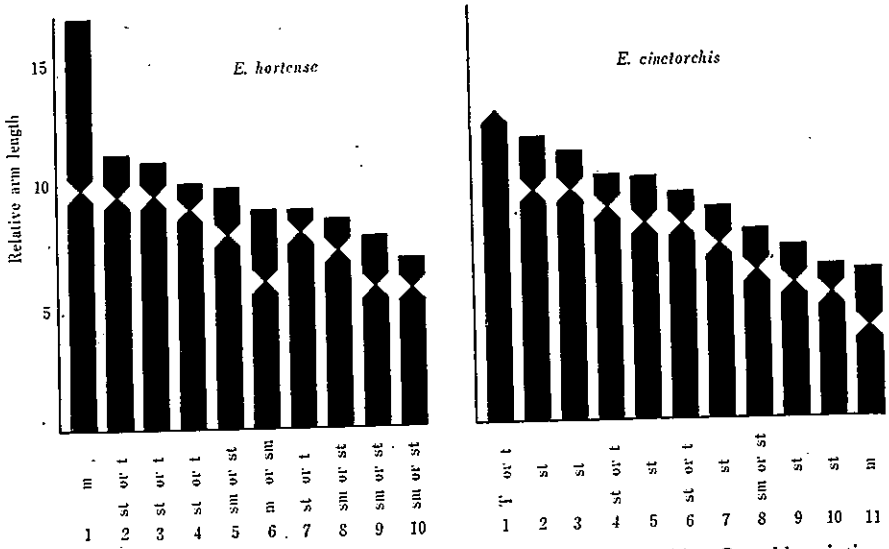

Fig. 1 Ideograms of chromosomes of *E. hortense* and *E. cinetorchis*. On abbreviations, see the text.

Table 1 Relative arm lengths (and arm ratios) of chromosome measurements of *E. hortense* and *E. cinetorchis*

	E. hortense		*E. cinetorchis*	
1	16.87±1.14	m	12.70±0.91	T or t
	(1.40±0.12)		(∞)	
2	11.24±0.58	st or t	11.60±0.45	st
	(5.60±2.37)		(4.26±0.73)	
3	10.93±0.48	st or t	11.00±0.45	st
	(7.73±2.52)		(5.89±1.61)	
4	10.03±0.57	st or t	10.00±0.44	st or t
	(8.28±4.92)		(6.31±1.89)	
5	9.84±0.56	sm or st	9.89±0.47	st
	(4.08±1.31)		(4.29±0.83)	
6	8.90±0.96	m or sm	9.23±0.49	st or t
	(2.09±0.64)		(5.88±1.47)	
7	8.88±0.70	st or t	8.59±0.50	st
	(9.02±4.65)		(4.77±0.84)	
8	8.48±0.63	sm or st	7.63±0.48	sm or st
	(5.67±3.00)		(3.64±0.90)	
9	7.74±0.58	sm or st	6.99±0.55	st
	(2.92±0.74)		(3.67±0.48)	
10	6.86±0.49	sm or st	6.20±0.46	st
	(5.18±2.79)		(4.40±0.99)	
11			6.11±0.44	m
			(1.51±0.19)	

On abbreviations, see the text.

in addition to the mean values, the terms showing the centromeric position in the table were adopted from the nomenclature recommended by Levan *et al.* (1964). The ideograms of the chromosomes in Fig. 1 are drawn on the basis of Table 1.

E. cinetorchis: In the mitotic metaphase figures of germ cells of *E. cinetorchis*, eleven pairs of chromosomes were recognized (Plate 1 A). They are composed of middle-sized and small-sized chromosomes, and each pair is slightly different in length. The longest pair of them have distinctive features; they have no short arms or very small ones, if any (telocentrics (T) or acrocentrics (t)). The pair numbers of 2, 3, 5, 7, 9, and 10 are subtelocentrics (st), the pair numbers of 4 and 6 are st or t, the pair number 8 is submetacentrics (sm) or st, and the pair number 11, the shortest one, is metacentrics (m) with their distinctive features (Plate 1C).

Meiotic metaphase figures were also recognized (Plate 1B), and many spermatozoa were observed in almost all the preparations. The chromosome number was 11, and the number of chiasma per chromosome was

probably 2, and each chromosome was round or had a magnifying glass shape. Such composition of the chromosomes corresponds to the eleven pairs of chromosomes in the mitotic metaphase.

E. hortense: The figures in the mitotic metaphase of germ cells of *E. hortense* were quite different from those of *E. cinetorchis*. As shown in Plate 2A, ten pairs of chromosomes were recognized. The largest pair of chromosomes showed the characteristics of the typical large-sized metacentric chromosomes. The other pairs were small- or middle-sized and showed a gradual difference in size. The pair numbers of 2, 3, 4, and 7 were st or t, the pair numbers of 5, 8, 9, and 10 were sm or st, and the pair number 6 was m or sm (Plate 2C).

Meiotic metaphase figures of germ cells were also observed (Plate 2B). Many spermatozoa were seen in the preparation. The chromosome number was ten. Among them was an extra large chromosome, and its chiasma number was more than four. The other choromosomes were X-shaped, γ-shaped or had the shape of a magnifying glass. The number of chiasma per chromosome was one or two. Such composition of the chromosomes corresponds to the ten pairs of chromosomes in the mitotic metaphase.

Discussion

The figures used in Table 1 show the measurement of the picture of chromosomes using the slide calipers. As the picture was not very clear, some error might occur in measurement, especially of shorter chromosomes. Therefore, such error must be considered when we present each chromosome in the nomenclatures. As the arm ratio of each chromosome is close to the limit of nomenclature, the expression "or" such as "m or sm" and "st or t" is employed.

The results of this study show the difference in chromosome number between *E. cinetorchis and E. hortense*. The chromosome number of *E. cinetorchis* is n=11, 2n=22, which corresponds that of *Parorchis acanthus* and *E. revolutum* (Rees, 1939; Churchill, 1950, 1951). Beaver (1937) reported that *T. cinetorchis* was synonym of *E. revolutum*. However, the karyotypical analysis was not made on *E. revolutum* and therefore karyotypical comparison of these species is not delt with in this study. The chromosome number of *E. hortense* is, on the other hand, n=10, 2n=20, which means one pair less than that of *E. cinetorchis*. This suggests that the basic chromosome number of the genus *Echinostoma* is n=11, 2n=22, and that *E. hortense* is the heteroploid with one pair of chromosomes attached to another pair. However, such proposal lacks in persuasive evidences because only a few karyotypes of the genus *Echinostoma* have been introduced up to now.

The karyotype of *E. cinetorchis* is widely different from that of *E. hortense*. In comparison of mitotic figures of chromosomes between *E. cinetorchis* and *E. hortense*, it is difficult to define the corresponding chromosome in each figure. The hypothesis that *E. hortense* with one pair of chromosomes attaching to another pair is the heteroploid of *E. cinetorchis* cannot give more concrete explanation as to which pair is attaching to which one. The only possible speculation is that various aberrations might take place in chromosomes. As the comparison of meiotic metaphase figures between *E. cinetorchis* and *E. hortense* also shows differences in the number of kiasma and its position, the relationship between these two species is not so close. Many species of the genus *Echinostoma* have been reported, and if the karyotypes of these species are clarified, the relationship between *E. cinetorchis* and *E. hortense* may be more fully explained.

It is well-known that the testis of *E. cinetorchis* differs in number and shape among the individuals. Some of *E. cinetorchis* have no testis (Ando and Ozaki, 1923). Some contain no spermatozoon in their seminal

vesicles (Terasaki *et al.*, 1979). One of the purposes of this study is to clarify the mechanism of reproduction and multiplication of *E. cinetorchis*. The results show that unlike *Paragonimus pulmonalis* and *Fasciola* sp. in Japan (Cho *et al.*, 1977; Moriyama *et al.*, 1979; Sakaguchi, 1980; Sakaguchi and Nakagawa, 1975; Sakaguchi and Tada, 1976, 1980; Sakaguchi and Yoneda, 1976; Terasaki, 1977, 1980a, 1980b, 1980c, 1980d), *E. cinetorchis* takes ordinary process of meiosis and normal spermatozoa are produced in the testis. However, the individuals which furnish no testis should produce no spermatozoa. No detail information concerning oogenesis or fertilization has been given. Further studies on *E. cinetorchis* may clarify its mechanism.

Summary

The difference of the karyotypes between *E. cinetorchis* and *E. hortense* was studied on adult materials which were obtained from the intestines of rats which had been fed with Japanese loaches. The specimens were prepared by the air-drying method. The results are as follows: (1) The chromosome number of *E. cinetorchis* is n=11, 2n=22; (2) The chromosome number of *E. hortense* is n=10, 2n=20; (3) Both species perform normal meiotic division and many spermatozoa were seen; and (4) The karyotypes of metaphase figures are entirely different between *E. cinetorchis* and *E. hortense*, and the relationship between these two species is discussed in view of their karyotypes.

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棘口吸虫2種, *Echinostoma cinetorchis* と *E. hortense* の核型比較

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日本産ドジョウをラットに生食させて得た棘口吸虫2種, *E. cinetorchis* および *E. hortense* を材料にair-drying 法で標本を作り核型分析を行なつた. 結果は次の通りである. (1) *E. cinetorchis* の染色体数は n=11, 2n=22 である. (2) *E. hortense* の染色体数は n=10, 2n=20 である. (3) 両種とも正常な減数分裂を行ない, 多数の精子を作る. (4) 両種の間には核型の上で大きな違いが見られる. なお, 両種間の核型の関係について論議した.

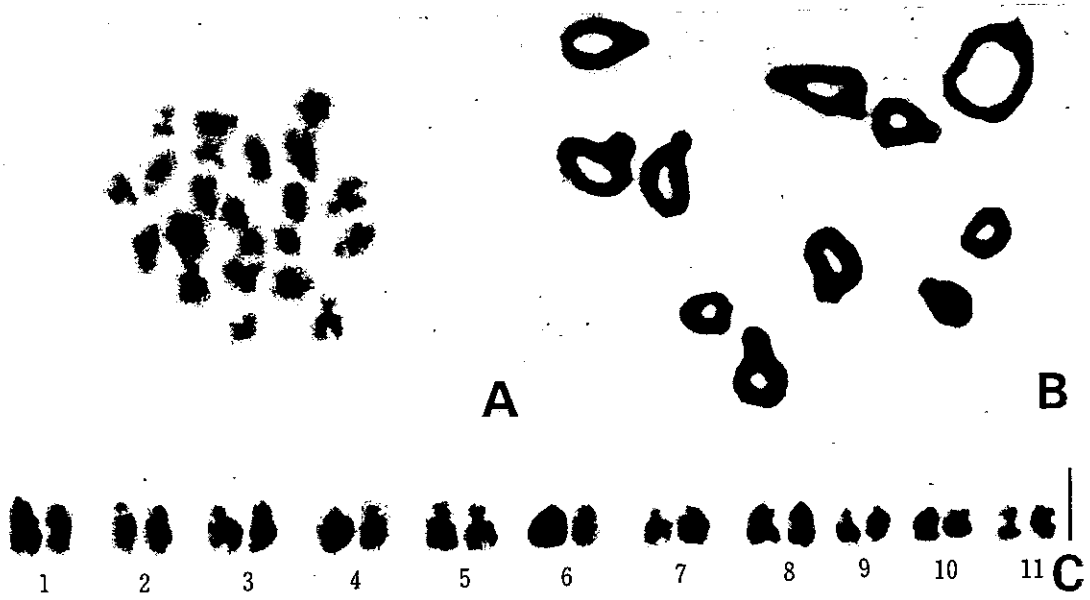

Plate 1 Chromosomes in cultured germ cells of *E. cinetorchis* prepared by a simple air-drying technique.

A: Mitotic metaphase with 22 chromosomes from the gonad.
B: Meiotic metaphase with 11 bivalents.
C: Karyotype plate made from the mitotic metaphase.
Numbers show the pair numbers of chromosomes. The scale indicates 5 μm.

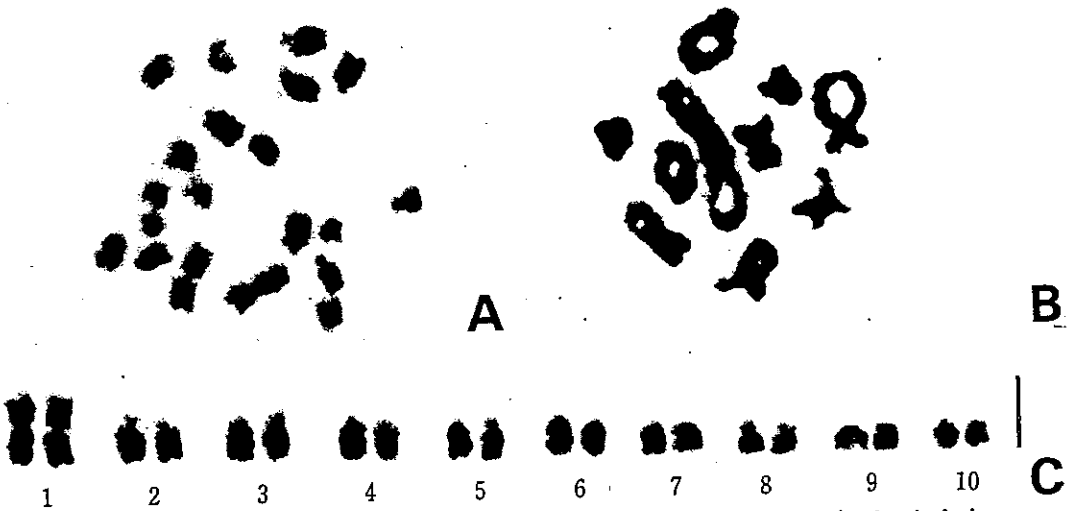

Plate 2 Chromosomes in cultured germ cells of *E. hortense* prepared by a simple air-drying technique.

A: Mitotic metaphase with 20 chromosomes from the gonad.
B: Meiotic metaphase with 10 bivalents.
C: Karyotype plate made from the mitotic metaphase.
Numbers show the pair numbers of chromosomes. The scale indicates 5 μm.