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Comparative Studies on the Karyotypes of *Paragonimus westermani* (s. str.) and *P. pulmonalis*

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There are some observations on the karyotype of lung flukes (the genus *Paragonimus*; Trematoda: Troglotrematidae). While Chen (1937) firstly reported that the chromosome number of *P. kellicotti* Ward, 1908 was 2n=16 and n=8, recently, Sakaguchi and Tada (1975, 1976a, 1976b), and Terasaki (1977, 1978) made cytological studies on the karyotypes of lung flukes, *P. westermani* (Kerbert, 1878), *P. ohirai* Miyazaki, 1939, *P. iloktsuenensis* Chen, 1940, *P. miyazakii* Kamo *et al.*, 1961, *P. sadoensis* Miyazaki *et al.*, 1968, and *P. peruvianus* Miyazaki *et al.*, 1969, and recognized that the chromosome number of five species except *P. westermani* was 2n=22 and n=11, and that their spermatogenesis was normal. On the other hand, the chromosome numder of *P. westermani* was 3n=33; no meiotic figure was recognized; spermatogenesis was abnormal; and its reproductive process might be parthenogenetic. Miyazaki (1977, 1978a, 1978b) observed wholly mounted or sectioned specimens of lung flukes collected in Asia, Africa, and Americas for the presence of spermatozoa in the seminal receptacle, and separated *P. westermani* into two types: One is bisexual type in which spermatozoa were present in the seminal receptacle and the other is parthenogenetic type in which spermatozoa were absent. He referred the former to *P. westermani* (s. str.) and the latter, *P. pulmonalis* (Baelz, 1880).

In this paper the author analyzes the karyotype of *P. westermani* collected from Akita Prefecture, and compares it with that of *P. pulmonalis* which has been already reported by the author (1977).

Materials and Methods

The materials used were metacercariae of *P. westermani* obtained from a crab, *Geothelphusa dehaani*, collected in Nishikimura, Semboku-gun, Akita Prefecture, Japan. The metacercariae were administered to two dogs orally and to a cat by abdominal injection. About five months after the administration, the infected animals were sacrificed, and twenty of mature lung flukes were recovered from worm cysts in their lungs.

A simple cell cultivation method of Ando and Uchida (1973) was modified (Terasaki, 1977), and germ cells of nine examples of *P. westermani* were used for experiments. The remained eleven flukes were used for other cytological and histological examinations. Finally 23 preparations were made from the flukes and were used for microscopic observation. These results were compared with karyotype of *P. pulmonalis* named by Miyazaki (1978b), while the entity was already reported by the author (1977) under the name of *P. westermani*.

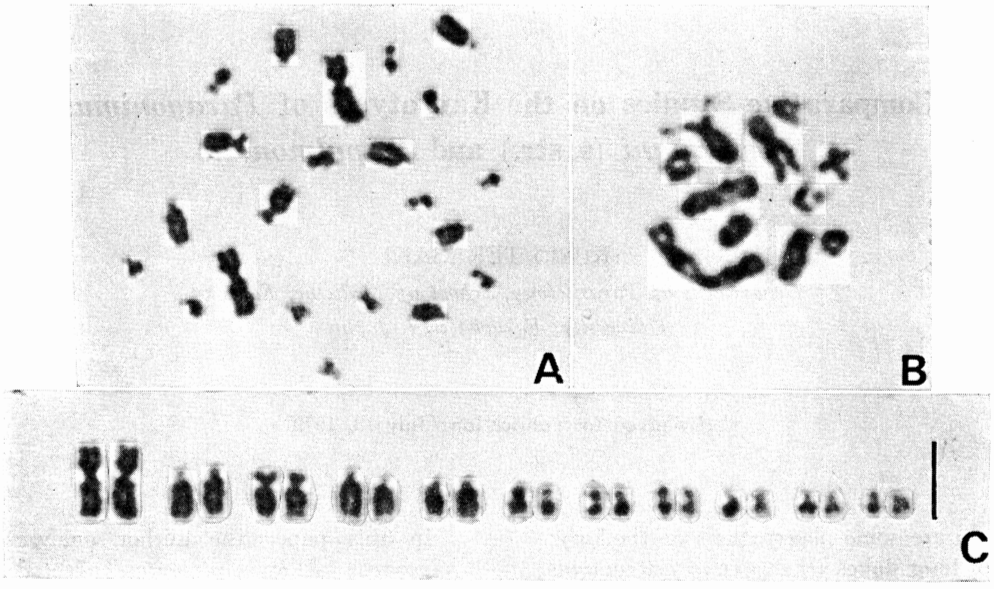

Fig. 1 Chromosomes in cultured germ cells of *P. westermani* prepared by a simple air-drying technique.
A: Mitotic metaphase with 22 chromosomes from the gonad.
B: Primary spermatocyte in diplotene to diakinesis with 11 bivalents.
C: Karyotype plate made from the mitotic metaphase (scale 5 μm).

Results

In the mitotic metaphase figures of germ cells of *P. westermani*, eleven pairs of chromosomes were recognized (Fig. 1, A), and could be divided into three groups (large, medium, and small) by their sizes. Just as in the previous reports (Terasaki, 1977, 1978), the averages and standard deviations of relative arm lengths and arm ratios of the chromosomes were calculated on the basis of 20 metaphase figures of mitosis (Table 1). Taking the maximum and minimum arm ratios calculated as above into consideration, the terms in the table show the centromeric position using the nomenclature recommended by Levan *et al.* (1964). The karyotype of the germ cells of *P. westermani* consisted of one pair of large-sized metacentrics (m), four pairs of small-sized subtelocentrics (st), three pairs of small-sized metacentrics (m) or submetacentrics (sm), and three pairs of small-sized submetacentrics (sm) or subtelocentrics (st) (Fig. 1, C).

On the other hand, meiotic metaphase figures were also recognized (Fig. 1, B), and many spermatozoa were seen in the preparations. The chromosome number was eleven, consisting of one of large-size, four of medium-size, and six of small-size. This composition of the chromosomes corresponded to eleven pairs of chromosomes in the mitotic metaphase.

Discussions

The results of the present study on *P. westermani* show that the chromosome number is 2n=22 and n=11, the germ cells undergo normal meiosis, and many spermatozoa are formed (Terasaki, 1980). These findings well resemble the results in other five congeneric species (*P. ohirai*, *P. iloktsuenensis*, *P. miyazakii*, *P. sadoensis*, and *P. peruvianus*) (Sakaguchi and Tada, 1975, 1976a, 1976b; Terasaki, 1977, 1978). In *P. pulmonalis* unlike *P. westermani*, the chromosome number was 3n=33 (triploid), no meiosis was recognized, and there were scarce spermatozoa

Table 1 Comparison of results of chromosome measurements between *Paragonimus westermani* and *P. pulmonalis*

Pair number	Relative arm length		Arm ratio		Terms showing centromeric position*
	P. westermani	*P. pulmonalis*	*P. westermani*	*P. pulmonalis*	
1	19.28±0.97	20.41±1.79	1.37±0.14	1.33±0.10	m
2	11.92±0.55	11.97±0.63	5.35±1.25	4.52±0.86	st
3	11.21±0.38	11.51±0.48	3.10±0.90	3.69±1.03	st
4	10.62±0.47	10.16±0.42	4.84±1.20	4.35±0.78	st
5	8.98±0.51	9.01±0.44	4.45±1.42	4.28±1.12	st
6	7.49±0.37	7.39±0.43	1.58±0.26	1.87±0.67	m or sm
7	6.69±0.36	6.75±0.63	3.06±0.73	3.02±0.84	sm or st
8	6.50±0.40	6.38±0.49	1.44±0.50	1.91±0.54	m or sm
9	6.11±0.37	5.98±0.51	2.83±0.48	3.35±1.15	sm or st
10	5.71±0.42	5.57±0.52	2.53±0.46	3.55±1.14	sm or st
11	5.48±0.36	5.11±0.55	1.38±0.17	1.89±0.71	m or sm

* These terms are after the nomenclature recommended by Levan *et al.* (1964), taking the maximum and minimum of arm ratios into consideration.
Underlines show the couples in which the sigificant difference is considered ($P<0.01$).

(Sakaguchi and Tada, 1976b ; Terasaki, 1977, 1980 ; Cho *et al.*, 1977). Nevertheless, relative arm lengths and arm ratios of the chromosomes of *P. westermani* show a great deal of similarities to those of *P. pulmonalis* (Fig. 2). In Fig. 2, a karyograph of *P. westermani* is drawn from average of relative arm lengths and arm ratios in Table 1 and the other from those in the previous report (Terasaki, 1977). Differences of the averages

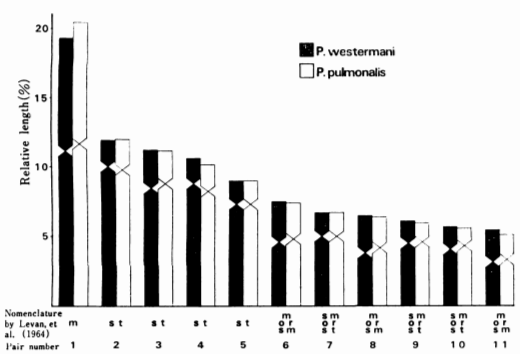

Fig. 2 Comparisons of karyograph between *Paragonimus westermani* and *P. pulmonalis*.
* These terms are used after the nomenclature recommended by Levan *et al.* (1964), taking the maximum or minimum of arm ratios into consideration.

of the relative arm length and arm ratios of each chromosome between the two species were surveyed by t-test. Couples of variances in which significant differences are seen, are underlined in Table 1. However, it is considered in view of Fig. 2 that these differences may be minute. Further, these two species are very similar in morphology of eggs, metacercariae, and adult flukes except their sizes (Miyazaki 1978b). Therefore it may be considered that *P. pulmonalis* is the autotriploid of *P. westermani* though there is no evidence found at present.

Summary

So-called "*Paragonimus westermani*" has been divided into two species (*P. westermani* and *P. pulmonalis*) by existence or scarcity of spermatozoa in seminal receptacle (Miyazaki, 1977, 1978a, 1978b). Using the germ cells, the karyotype of *P. westermani* is analyzed by cell cultivation method and is compared with that of *P. pulmonalis* which have been well investigated (Terasaki, 1977).

In *P. westermani*, chromosome number is $2n=22$ and $n=11$, and the karyotype consists of the chromosomes with one pair of large-

sized metacentrics, four pairs of medium-sized subtelocentrics, three pairs of small-sized metacentrics or submetacentrics, and three pairs of small-sized submetacentrics or subtelocentrics. There are a great deal of similarities between the karyotype of this species and *P. pulmonalis* except the chromosome number. In *P. westermani*, meiotic figures are also observed and many spermatozoa are recognized unlike *P. pulmonalis*. These results show a strong resemblance to those of five congeneric spesies (*P. ohirai*, *P. iloktsuenensis*, *P. miyazakii*, *P.sadoensis*, and *P. peruvianus*) as well as their karyotypes.

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ウエステルマン肺吸虫とベルツ肺吸虫の核型の比較

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肺吸虫の染色体の研究 (Sakaguchi and Tada, 1975, 1976a, 1976b ; Terasaki, 1977) から，ウエステルマン肺吸虫は精子形成に異常のあることがわかり，宮崎 (1977, 1978a, 1978b) はウエステルマン肺吸虫について，精子形成に異常のあるものの他に異常を認めないものの２つの型があり，前者をベルツ肺吸虫として，ウエステルマン肺吸虫と区別した．ベルツ肺吸虫の核型はすでに明らかにされ，3n＝33 の３倍体であることがわかつている (Sakaguchi and Tada, 1976b ; Terasaki, 1977).

著者は，ウエステルマン肺吸虫とされている秋田県産のサワガニから分離したメタセルカリアをイヌおよびネコに感染させ，得た成虫の生殖細胞について染色体分析を行なつた．その結果，ウエステルマン肺吸虫の染色体数は 2n＝22, n＝11 であり，染色体は大，中，小の３つのグループに分けられた．生殖細胞の体細胞分裂中期像は，１対の大型 metacentrics, ４対の中型 subtelocentrics, ３対の小型 metacentrics または submetacentrics, および３対の小型 submetacentrics または subtelocentrics よりなつていた．

Relative arm length や arm ratio を計算し，さきに報告した (Terasaki, 1977) ベルツ肺吸虫の核型と比較した結果，両者は非常によく似ており，ベルツ肺吸虫はウエステルマン肺吸虫の同質３倍体であると考えられたが，現在のところ確実な証拠はない．また，今回観察したウエステルマン肺吸虫では．減数分裂像が認められ，精子形成に異常を認めず，他種肺吸虫（大平肺吸虫，小型大平肺吸虫，宮崎肺吸虫，佐渡肺吸虫，およびペルー肺吸虫）と同様であつた．核型もそれらの肺吸虫のものとよく似ていた．