

A Comparative Karyotype Study of Lung Flukes, *Paragonimus ohirai* and *P. miyazakii*

YUJI SAKAGUCHI and ISAO TADA

Department of Medical Zoology, Kanazawa Medical University,
Uchinada, Kahoku-gun, Ishikawa, Japan

(Received for publication; Nov. 14, 1975)

A considerable number of reports has been available in the literature on the chromosomes of parasitic trematodes (cf. Makino 1956), while the karyotypic details of those species have remained unexplored. In view of the basic importance in biology and medicine, we commenced karyological investigations of lung flukes distributing in Japan using modern cytogenetic techniques.

The present study deals with the karyotype analysis of two species of lung flukes belonging to the genus *Paragonimus*.

Materials and Methods

The metacercariae of *Paragonimus ohirai* and *P. miyazakii* were collected from the intermediate hosts, *Sesarma dehaani* obtained in Kinosaki of Hyogo Prefecture and *Potamon dehaani* captured in the suburbs of Nagasaki and Iwakuni, respectively. Adult specimens of *P. ohirai* were recovered from the lungs of rats after 40 to 50 days of oral inoculations of the metacercariae. The adult worms of *P. miyazakii* grown in the lungs of dogs for 40 to 60 days were also used. The chromosomes were studied on the ovary and testis of the adult specimens thus obtained. Air-dried slides were prepared according to the method of Takagi and Oshimura (1973) and stained with 5% Giemsa solution for 10-15 minutes. Karyotypes were arranged in accordance with the morphological criteria proposed by Levan *et al.* (1964).

Results

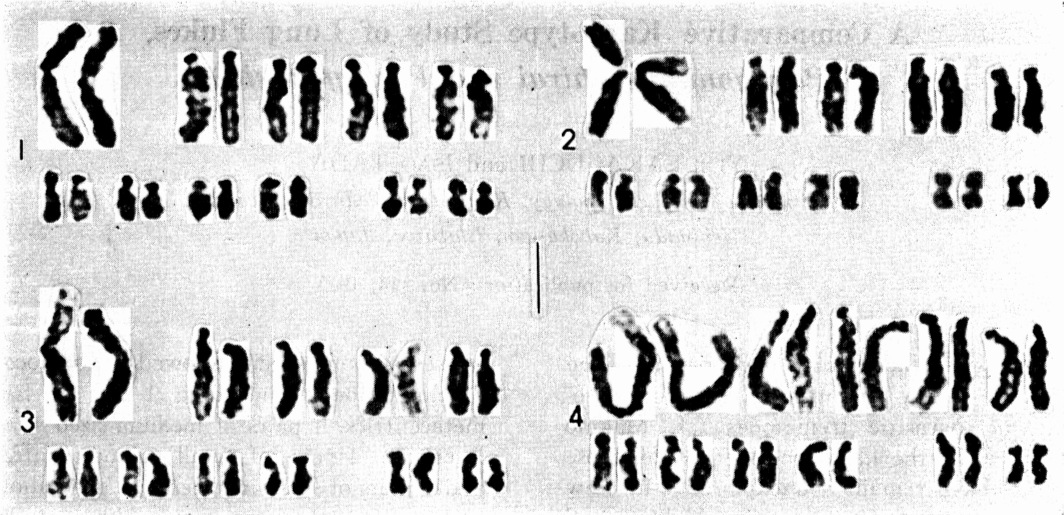
The spermatogonial and oogonial meta-

phases of both species showed unequivocally 22 chromosomes, including 1 pair of large metacentrics, 4 pairs of medium-sized subtelocentrics, 4 pairs of small submetacentrics, and 2 pairs of small metacentrics. As shown in Figs. 1-4, the two species here studied were karyotypically indistinguishable on the morphological basis alone. No heteromorphic pairs were found in both species. On the basis of 10 spermatogonial metaphase plates from each species, relative arm lengths and centromeric indices were compared between the two species (Table 1). The statistical test revealed no difference in those values of the corresponding individual chromosome pairs between both species except for the pair Nos. 2, 3 and 7.

Discussion

The present results confirmed our previous observations on the chromosome numbers of $2n$, 22 and n , 11 in *P. ohirai* and *P. miyazakii* (Sakaguchi and Tada, 1975 a, b and c), and further substantiated the karyotypic similarities of both species. Only one species of the lung fluke, *Paragonimus kellicotti*, has ever been studied chromosomally (Chen, 1937) which showed $2n$, 16 and n , 8 in the sectioned preparations (cited from Makino, 1956). We studied the chromosomes of another species of lung fluke, *Paragonimus westermani* by the air drying method. The diploid chromosome number for this species was 33 (Sakaguchi and Tada, 1975 b). This may represent a triploid form of the genus, though further studies are needed.

Although the statistically significant difference in the arm indices of the 3 pairs



Figs. 1-4 Karyotype analysis of Spermatogonial (1, 3) and oogonal (2, 4) metaphases from *Paragonimus ohirai* (1, 2) and *P. miyazakii* (3, 4). Bar indicates 5μ

Table 1 The results of chromosome measurements in *P. ohirai* (*P.o*) and *P. miyazakii* (*P.m*)

Pair No.	Species	Relative length	Arm ratio	Centromeric index
1	<i>P.o</i>	18.7 ± 0.67	1.28 ± 0.07	44.0 ± 1.30
	<i>P.m</i>	19.9 ± 1.07	1.38 ± 0.13	42.2 ± 2.23
2	<i>P.o</i>	12.7 ± 0.35	5.30 ± 0.45	16.0 ± 1.18
	<i>P.m</i>	12.4 ± 0.49	7.00 ± 0.92 *	12.6 ± 1.27 *
3	<i>P.o</i>	11.9 ± 0.42	4.23 ± 0.26	19.2 ± 0.98
	<i>P.m</i>	11.5 ± 0.38	5.79 ± 0.71 *	14.9 ± 1.61 *
4	<i>P.o</i>	11.2 ± 0.31	4.56 ± 0.72	18.3 ± 2.33
	<i>P.m</i>	10.7 ± 0.39	5.21 ± 0.73	16.3 ± 1.76
5	<i>P.o</i>	10.2 ± 0.48	4.73 ± 0.78	17.8 ± 2.25
	<i>P.m</i>	9.8 ± 0.34	5.15 ± 0.52	16.4 ± 1.44
6	<i>P.o</i>	6.8 ± 0.24	2.02 ± 0.23	33.3 ± 2.52
	<i>P.m</i>	6.5 ± 0.46	2.34 ± 0.26	30.2 ± 2.38
7	<i>P.o</i>	6.2 ± 0.20	2.46 ± 0.23	29.0 ± 1.96
	<i>P.m</i>	6.2 ± 0.30	3.03 ± 0.26 *	25.0 ± 1.65 *
8	<i>P.o</i>	5.7 ± 0.25	2.62 ± 0.25	27.8 ± 1.91
	<i>P.m</i>	5.9 ± 0.36	2.79 ± 0.19	26.4 ± 1.26
9	<i>P.o</i>	5.7 ± 0.28	1.80 ± 0.38	36.4 ± 4.57
	<i>P.m</i>	6.2 ± 0.52	2.05 ± 0.39	33.3 ± 4.45
10	<i>P.o</i>	6.0 ± 0.30	1.15 ± 0.08	46.6 ± 1.76
	<i>P.m</i>	6.1 ± 0.40	1.19 ± 0.15	45.8 ± 3.05
11	<i>P.o</i>	5.0 ± 0.64	1.17 ± 0.12	46.3 ± 2.46
	<i>P.m</i>	4.9 ± 0.58	1.24 ± 0.14	44.8 ± 2.63

* Difference significant ($P < 0.01$)

between the present 2 species could be explained by pericentric inversions, this might be accounted for the mensural artifacts due to incorrect homologue matching of

certain morphologically similar members. Our present emphasis is, therefore, placed on the fact that *P. miyazakii* is closely related to *P. ohirai* from the karyotypic

viewpoint.

Summary

Karyotypes were analyzed and compared to each other in 2 species of lung flukes, *Paragonimus ohirai* and *P. miyazakii* on the basis of air-dried preparations of spermatogonial and oogonial metaphase plates. Both species had 22 chromosomes and were karyotypically very similar. No heteromorphic pairs were observed in both the male and female complements.

Acknowledgements

The authors would like to thank Prof. M. Sasaki and Emeritus Prof. S. Makino of Hokkaido University for their helpful suggestions and critical reading of the manuscript. The authors are indebted to Prof. D. Katamine and his staffs in Nagasaki University and to Prof. H. Yoshimura of Kanazawa University for providing a part of the specimens. Finally, the authors are very grateful to Miss Y. Iwa and Miss S. Kawaguchi of our Department for the preparation of the manuscript and the assistance in the experiments.

References

- 1) Chen, P. D. (1937) : The germ cell cycle in the trematode, *Paragonimus kellicotti* Ward. Trans. Am. Micro. Soc., 56, 208-236.
- 2) Levan, A., Fredga, K. and Sandberg, A. A. (1964) : Nomenclature for centromeric position on chromosomes. Hereditas., 52, 201-220.
- 3) Makino, S. (1956) : A Review of the Chromosome Number in Animals. Hokuryukan, Tokyo. 300 pp.
- 4) Sakaguchi, Y. and Tada, I. (1975 a) : Studies on the Chromosome of Helminths, (1), A comparative study on karyotype of *Paragonimus ohirai* and *Paragonimus miyazakii*. Jap. J. Parasitol., 24, 42.
- 5) Sakaguchi, Y. and Tada, I. (1975 b) : Studies on the Chromosome of Helminths (2), A comparative study on the karyotype of three species of lung flukes. Jap. J. Parasitol., 24, Suppl., 62.
- 6) Sakaguchi, Y. and Tada, I. (1975 c) : Chromosomes of two species of lung flukes, *Paragonimus ohirai* and *P. miyazakii*. Chromosome Inform. Serv., 19, 21-23.
- 7) Takagi, N. and Oshimura, M. (1973) : Fluorescence and Giemsa banding studies of the allocyclic x chromosome in embryonic and adult mouse cells. Exp. Cell Research., 78, 127-135.

大平肺吸虫と宮崎肺吸虫の核型比較

坂口祐二・多田 功

(金沢医科大学医動物学教室)

大平肺吸虫及び宮崎肺吸虫の染色体をエアードライ法で作製したプレパラートで観察し、その核型分析を行った。得られた成績は次の通りである。

1) 大平肺吸虫と宮崎肺吸虫の染色体数は共に $2n=22$ で、大形の中部着糸型の染色体1対、中形の次端部着糸型4対、小形の次中部着糸型4対及び小形の中部着糸

型の染色体2対から構成されている。

2) 両種の核型は No. 2, 3 及び 7 の 3 対の染色体で、その形が若干異なる。

3) 大平肺吸虫と宮崎肺吸虫は染色体数及びその核型からみて分類学的に極めて近接した関係にある。