

Chromosome Analysis on a South American Lung Fluke, *Paragonimus peruvianus*

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There have been a few studies on the karyotype of the lung fluke (the genus *Paragonimus*). Chen (1937) reported that the chromosome number of *P. kellicotti* Ward was $2n=16$ and $n=8$ without showing its karyotype. In recent years, Sakaguchi and Tada (1975, 1976a) clearly showed the karyotypes of *P. ohirai* Miyazaki and *P. miyazakii* Kamo *et al.* to be $2n=22$ and $n=11$ by an air drying method (Takagi and Oshimura, 1973). They (1976b) found that the chromosome number in *P. westermani* (Kerbert) was thirty three and considered that the fluke might represent a triploid form. Terasaki (1977) subsequently conducted comparative studies on five species of the lung fluke in Japan (*P. westermani*, *P. ohirai*, *P. iloktsuenensis* Chen, *P. miyazakii*, and *P. sadoensis* Miyazaki *et al.*) analyzing the karyotype by a method modified from a simple cell cultivation method (Ando and Uchida, 1973) and reported the results as follows. While four species except *P. westermani* closely resembled one another in their karyotypes especially in chromosome number $2n=22$ and $n=11$, the chromosome number of *P. westermani* was demonstrated as $3n=33$

(triploidy) in spite of the difficulty for the karyotype analysis. Its karyotype was not distinguishable within the genus *Paragonimus* including the other four species and it suggests that the reproductive process of *P. westermani* might be parthenogenetic.

In the present report, the karyotype of *P. peruvianus* Miyazaki *et al.*, 1969 is analyzed by a simple cell cultivation method and is compared with the karyotypes of the Japanese congeneric lung flukes. The locality of this fluke is geographically distant from Japan and the fluke is biologically much different from the Japanese lung flukes, e. g. the absence of cyst membrane in the metacercaria (Miyazaki *et al.*, 1971; Tantalean *et al.*, 1974) and the occurrence of penetration at esophagus and stomach in the final host (Tantalean *et al.*, 1974).

Materials and Methods

Metacercariae of *P. peruvianus* were provided by the Scientific Expedition sponsored by the Japanese Ministry of Education (Representative: Prof. Ichiro Miyazaki, School of Medicine, Fukuoka University). They were obtained from crabs, *Pseudothelphusa chilensis*, known to be the intermediate host, which were collected by the members of the Scientific Expedition at Tabacal in the Condebamba Valley, Department of Cajamarca, Peru. The metacercariae were administered

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to mature dogs orally or to immature cats by abdominal injection. On the fiftieth to the one hundredth day after the administration, the infected animals were sacrificed, and the mature lung flukes were recovered from the worm cysts of the lung.

A simple cell cultivation method of Ando and Uchida (1973) was modified (Terasaki, 1977), and germ cells of 12 examples of *P. peruvianus* were used for experiments. Finally 32 preparations were made from the flukes and were used for microscopic observation.

Results

In the mitotic metaphase figures of germ cells of *P. peruvianus*, eleven pairs of chromosomes were unequivocally recognized (Fig. 1, A), and they could be divided into three groups (large, medium, and small) by their sizes. As same as in the previous report (Terasaki, 1977), 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

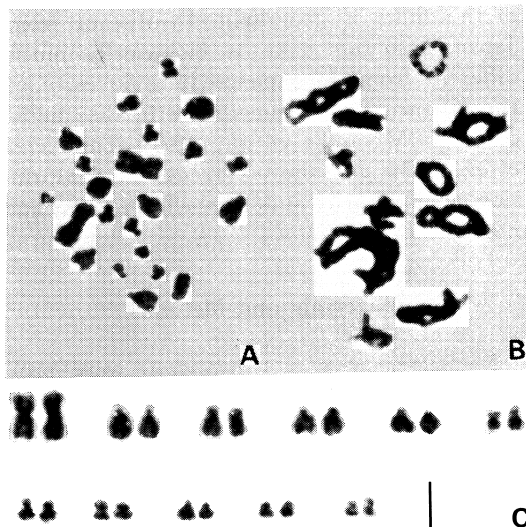


Fig. 1 Chromosome of *Paragonimus peruvianus*.

- A: Metaphase figure in mitosis.
- B: Metaphase figure in meiosis.
- C: Karyotype plate (scale 5 μ m).

mitosis (Table 1). From arm ratios calculated, using the nomenclature recommended by Levan *et al.* (1964), to be taken into consideration to maximum or minimum of arm ratios by the author, the karyotype of the germ cells of *P. peruvianus* consisted of one pair of large-sized metacentrics (m), four pairs of medium-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 sperms were seen in the preparations. Their chromosome number was eleven, consisting of one of large-size, four of medium-size, and six of small-size. This corresponded to eleven pairs of chromosomes in the mitotic metaphase.

Discussion

The results of the present study on *P. peruvianus* show that the chromosome number is $2n=22$ and $n=11$, the germ cells have the period of meiosis, and many sperms are formed. These findings well resemble the

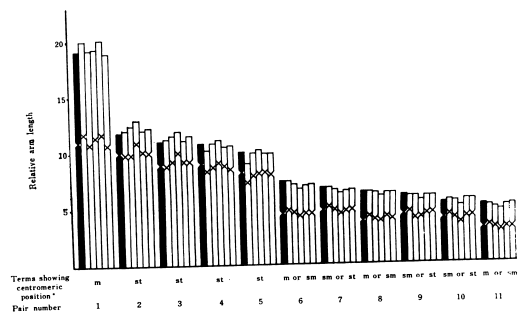


Fig. 2 Comparisons of chromosomes among the lung flukes. In each group of chromosomes, black one shows *Paragonimus peruvianus* and white ones show *P. westermani*, *P. ohirai*, *P. iloktsuenensis*, *P. miyazakii*, and *P. sadoensis* from left.

* These terms are used after the nomenclature recommended by Levan *et al.* (1964), to be taken into consideration to maximum or minimum of arm ratios by the author.

Table 1 Results of chromosome measurements in *Paragonimus peruvianus*

Pair number	Relative length	Arm ratio	Terms showing centromeric position*
1	19.05±1.23	1.37±0.08	m
2	11.80±0.47	5.68±1.03	st
3	11.06±0.40	4.12±0.96	st
4	10.76±0.33	5.25±1.18	st
5	10.03±0.48	4.36±0.74	st
6	7.36±0.45	1.54±0.27	m or sm
7	6.76±0.32	2.54±0.39	sm or st
8	6.44±0.38	1.46±0.30	m or sm
9	6.14±0.47	2.61±0.46	sm or st
10	5.41±0.53	2.84±0.54	sm or st
11	5.19±0.48	1.51±0.22	m or sm

* These terms are used after the nomenclature recommended by Levan *et al.* (1964), to be taken into consideration to maximum or minimum of arm ratios by the author.

Table 2 Results of t-test on averaged relative arm lengths of chromosomes between *Paragonimus peruvianus* and each species of the lung flukes in Japan

	<i>P. westermani</i>	<i>P. ohirai</i>	<i>P. iloktsuenensis</i>	<i>P. miyazakii</i>	<i>P. sadoensis</i>
<i>P. peruvianus</i>	4,5	2,3	2,3,6,7,8,9,11	4	3

The relative arm lengths of the Japanese lung flukes are shown in the previous report (Terasaki, 1977).

Figures indicate pair numbers of chromosomes in which significant differences ($P < 0.01$) are recognized.

Table 3 Results of t-test on averaged arm ratios of chromosomes between *Paragonimus peruvianus* and each species of the lung flukes in Japan

	<i>P. westermani</i>	<i>P. ohirai</i>	<i>P. iloktsuenensis</i>	<i>P. miyazakii</i>	<i>P. sadoensis</i>
<i>P. peruvianus</i>	2,4,8	1,2,4	6,7	8	2,4

The arm ratios of the Japanese lung flukes are shown in the previous report (Terasaki, 1977).

Figures indicate pair numbers of chromosomes in which significant differences ($P < 0.01$) are recognized.

results in the other four Japanese congeneric species (*P. ohirai*, *P. iloktsuenensis*, *P. miyazakii*, and *P. sadoensis*) except *P. westermani* (Sakaguchi and Tada, 1975, 1976a, 1976b; Terasaki, 1977). Relative arm lengths and arm ratios of the chromosomes of *P. peruvianus* also show a great deal of similarities to those of the Japanese species (Fig. 2). In Fig. 2, diagram of *P. peruvianus* is drawn from averages of relative arm lengths and arm ratios in Table 1 and the others drawn

from these in the previous report (Terasaki, 1977). Differences of the averages of the relative arm lengths and arm ratios of each chromosome between *P. peruvianus* and each species of lung flukes in Japan were surveyed by t-test and the results are shown in Tables 2 and 3. The pair numbers in which significant differences are seen between two variances, are shown in the tables. However, it is considered in view of Fig. 2 that these differences may be small. Those members

of the genus *Paragonimus* may, therefore, have a great deal of similarities in their karyotypes in spite of their biological difference and geographical distance. In contrast to the above findings, Chen (1973) reported that chromosome number of *P. kellicotti* was $2n=16$ and $n=8$. However, he did not show its karyotype clearly since his observation was based on sectioned preparations.

Summary

The karyotype of chromosomes obtained from the germ cells of *Paragonimus peruvianus* is analyzed by cell cultivation method and is compared with those of five species of the Japanese lung flukes which have been well investigated.

In *P. peruvianus*, 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. The meiotic figures are also observed and many sperms are recognized unlike in specimens of *P. westermani*. These results have strong resemblance to those of four species of the Japanese lung flukes except *P. westermani*.

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ペルー肺吸虫の染色体

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日本産肺吸虫の染色体については、すでに5種とも分析がなされたが、外国産の肺吸虫については、まだ明確な分析はなされていない。著者は、昭和51年度文部省海外学術調査隊(代表者:宮崎一郎)から分与されたペルー肺吸虫(*Paragonimus peruvianus*)のメタセルカリアをイヌまたはネコに感染させ、得た成虫の生殖細胞について染色体分析を行った。結果は $2n=22$, $n=11$ で大, 中, 小の3つのグループに分けられた。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 よりなっていた。Relative arm length や arm ratio を計算し, 日本産肺吸虫の染色体(ウエステルマン肺吸虫は $3n=33$, 他の4種は $2n=22$)と比較した結果, ペルー肺吸虫は日本産のものによく似ており, 染色体だけでは区別が困難であった。