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Karyotypic Studies of Lung Flukes, *Paragonimus iloktsuenensis, P. sadoensis* and *P. westermani,* with Special Reference to Gametogenesis in *P. westermani*

YUJI SAKAGUCHI AND ISAO TADA

Department of Parasitic Diseases, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860, Japan

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Previous cytological investigations of 4 species of lung flukes, *Paragonimus ohirai, P. miyazakii, P. iloktsuenensis* and *P. sadoensis* revealed that all of them were provided with 22 (2n) and 11 (n) chromosomes (Sakaguchi and Tada, 1975a, b; Sakaguchi *et al.*, 1977a, b). There was karyotypically a close similarity between *P. ohirai* and *P. miyazakii* (Sakaguchi and Tada, 1976a, b, c). Further, the present authors discovered the triploidy of *P. westermani* which had 33 chromosomes comprising 3 sets of 11 basic chromosomes (Sakaguchi and Tada, 1975a, 1976b) and they reported the lack of pairing form in germ cells and abnormality in spermatogenesis (Sakaguchi, 1977). Terasaki (1977) confirmed the findings reported by the present authors using the simple cell cultivation method of Ando and Uchida (1973). Cho *et al.* (1977) studied gametogenesis of *P. westermani* and estimated the probable parthenogenetic reproduction of this species.

The present paper deals with the results of recent karyotypic studies of *P. iloktsuenensis, P. sadoensis* and *P. westermani,* and the findings with regard to spermatogenesis, oogenesis and fertilization in *P. westermani.*

Materials and Methods

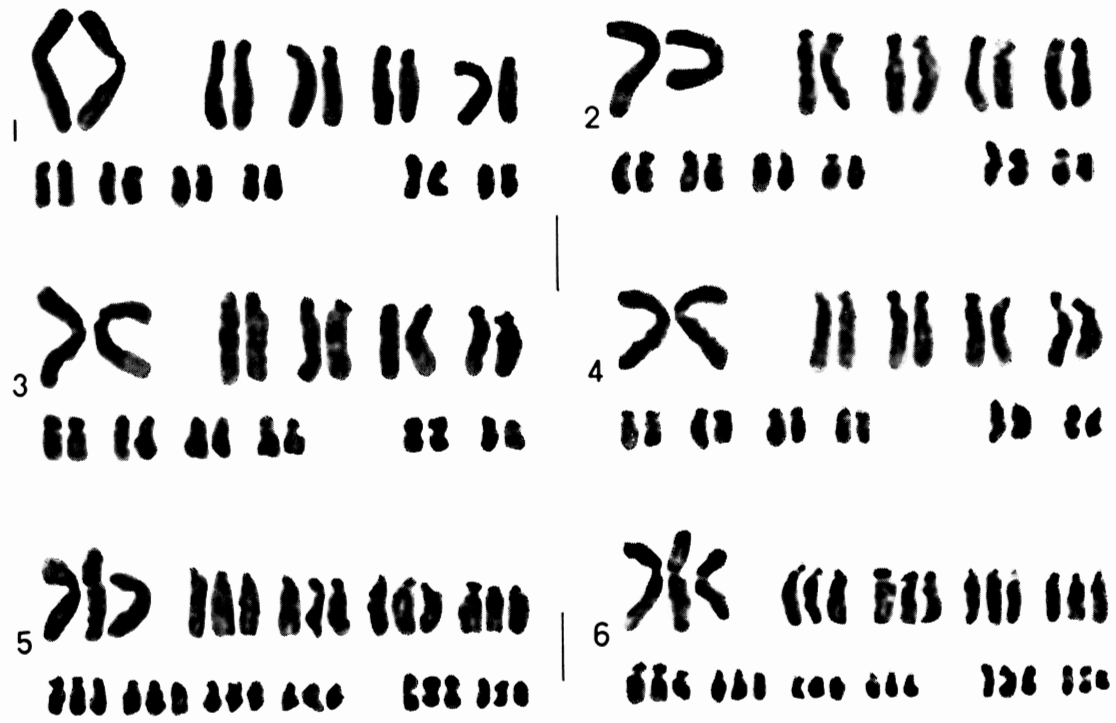
The metacercariae of *P. iloktsuenensis* (*P. i.*), *P. sadoensis* (*P. s.*) and *P. westermani* (*P. w.*) were recovered from 3 species of crabs; *Sesarma dehaani* (Sendai, Kagoshima Prefecture), *Potamon dehaani* (Sado Is., Niigata Pref.) and *Eriocheir japonicus* (Matsuura, Nagasaki Pref.), respectively. The adult worms of *P. i.* and *P. s.* were obtained from rat lungs 40-50 days after the oral inoculation of individual metacercariae, while those of *P. w.*, from dog lungs, 60-90 days after inoculation.

The chromosomes were studied in the gonadal tissues of adult specimens thus obtained. Air-dried slides were prepared according to the method of Takagi (1971) and stained with Giemsa's solution. Chromosomes were classified in accordance with the system proposed by Levan *et al.* (1964).

Adult *P. w.* was fixed in ethanol-acetic acid solution (3 : 1) for 24 hours, transferred to 70% ethanol and stained with alcohlic hydrochloric acid-carmin for 24 hours (Snow, 1963). Testicular tissue (from testis to seminal vesicle) and ovarian tissue (from ovary to uterus) were dissected out from the flukes. The specimens were placed on a slide glass and squashed with a thumb under a coverslip. The squash preparations were used for the observation of gametogenesis, fertilization and egg-formation.

Results

The chromosome number of spermatogonial and oogonial metaphase was clearly 22 (2n)



Explanation of Figures

Figs. 1–6 Karyotype analysis of spermatogonial (1, 3 and 5) and oogonial (2, 4 and 6) metaphases from *Paragonimus iloktsuenensis* (1 and 2), *P. sadoensis* (3 and 4) and *P. westermani* (5 and 6). Bar indicates 5 μm.

in both *P. i.* and *P. s.* The Karyotype consisted of 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 arrangement of chromosomes by size showed a karyotypic resemblance between these two species. No heteromorphic pairs were seen in both species. On the other hand, the chromosome number of both the testicular and ovarian tissues of *P. w.* at their metaphase was 33, which comprised 3 large metacentrics, 12 medium-sized subtelocentrics, 12 small submetacentrics and 6 small metacentrics. These chromosomes build up a triploid form with 3 sets of 11 basic chromosomes (Figs. 5, 6). No karyotypic difference was observed between the spermatogonial and oogonial cells.

The relative arm length, arm ratio and centromeric index of 10 spermatogonial metaphase plates of the flukes are shown in Table 1. Comparisons of the chromosomes at their identical number revealed statistical difference at the No. 11 chromosome alone between *P. i.* and *P. s.*; at Nos. 2, 3, 4, 9, 11 chromosomes between *P. i.* and *P. w.*; and at Nos. 2, 3, 4 chromosomes between *P. s.* and *P. w.*, respectively. All the other chromosomes compared interspecifically were similar to each other.

Aberrant spermatogenesis was seen in the testes of *P. w.* and no intact sperm was found. However, very few flukes had abnormal spermatozoa as shown in Fig. 7. The chromosomes in the primary oocytes in ovaries and oviducts were asynaptic and were recognized as univalents (Fig. 8).

Oocytes showed the emission of a polar body in mitotic division (Figs. 9, 10). Thereafter, in the beginning portion of the uterus, the oocytes encountered several or some dozen vitelline cells and began to form thin-capsuled eggs (Fig. 11). No spermatozoa, how-

Table 1 The chromosomes of *Paragonimus iloktsuenensis* (*P. i.*), *P. sadoensis* (*P. s.*) and *P. westermani* (*P. w.*)

Chromosome No.	Species	Relative length	Arm ratio	Centromeric index
1	*P. i.*	19.3±0.15	1.31±0.02	43.3±0.40
	P. s.	19.0±0.16	1.29±0.02	43.7±0.31
	P. w.	18.9±0.35	1.34±0.03	42.7±0.62
2	*P. i.*	13.3±0.14	6.06±0.18	14.6±0.20 } *
	P. s.	13.0±0.14	5.57±0.08 } *	15.2±0.19 } *
	P. w.	12.2±0.14	6.62±0.16 }	13.2±0.27 }
3	*P. i.*	11.7±0.11	4.98±0.12 }	16.8±0.33 }
	P. s.	11.6±0.10	4.54±0.13 } *	18.2±0.41 } *
	P. w.	11.7±0.17	3.97±0.13 }	20.7±0.48 }
4	*P. i.*	11.0±0.14	4.66±0.13 }	17.8±0.42 }
	P. s.	11.1±0.15	4.83±0.13 } *	17.2±0.38 } *
	P. w.	10.9±0.15	5.81±0.19 }	14.8±0.39 }
5	*P. i.*	10.6±0.11	4.59±0.10	18.0±0.34
	P. s.	10.3±0.12	4.47±0.10	18.3±0.32
	P. w.	9.8±0.14	4.17±0.21	19.7±0.90
6	*P. i.*	6.6±0.09	2.49±0.08	28.8±0.71
	P. s.	6.7±0.07	2.27±0.07	30.7±0.61
	P. w.	7.5±0.09	2.49±0.06	28.8±0.63
7	*P. i.*	6.2±0.08	2.65±0.10	27.6±0.78
	P. s.	6.3±0.07	2.49±0.05	28.7±0.39
	P. w.	6.5±0.14	2.62±0.10	27.8±0.76
8	*P. i.*	5.7±0.08	2.76±0.01	26.6±0.10
	P. s.	5.9±0.07	2.61±0.06	27.8±0.51
	P. w.	5.7±0.12	2.89±0.09	25.9±0.66
9	*P. i.*	5.2±0.03	2.21±0.06 }	31.2±0.57
	P. s.	5.6±0.10	1.93±0.08 } *	34.4±1.00
	P. w.	5.3±0.11	1.78±0.10 }	36.4±1.36
10	*P. i.*	6.0±0.09	1.13±0.01	47.0±0.29
	P. s.	5.6±0.08	1.17±0.01	46.2±0.17
	P. w.	6.6±0.16	1.16±0.03	46.5±0.63
11	*P. i.*	4.5±0.10	1.44±0.03 }	41.1±0.49 }
	P. s.	5.4±0.18	1.25±0.02 } *	44.5±0.32 } *
	P. w.	5.1±0.17	1.24±0.03 }	44.7±0.52 }

* Difference significant ($P<0.01$)

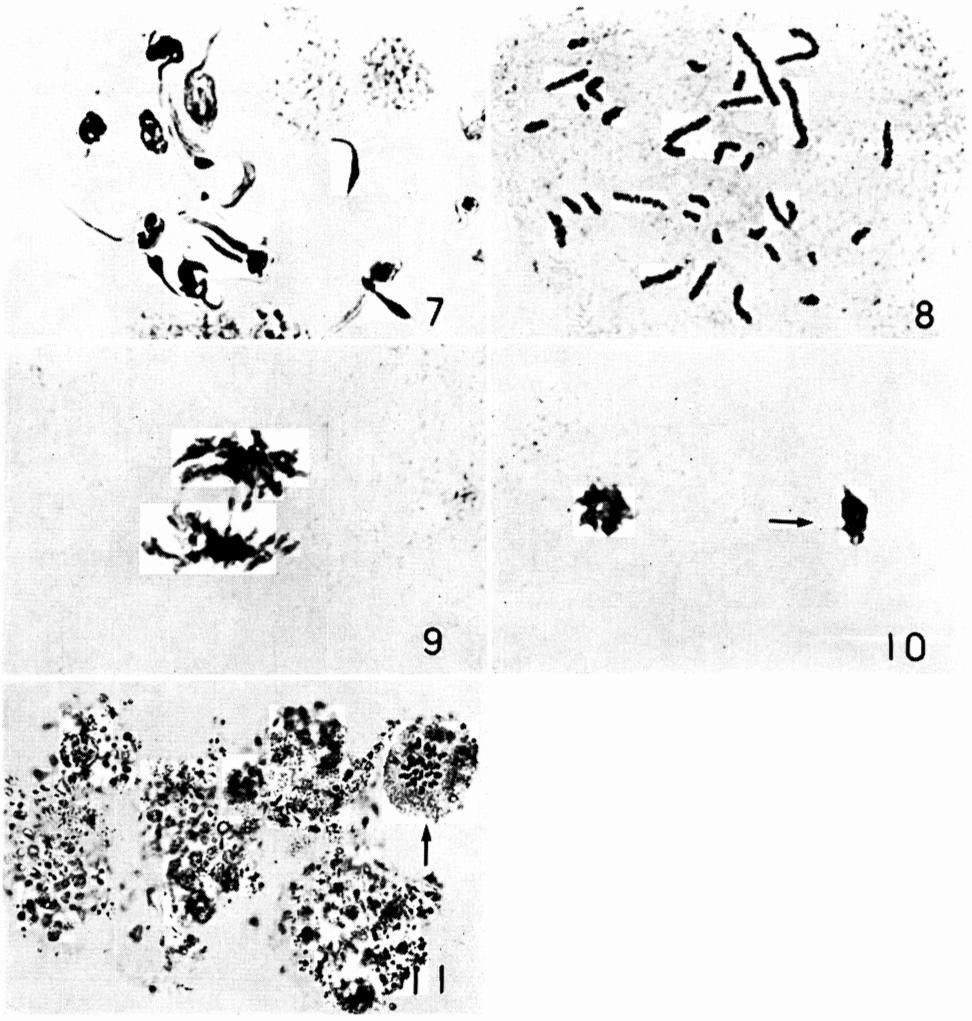
ever, were found in the cytoplasm of oocytes at any stages of oogenesis and even in young eggs.

Discussion

The present study confirmed our previous conclusion that the chromosomes of both *P. i.* and *P. s.* were 22 (2n) and 11 (n) and that of *P. w.*, 33 (3n) (Sakaguchi and Tada, 1975 a, b, 1976a). Although karyotypic similarity of *P. i.* with *P. s.* was reported briefly (Sakaguchi *et al.*, 1977a, b), this evidence was demonstrated in the present study through various indices (Table 1). Each set of the trimorphic chromosomes of *P. w.* corresponded strictly to that of *P. i.* and *P. s.*. The differences shown among the arm indices of several pairs among these 3 species could be accounted for the pericentric inversion. However, it should be considered also that the mensural artifacts occurred due to incorrect homologue matching of certain morphologically similar members.

It should be noted that there is close similarity in the karyotype not only between *P. i.* and *P. s.*, but also between these two species and *P. o.* and *P. m.* which were previously reported by us (Sakaguchi and Tada, 1976 a, c). This finding confirms the results of karyotype analysis by Terasaki (1977), too.

In all the testes of *P. w.* examined, both



Explanation of Figures

Fig. 7 Abnormal spermatozoa found in the testis of *P. westermani* (Ca ×1,100).
Fig. 8 Primary oocyte showing 33 univalent chromosomes (Ca ×1,100).
Fig. 9 Mitotic division of a primary oocyte (anaphase) (Ca ×1,100).
Fig. 10 Emission of a polar body (arrow) in a primary oocyte (telophase) (Ca ×1,100).
Fig. 11 Primary oocyte provided with 33 chromosomes (arrow) and surrounding vitelline cells (Ca ×440).

spermatogenesis and spermiogenesis were aberrant and no spermatozoa were found. This evidence supported the author's preliminary description (Sakaguchi, 1977) and the finding by Cho *et al.* (1977). Based on this evidence, the change of the chromosomes in the course of oogenesis and the presence or absence of fertilization were carefully investigated in order to substantiate probable parthenogenesis. The study revealed that all the chromosomes in the primary oocyte were characteristically asynaptic comprising 33 univalents and the oocytes divided mitotically and developed to triploid eggs. In other words, it may be concluded that the reproduction of *P. w.* of parthenogenetic nature. This conclusion coincides with that of Cho *et al.* (1977) who demonstrated the mechanism of triploid formation in *P. w.* A similar mechanism is probably functioning in

the triploid formation of *Fasciola* sp. from Japan and Korea and *F. gigantica* from Hawaii, whose triploid chromosome number was 30 (Sakaguchi and Nakagawa, 1975; Sakaguchi and Yoneda, 1976; Sakaguchi *et al.*, 1979; Sakaguchi and Ueno, 1979).

Recently Miyazaki (1978) discovered the presence of the diploid form of *P. w.* in Japan and he recommended that the triploid form of the fluke be called *P. pulmonalis* (Baelz, 1880). According to this criterion, the fluke we dealt with is *P. pulmonalis*. This should be taken into consideration in future studies.

Summary

The spermatogonial and oogonial metaphase plates of *Paragonimus iloktsuenensis*, *P. sadoensis* and *P. westermani* were investigated karyotypically for the comparison of these flukes using an air-drying method. The gametogenesis and fertilization of the triploid form of *P. westermani* were studied in order to examine the parthenogenetic nature of reproduction in this species. The following are the results obtained:

1) The chromosome number of both *P. iloktsuenensis* and *P. sadoensis* were 22 (2n) and these two species are very close from the karyotypic viewpoint. No heteromorphic pairs were found between male and female complements in these two species.

2) The chromosome number of *P. westermani* was 33 (3n) which comprised 3 sets of 11 basic chromosomes.

3) The primary oocyte of *P. westermani* divided mitotically and resulted in a triploid egg which developed parthenogenetically.

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小型大平肺吸虫，佐渡肺吸虫，ウエステルマン肺吸虫の核型及び ウエステルマン肺吸虫の配偶子形成について

坂口祐二　多田　功
（熊本大学医学部寄生虫病学教室）

小型大平肺吸虫，佐渡肺吸虫及びウエステルマン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，その核型分析を行つた．又ウエステルマン肺吸虫については配偶子形成と受精の有無を観察した．得られた成績は次の通りである．

1) 小型大平肺吸虫と佐渡肺吸虫の染色体数は共に 22 (2n)で，その核型は極めて似かよつている．

2) ウエステルマン肺吸虫の染色体数は 33(n) で，11 本の染色体を基数として三セットからなる．

3) ウエステルマン肺吸虫の第一卵母細胞は有糸分裂によつて分割し，単為生殖的に三倍体の虫卵を形成する．