

## Karyotype and Gametogenesis of the Common Liver Fluke, *Fasciola* sp., in Japan

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The cytology of genus *Fasciola* has been a matter of confusion with divergent opinions. The diploid chromosome numbers of *F. hepatica* so far reported were 8 (Schubmann, 1905), 12 (Schellenberg, 1911), 16 (Govaert, 1960) and 20 (Dehorne, 1911; Sanderson, 1953) and that of *F. gigantica* varied from 16 (Srivastava and Jha, 1964) to 20 (Venkat-Reddy and Subramanyam, 1973). All the studies were made on sectioned materials except those of Sanderson (1953) and Venkat-Reddy and Subramanyam (1973).

We have reported the presence of two types of chromosome numbers, 20 and 30, in the Japanese common liver fluke (*Fasciola* sp.) (Sakaguchi and Nakagawa, 1975; Sakaguchi and Yoneda, 1976). These two types were well discriminated by the body size. It was suggested that the specimens with 30 chromosomes were of triploid form (Sakaguchi and Yoneda, 1976). In these two types, spermatogenesis was entirely aberrant in testis with no evidence of synaptic pairing in spermatocyte (Sakaguchi, 1977). Moriyama *et al.* (1979) confirmed our findings and reported the presence of mixoploid type with a 20/30 mosaic constitution. Kayano (1978, personal communication,) suggested that the reproduction of Japanese *Fasciola* sp. might be parthenogenetic.

The present paper deals with detailed

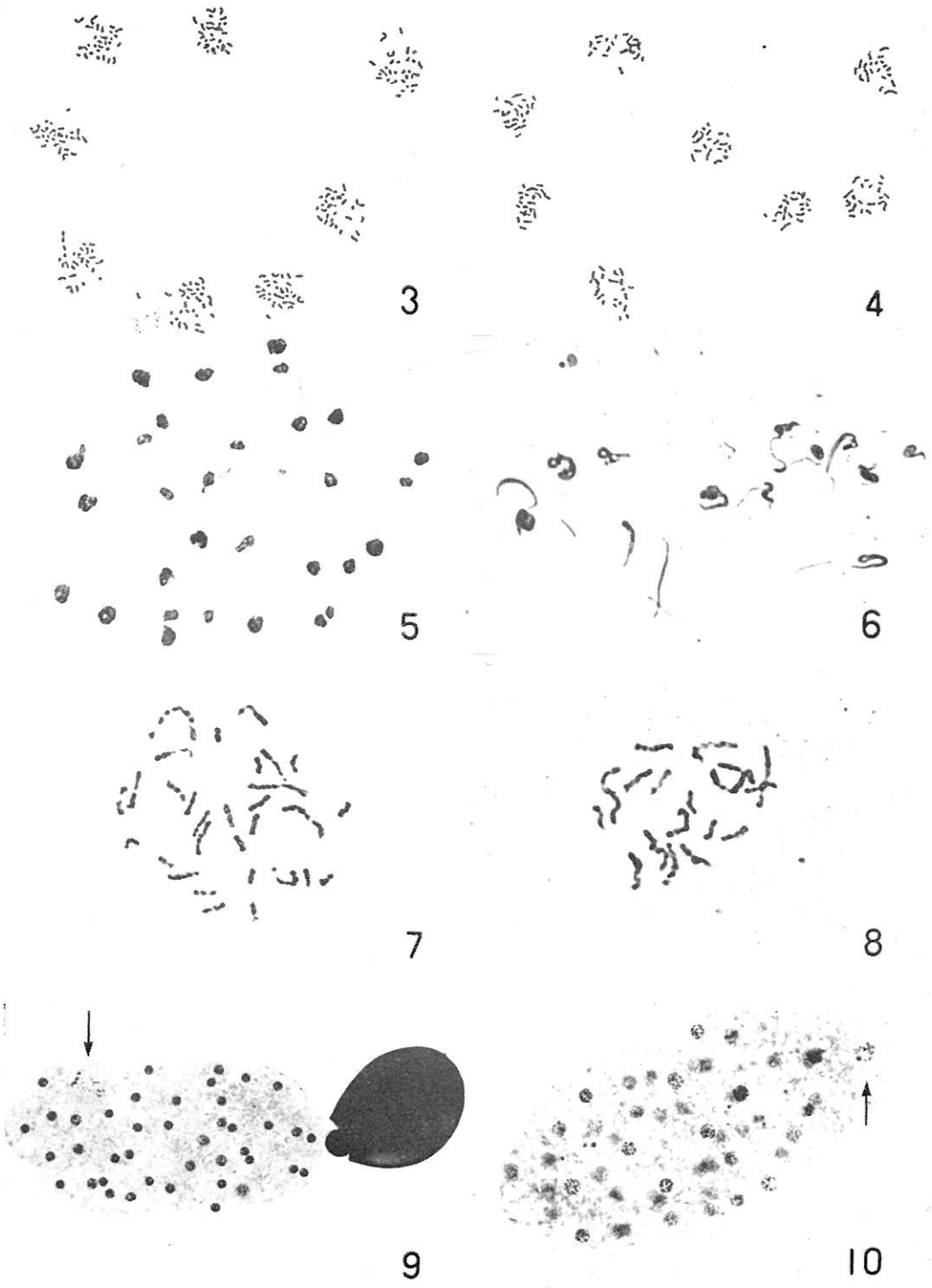
karyotypes of the two types of Japanese liver fluke (*Fasciola* sp.), with special attention to the spermatogenesis, oogenesis and process of egg-formation.

### Materials and Methods

Mature worms of *Fasciola* sp. were obtained from cattle at slaughter houses in Kanazawa and Kumamoto Cities. The host animals were from various places such as Ishikawa, Toyama, Niigata, Nagano, Saitama, Gumma, Chiba, Ōita and Kumamoto Prefectures.

The chromosomes were studied on the gonadal tissues following the air-dry method of Takagi (1971) with Giemsa staining. Chromosomes were classified in accordance with the system proposed by Levan *et al.* (1964).

Some specimens were fixed in methanol-acetic acid (3:1), transferred to 70% ethanol and stained with alcoholic hydrochloric acid-carmin (Snow, 1963). Testicular tissues (from testis to seminal vesicle) and ovarian tissues (from ovary to uterus) were dissected out from the flukes, placed on a glass slide and squashed with a thumb under a cover slip. The squash preparations were used for observations of gametogenesis.



reproduction of *Fasciola* sp. is of parthenogenetic nature, as has been implicated by Kayano (1978, personal comm.)

We have examined chromosomes of *Fasciola* sp. from other localities such as Korea and India (Assam) and those of *F. gigantica* from Hawaii, and found that they also had 20 or 30 chromosomes (Sakaguchi *et al.*, 1978, 1980; Sakaguchi and Ueno, 1979). It seems probable that they also represent parthenogenetic forms, though not confirmed. On the contrary, we recently found that in *F. hepatica* obtained from Australia, England, and North America and in *Fasciola* sp. from Thailand, India (Bombay), Pakistan and Puerto-Rico examined, both spermatogenesis and oogenesis proceeded normally with mature sperm and eggs, and the chromosomes in primary oocytes were all synaptic (Sakaguchi and Ueno, 1977, 1978; Sakaguchi and Kusano, 1979; Sakaguchi *et al.*, 1980). These local forms were all diploid which were thought to produce eggs through ordinary sexual reproduction as frequently seen in many other trematode species.

The presence of two different reproduction mechanisms in the genus *Fasciola* is of particular interest and further studies of liver flukes are requested on the cytogenetic and taxonomic aspects, as well as from the geographical point of view.

## Summary

Karyotype and gametogenesis of Japanese common liver fluke (*Fasciola* sp.) were investigated by means of the air-drying and squashing methods.

Japanese *Fasciola* sp. was classified into three types based on their chromosomal complements; individuals with 20 or 30 chromosomes and with a 20/30 mosaic constitution. Worms having 30 chromosomes represent a triploid form with 3 sets of 10 basic chromosomes, while those with 20 chromosomes were diploid and mosaic individuals were 2n/3n mixoploid. No karyotypic difference was found between spermatogonial and oogonial cells in these three types, except for the different level of ploidy. Gametogenesis was entirely aberrant in both testes and ovaries with no evidence of synaptic pairing in both spermatocytes and oocytes. The diploid and triploid eggs were assumed to develop parthenogenetically.

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## Explanation of Figures

- Fig. 3 Eight primary spermatocytes each composed of 30 univalent chromosomes. Ca  $\times$ 245.  
 Fig. 4 Eight primary spermatocytes each composed of 20 univalent chromosomes. Ca  $\times$ 245.  
 Fig. 5 Abnormal spermatids found in the testis of *Fasciola* sp. (3n=30 type) Ca  $\times$ 700.  
 Fig. 6 Abnormal spermatozoa found in the testis of *Fasciola* sp. (3n=30 type) Ca  $\times$ 700.  
 Fig. 7 Primary oocyte with 30 univalents. Ca  $\times$ 700.  
 Fig. 8 Primary oocyte with 20 univalents. Ca  $\times$ 700.  
 Fig. 9 Primary oocyte with 30 chromosomes (arrow) and vitelline cells pushed out of egg-shell. Ca  $\times$ 110.  
 Fig. 10 Primary oocyte with 20 chromosomes (arrow) and vitelline cells pushed out of egg-shell. Ca  $\times$ 110.

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## 日本産肝蛭の核型と配偶子形成

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日本産肝蛭の核型と配偶子形成過程をエアードライ法と押しつぶし法を用いて観察した。

日本産肝蛭はその染色体数からそれぞれ20本の個体、30本の個体及び20/30本のモザイク個体の三型に分けられる。染色体数が30本の個体は10本の染色体を基数として三セットからなる三倍体である。又染色体数が20本の個体は二倍体で、モザイク個体は  $2n/3n$  の混数体で

ある。三型の出現頻度は二倍体個体が16.7%、三倍体個体、75.8%、モザイク個体は7.5%であった。三型とも精原細胞と卵原細胞の核型には差違が認められない。

二倍体の個体と三倍体の個体はいずれも配偶子形成異常を示し、精母細胞及び卵母細胞で染色体対合がみられない。二倍体及び三倍体の虫卵は単為生殖的に發育するものと思われる。

