



Title	Studies on the Sex and Chromosomes of the Oriental Human Blood Fluke, <i>Schistosomum japonicum</i> Katsurada (With 16 Textfigures and 3 Tables)
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Studies on the Sex and Chromosomes of the
Oriental Human Blood Fluke,
Schistosomum japonicum
Katsurada¹⁾

By

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(With 16 Textfigures and 3 Tables)

The family Schistosomidae is the only group of trematodes in which the sexes are separate and dimorphic; the female is usually found held in the gynaecophoric canal of the male which is formed by the curling up of the sides of the post-acetabular region. Such extreme sexual difference develops only in the adult found within the final mammalian host. It must be considered, however, that the sex of the schistosome is differentiated at least in the cercariae stage within the intermediate host. For, if the cercariae of *Schistosomum japonicum* from a single infected snail are artificially introduced into the final host, the recovered flukes are usually, but not always, of the same sex (Katsurada, 1915; Tanabe, 1919; Tojihara, 1919; Faust, 1927; Sagawa, Ogi and Sumigoshi, 1928; Severinghaus, 1928; Tanaka, 1929; and Sugiura, 1933). Furthermore, since the cercariae develop parthenogenetically from the germ cells of the secondary sporocysts and the latter in turn develop in the same manner from those of the primary sporocysts which directly develop from the miracidia, it is theoretically expected that the sex of this fluke would be determined by a chromosomal mechanism at the time of the fertilization of the eggs. According to Lindner (1914), the

1) Contribution No. 103 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

Egyptian species, *Schistosomum haematobium*, contains 14 chromosomes in the spermatogonium and six autosomes plus two small sex chromosomes in the primary spermatocyte, the latter passing undivided to one pole of the spindle, so that one half of a spermatid has eight chromosomes and the other half six chromosomes. Severinghaus (1928) recently described a similar chromosomal complex for *Schistosomum japonicum*. On the other hand, Faust and Meleney (1924) found in the latter form a single X-chromosome which goes to one pole in the primary spermatocyte division, producing as a result two kinds of secondary spermatocytes, of which one contains eight chromosomes and the other seven. In this respect the Japanese species was distinguished by them from the Egyptian representative. Thus these authors stand in complete agreement with the point of view that the male is heterogametic, though there still exists a difference of opinion upon the sex chromosome. Their conclusion however was based on figures which are not absolutely convincing. Unfortunately their observations have been almost confined to the male side only and little attention was paid to the female cell which has important significance regarding the problem of sex-determination. Under these circumstances the present work was undertaken to obtain more exact figures of chromosomes, with which the previous conclusion could be examined, and to ascertain, on the other hand, whether the corresponding sex chromosomes found in the male cell are actually present in the female or not. Further, this opportunity will be embraced for the presentation of a brief discussion on certain phases of the sexual phenomenon of *Schistosomum japonicum*, based in part on our own observations and the experimental data reported by previous investigators.

Before going further the authors wish to express cordial thanks to Professor Kan Oguma for his helpful criticism and revision of the manuscript. In consulting literature necessary for this study, the authors are indebted to the kindness of Professors S. Goto, K. Kawamura and M. Suzuki.

I. The chromosomes of *Schistosomum japonicum*

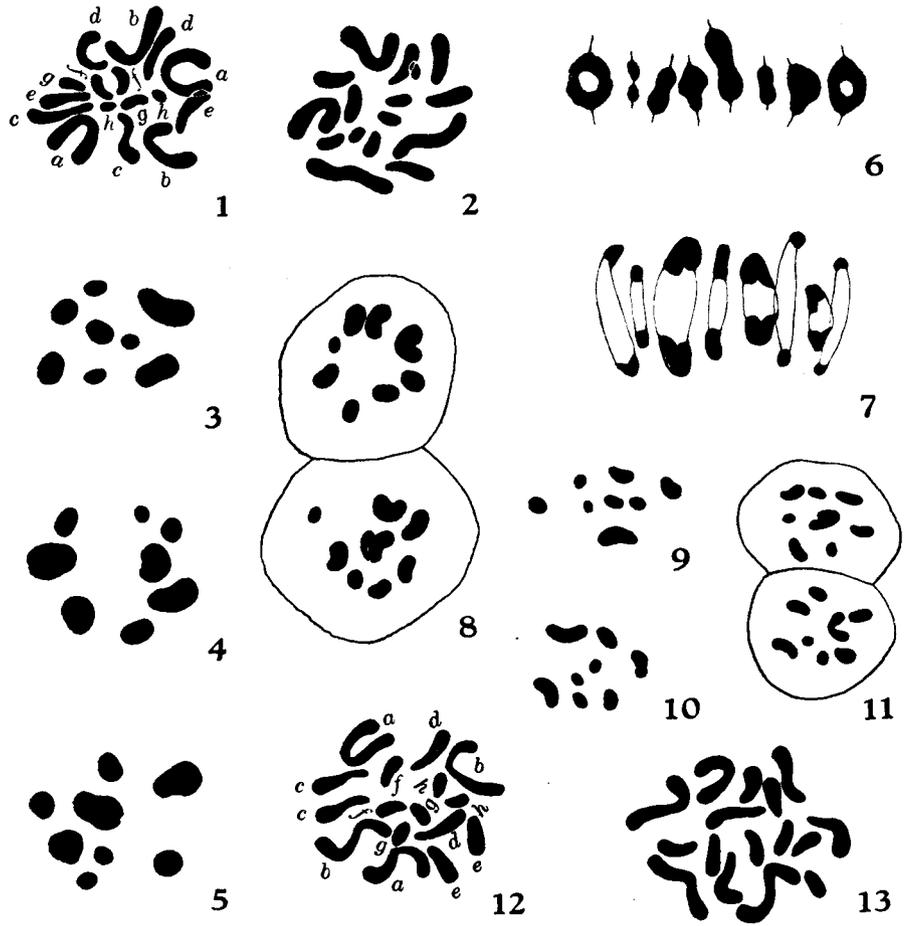
For the present material a large number of flukes in different stages of maturity have been obtained by autopsies of many artificially infected rabbits with the generous assistance of Dr. S. Sugiura, who has practised as a physician in Saijo-Mura near Kofu in Yamanashi Prefecture, one of the endemic areas of Schistosomiasis japonica, and has engaged in ecol-

gical studies on the intermediate snail of this disease, *Onchomelania nosophora*. The flukes were fixed in Flemming's strong solution without dissection. The sections were cut 10 micra thick and stained with iron-alum haematoxylin as usual. Though various stages of infection were examined, worms of from forty to fifty days after infection seem to be the most satisfactory for the study of chromosomes either in the male or in the female.

The chromosome of the male (Figs. 1-11). The spermatogonium contains 16 chromosomes as seen in the metaphase polar view of division (Figs. 1-2). The mode of arrangement shows a typical rosette, as found in other forms of animals, scattered radially on the equatorial plate; the larger chromosomes always take the peripheral positions surrounding those of smaller size in the central part of the spindle.

As noted above, the number of chromosomes in the spermatogonium is even. Therefore, if the sex chromosomes exist there it may only be accounted for by supposing that they are either of the X-Y type or the XX-O type. With this idea, supposed homologous pairs were checked according to their shape and size. Fortunately in the present species the individual chromosomes are distinguishable from one another by their characteristic shape and relative length, and so identification of the homologous mates is made with absolute clearness. A result of such an attempt is shown in Fig. 1, in which homologous mates are indicated with the letters *a* to *h*. Fig. 14 shows the serial alignment of the same from left to right graduated in order of size. As is obvious in these demonstrations the diploid garniture is composed of two homologous pairs of atelomitic V-shaped chromosomes, of which one pair has a quite median fibre attachment while the other has a submedian, and six homologous pairs of telomitic rod-shaped ones, gradually diminishing in length. Thus the present observation has failed to find out any kind of sex-chromosomes at least by any identifiable differences in form.

As is naturally expected from the evidence found in the spermatogonium, there are produced eight bivalent chromosomes as the reduced condition in the primary spermatocyte (Figs. 3-5). They assume a thick, oblong shape in polar view, showing a gradatory size diminution corresponding to that in the spermatogonium. When viewed from one side of the spindle, two larger bivalents appear as vertical rings (see Fig. 6). It is certain that these are the tetrads descended from two pairs of the atelomitic chromosomes already observed in the sperma-



	a	b	c	d	e	f	g	h	
♂									14
♀									15

S. Makino del.

togonium. The remaining six bivalents assume a lozenge or dumbbell shape in the lateral aspect.

In order to examine, next, whether any chromosomes with particular behavior in division are actually present, the reader's attention will be called to the meta-anaphasic figures shown in Figs. 6-8. Fig. 6 shows the side view of the primary spermatocyte metaphase and Fig. 7 is the same in the anaphase. In both figures there is found no one chromosome which presents any different behavior in separation from ordinary tetrads. Fig. 8 represents the polar view of the sister groups in the primary spermatocyte anaphase found in a single section, in each of which eight chromosomes correspond exactly to each other in pairs. Thus the present observation results in the conclusion that all eight chromosomes are to be considered as ordinary tetrads and eventually there exists no size difference between the separated halves from which a tetrad is composed.

The symmetrical distribution of the chromosomes in the primary spermatocyte division gives rise to only one kind of secondary spermatocyte, which contains, without exception, eight chromosomes corresponding in size to the primary spermatocyte chromosomes, but in dyad condition (Figs. 9-10). Fig. 11 shows the polar view of the secondary spermatocyte anaphase found in a single section in which both sister sets contain eight chromosomes corresponding in size and shape with one another.

In conclusion, therefore, the present workers are unable to accept the view of the existence either of XY or double X's in the male cells in spermatogenesis of *Schistosomum japonicum*.

The chromosomes of the female (Figs. 12-13). In the ovaries, many oögonia were found in process of division. The chromosomes were preserved sufficiently well to count their complete number and to compare the morphological characters (Figs. 12-13). The number

Figs. 1-15. Chromosomes of *Schistosomum japonicum*. (4200 ×). 1-2, metaphase polar views of the spermatogonia. 3-5, metaphase polar views of the primary spermatocytes. 6, side view of the primary spermatocyte metaphase, showing all elements serially arranged. 7, side view of the primary spermatocyte anaphase. 8, polar view of the complete sister groups in the primary spermatocyte anaphase. 9-10, metaphase polar views of the secondary spermatocytes. 11, polar view of the complete sister groups in the secondary spermatocyte anaphase. 12-13, metaphase polar views of the oögonia. 14, serial alignment of the spermatogonial chromosomes, from Fig. 1. 15, the same of the oögonial chromosomes, from Fig. 12.

of chromosomes is 16 in an oögonium, the same number as in spermatogonium; this has been determined beyond question in a sufficient number of cases in which no conflicting evidence was involved. With the same idea as the authors carried out in the observation of the male cell, the supposed homologous pairs were checked by comparing their shape and size, and there are obtained eight pairs of mated chromosomes, any solitary chromosome or a pair of unequal size being quite lacking (see Figs. 12 and 15). In other words, the complex is composed of two pairs of atelomitic and six pairs of telomitic chromosomes, as readily demonstrated in Fig. 15. It is obvious, then, that the present observation has disproved the occurrence of any special chromosomes in the oögonium, and further that the female diploid group is composed of chromosomes with corresponding relation to those of the male, as readily suggested from Figs. 14 and 15, in which the morphological characters of chromosomes can be most clearly compared between the two sexes.

Remarks. So far as the present observations go, it was absolutely impossible to discern the sexes of *Schistosomum japonicum* by any particular chromosomes either in form or in behavior as shown in the foregoing description. This is essentially different from the opinion of the previous authors who claim male heterogamety in respect to the sex-chromosome.

Lindner's observations (1914) on *Schistosomum haematobium* seem to warrant the following conclusion; there are 14 chromosomes in the spermatogonium and eight in the primary spermatocyte metaphase, of which six chromosomes are larger sized and always take a peripheral arrangement surrounding two smaller chromosomes in the central part of the spindle. The former divide normally, but the latter two pass undivided to one pole, so that one half of the secondary spermatocyte carries six chromosomes and the other half eight. Since all the chromosomes divide normally in the second division there are formed two types of sperms, the one having six chromosomes, the no X-class, and the other eight chromosomes, the X-class, which possesses six autosomes plus two smaller X-chromosomes. Recently a quite similar condition of chromosomes has been reported by Severinghaus (1928) to exist in *Schistosomum japonicum*. For instance, the spermatogonial chromosomes given by him show a quite different appearance from those observed in the present case, as they are all represented by small dot-like ones with a

somewhat oblong outline without marked size variation, in spite of the fact that in the metaphase of the primary spermatocyte the chromosomes show various sizes and shapes. This fact indicates that Severinghaus's observation on this line was not absolutely correct so that the conclusions therefrom can hardly be accepted without hesitation. One must consider then, the problem that two small chromosomes surrounded by six at the metaphase of the primary spermatocyte may represent the sex-chromosomes as he so described. From the authors' experience it becomes clear that there are often found cases in which some displaced tetrads have been misinterpreted as heterochromosomes in various kinds of animals when the fixation is not adequate. The two small chromosomes in question in Lindner's figure, which were taken for X's by him, seem to correspond with all probability to two small tetrads in *Schistosomum japonicum* as ascertained by the present observation. As a matter of fact, these small tetrads tend easily to be displaced out of the equatorial plate, by some mechanical effect of the fixing reagent due probably to their diminished volume. On the contrary, however, these two small tetrads in material of good fixation show no such displacement and are divided in each case into equal component halves just like the remaining larger ones. At the same time, similar criticism should be applied also to Severinghaus's observations on *Schistosomum japonicum*, which entirely agrees in every respect with those of Lindner.

In *Schistosomum japonicum* Faust and Meleney (1924) observed seven tetrads and one dyad in a reduced condition, the latter passing undivided to one pole of the first division and separating from each other in the second division, so that spermatids of two types are formed, one having eight chromosomes and the other seven. Although they stated "the observations on the spermatogenesis of *Schistosomum japonicum* follow those of Lindner of *S. haematobium* very closely and agree with his studies point for point, except that the reduced number is respectively eight and seven, instead of eight and six, indicating a specific difference in the chromosome complex of these two related species," their descriptions of the spermatogenetic stages do not agree in many respects with those of Lindner, and their figures contain too many dubious points to allow any further discussion.

In brief, the chromosomes of *Schistosomum japonicum* do not show morphologically any sexual difference, so far as the scope of the present observations is concerned, and therefore, it is impossible to determine which sex is cytologically heterogametic.

II. The sexual difference of *Schistosomum japonicum* cercariae

Cort (1921) found two distinct size types in a species of schistosome cercariae from *Planorbis trivolis* from Douglas Lake, Michigan, and assumed it as a sexual difference. Miller (1927) also reported the same fact in *Cercariae tuckerensis*. In the cercariae of *Schistosomum japonicum*, however, no one has been able to detect any sexual difference, notwithstanding that the sexes are theoretically expected to be differentiated early in that stage of development, from the fact of the occurrence of unisexual infection in experimented animals artificially infected with cercariae from single snails. In order to find the size difference, Takahashi (1928) has recently made a series of measurements on the cercariae of this species separately obtained from single infected snails, but no significant difference was detected between the cercariae of one snail and those of another. Such a method of measurement, however, seems to be unsuitable for finding the sexual difference, if present, in such a minute structure. It remains therefore only a possibility that the sexual difference, if it exists, would be found by a close examination on cercariae, whose sexes are identified beforehand.

For this reason, in the summer of 1930, with the enthusiastic co-operation of Dr. Sugiura, a number of rabbits were artificially infected with cercariae from single infected snails. In this experiment, to avoid the possibility of an accidental adhesion of cercariae from one snail to the body surface of another, all the snails were first dried in the air for a while, and then singly placed in a small glass vial partly filled with water and allowed to remain for a fortnight, during which the water contained in the vial was changed two to three times. The isolated snails were singly placed on a slide and covered with another one and then crushed by compressing with the finger. By examination under the low power microscope, infected snails could be distinguished at a glance, for the cercariae are very active. Out of 36 snails examined in this way a heavy infection was found in 22 snails, of which 20 were used for the experiment. A large portion of the cercariae from each infected snail together with fragments of its tissue and a small amount of water were smeared on a small area of exposed ventral skin of a rabbit which was tied on a board with a string. All the experimental animals were killed from 35 to 117 days after operation. At autopsies the flukes were found, from fifteen to three hundred in number, in the vessels of the portal system. Three

kinds of infection were to be distinguished: a male and female or bisexual infection in four rabbits, and a male and a female unisexual infection in eight respectively. The detailed description of this infection experiment has already appeared in Dr. Sugiura's paper (1933) with the results of his own subsequent research concerning the sex problem of the present species.

In every case of the infection experiments above mentioned a portion of the cercariae with the adhered fragments of each snail was fixed in saturated aquatic solution of sublimate or Flemming's solution and preserved in 80% alcohol for future study. At the same time a number of the cercariae were moderately narcotized with a dilute aquatic solution of cocaine hydrochloride, and mounted with glycerine after being stained with aceto-carmin. The sex of these preserved cercariae from each infected snail was determined by examination on the sex of the flukes obtained at autopsy from rabbits which had previously become infected with cercariae from the same snail. For measurement 10 well preserved cercariae were selected for each unisexual infected snail used in experiment and drawn with a camera lucida under magnification of 320 times.

Table 1. Length and breadth of body proper and tail trunk, and length of furci of the male and female cercariae; average of 10 individuals for each infected snail.

Infected snails. No.	Cercariae				
	Sex	Number	Length & breadth of body proper	Length & breadth of tail trunk	Length of furci
1	♀	10	148-62	108-21	48
2		10	155-64	111-21	49
3		10	149-60	119-21	51
13		10	162-65	111-21	49
16		10	152-61	113-20	51
17		10	163-60	112-20	49
20		10	171-65	119-20	53
22		10	157-56	108-20	49
5		♂	10	152-63	116-21
6	10		158-70	111-20	51
9	10		153-58	115-21	51
10	10		172-62	120-22	50
12	10		156-56	113-20	50
21	10		164-64	116-22	50
23	10		162-62	120-21	53
19	10		164-57	112-22	50

Perusal of the figures in Table 1 shows no significant differences between the male and female cercariae in the length and breadth of the body proper (1) and of the tail trunk (2), and the length of the furci (3). Our specimens are somewhat small in body length, but large in breadth of body, compared with those of the previous authors (Table 2). This is probably due to the different methods of measurement used by the different authors and not to any actual size variation.

Table 2. Measurement of the cercariae of *Schistosomum japonicum*.

Authority	Cercariae			Remarks
	Number of individuals	Length and breadth of body	Length and breadth of tail	
Suzuki (1914)	87	165-22 ^μ	165-22 ^μ	Fixed with osmium.
Narabayashi (1916)	52	164-39	133-15	Stained with neutral red in vitro.
Faust (1920)	59	126-46	145	
Takahashi (1928)	87	179-52	155-25	Narcotized with cocaine hydrochloride.
Ikeda (1935)	80	157-62	112-21	Ditto.
	80	160-62	115-21	

The genital primordium of the cercariae of *Schistosomum japonicum* is extremely simple, consisting of a single cluster of small cells with a large nucleus, situated directly behind the ventral sucker (Faust and Meleney, 1924; Takahashi, 1928). In the microscopical observations on total and sectioned preparations of the male and female cercariae, special attention has been paid to the structure of the genital primordium; that is, to its size and shape, and to the number, arrangement and inclusion of the cells. Unfortunately these efforts were not successful.

III. The proportion of bi- and unisexual infections in the intermediate hosts

From the experiments where the cercariae of *Schistosomum japonicum* from a single snail are used to infect a mammalian host, it becomes clear that there are three kinds of infected snails, one of which carries both male and female cercariae, and the other two only

male or female cercariae respectively. Reviewing the experimental data reported by the previous authors as seen in Table 3, although the proportion of these kinds of infections varies somewhat in different cases, unisexual parasitism occupies the major part of the infected snails examined. Further, it is noticeable that in the unisexual parasitism the male and female infections occur in approximately the same number, with the exception of Faust's experiment where all the infected animals yielded immature male worms only. He utilized cercariae obtained from *Onchonelania hupensis* which were collected from several localities in the adjacent vicinity of Soochow, China during the dry season from March to September, and stated that "the dry condition prevailing in the collected area may have been more severe on the female than on the male parthenitae within the snails".

Table 3. Proportion of the uni- and bisexual infections in the intermediate host.

Authority	Animals exposed to infection	Number of infected snails			Ratio between uni- and bisexual infection	Per cent of infection
		♀ ♂	♀	♂		
Tanabe (1919)	Rabbit and guinea pig	5	14	12	16 : 84	3-5
Faust (1927)	Rabbit and dog	0	0	5*	0 : 100	0.2
Severinghaus (1928)	Hamster	3	12	12	11 : 89	1-4
Tanaka (1929)	Rabbit	2	7	3	17 : 83	1-2
Sugiura (1933)	Rabbit	9	16	21	25 : 75	60
		19	49	54	16 : 84	

* Faust used cercariae collected from 15 infected snails to infect 11 mammalian hosts, out of which four were submitted to infection with cercariae obtained together from two snails respectively, and the remaining seven with those derived from each single snail. In the latter series of experiments the flukes at autopsy are found in five hosts, but none in two.

If the miracidia are half male and half female producing, chance invasion of a single miracidium into a snail would give only unisexual parasitism, one half of the snails harbouring male and the other half female cercariae. Conversely, when all the snails are attacked at random with two or more miracidia it would be theoretically expected

that the bisexual infections would predominate to a great extent over the unisexual infections. In the results of artificially infecting experiments of the former investigators, however, the bisexual parasitisms occupy only 16 per cent of the snails examined. It is conceivable therefore that the invasions of two or more miracidia into a snail seldom occur in the natural habitat. We will not claim of course that there is any kind of barrier within the snails to prevent them from the additional invasion after being attacked by a miracidium, because, Faust and Meleney (1924), for instance, really found that some of the snails submitted to heavy infection were invaded by two to several dozen miracidia. The condition seems to indicate a numerical correlation between the miracidia and the snails exposed to the former. If the miracidia are present in a large number or the snails in a small number, a heavy invasion would take place. Moreover, another interpretation of the predominance of the unisexual parasitism in the infected snails might be given from another point of view. Yasuda (1915) stated in his study on the development of the parasitic progeny of *Schistosomum japonicum* within the experimentally infected snails that he could not trace back to the early stage of redia only, on account of the death of all the snails caused by heavy invasion of miracidia. At present we have no actual data on this point, however, and it is naturally supposed that the degree of mortality is higher in the snails invaded by double or more miracidia than in those infected by a single one. If so, the snails carrying only male or female parasitic progeny would occupy the greater part of the survived infected ones.

IV. Development and Growth of the Fluke Under Unisexual Infection

Of greater interest is the fact that when the male or female of *Schistosomum japonicum* are allowed to develop in the mammalian host in the absence of the opposite sex, their growth is extremely retarded (Fig. 16). Severinghaus (1928) states that the male when present alone does not develop to normal size but shows a normal development of the gonad, while the independent females fail to develop both as regards somatic and germ structures. Further he describes how the males living without the female attain almost normal size 65 days after infection and then become smaller again with advancing age, while the solitary females reach only one-fifth the body length of the normal

worms at 50 days and then afterwards change very little through subsequent ageing. Conversely, according to Sugiura (1933) the decrease of the body length with advancing age is more pronounced in the female than in the male.

Severinghaus (1928) further made an excellent experiment where the immature solitary females completely develop within a relatively short interval when the animal carrying them is re-infected with the male-producing cercariae. Sugiura (1933) also found the same phenomenon on the male as well as on the female side. This fact leads to an assumption, as pointed out by Severinghaus, that the normal development of the male or female

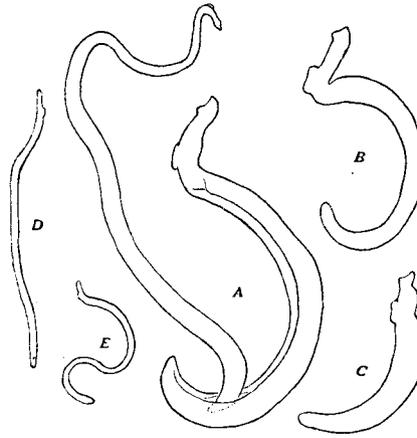


Fig. 16. *Schistosomum japonicum* under the bisexual (A) and unisexual (B-E) infections. A, male and female in copula, age 66 days. B, male, 66 days. C, male, 117 days. D, female, 37 days. E, female, 66 days. All figures drawn at table height with camera lucida. $\times 10$.

schistosome within the mammalian host depends upon some certain hormone produced by worms of the opposite sex. In connection with this the painstaking experiments independently undertaken by Sagawa, Ogi and Sumigoshi (1928) should be mentioned here. They made an extraction of the male and female flukes by crushing and grinding a large number of specimens obtained from artificially infected animals (rabbits), and repeatedly injected it with a small amount of physiological saline water into the blood vessel of the ear of the experimental animal known by control to be carrying male or female worms alone. Further, they injected the blood serum obtained from known male or female carriers into infected rabbits known to be carrying the opposite sex. So far as their experiments went, no sign of growth was found either in male or in female flukes within the hosts. Recently also, Sugiura (1933) made such injecting experiments in more detail with the extract of mature worms and the blood serum of infected rabbits, but he arrived at a negative result. It seems to the present workers that the injecting method employed by these authors, is not reliable for testing the presence of hormonal correlation between the male and female flukes within the

mammalian host. For, at autopsy of infected animals there are frequently found undersized flukes besides fully developed ones. For instance, in one of the series of Sugiura's experiments where rabbits were infected with cercariae from a single snail, a rabbit (No. 7) yielded 22 paired flukes with the normal body length and 44 solitary dwarf males about 3.8 mm. long, the same body length as that of the male under the unisexual condition for the same duration (rabbit No. 5). Rabbit No. 4 also yielded the normal and the dwarf males at autopsy. Further he found in another series of experiments that one of the rabbits which was re-infected with the male-producing cercariae at the 140th day after the first infection with the female cercariae yielded at autopsy a number of solitary dwarf females 3 to 3.5 mm. long, the same as that of the atrophied ones under the unisexual condition for 180 days, besides paired worms of almost normal sizes. It is probable that the occurrence of such solitary dwarf worms under the bisexual parasitism is due to the paucity of the mates, and consequently that the complete development of male and female flukes does not merely depend upon the co-habitation with the opposite sex in the same host, but rather upon the mutual contact with the opposite sex or continual copulation, the cause of which however is obscure at present.

V. Summary

1. The chromosome-complex of *Schistosomum japonicum* consists of 16 chromosomes, of which two pairs are atelomitic V-shaped chromosomes and the remaining six pairs are telomitic rod-shaped showing intergrading size.

2. Any special chromosomes, acceptable for XY or 2 X's were not absolutely discernible in both sexes of this dioecious trematode.

3. No morphological sex difference was detected between the male and female cercariae of *Schistosomum japonicum*, although their sexes would be theoretically expected to be differentiated from the evidence of infection experiments where mammals invaded by cercariae from single snails usually yield worms of one sex.

4. The cause of the predominance of the unisexual parasitism in the intermediate host, *Onchomelania*, was considered to some extent.

5. The male and female of *Schistosomum japonicum* living under the unisexual infection within the final host fail to mature respectively, the cause of which was briefly discussed.

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