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CHROMOSOMA

Karyotype Evolution and Sex Chromosome Differentiation in Schistosomes (Trematoda, Schistosomatidae)

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Abstract. The morphology of C-banded metaphase chromosomes has been studied in two hermaphroditic and ten gonochoristic digenetic trematodes (schistosomes). Comparison of numbers and morphology of chromosomes indicates that the karyotype of primitive trematodes probably was composed of 10 (or 11) pairs of telocentric or subtelocentric chromosomes, and reduction of chromosome numbers in advanced species resulted from centromeric fusion rather than elimination of chromosomes. Observation of heteromorphic chromosomes in a hermaphroditic trematode (*Spirorchis*) suggested a differentiation of "pre-sex" chromosomes in species ancestral to dioecious trematodes which possess distinctly differentiated sex chromosomes. Our results indicate that differentiation of Z and W chromosomes in the gonochoristic trematodes resulted from: (a) partial constitutive heterochromatinization of the W chromosome (*Schistosoma mansoni* and *S. haematobium* complexes, African schistosomes), (b) deletion of part of the W (*S. japonicum* and *S. mekongi*, Asian schistosomes), and (c) translocation of part of one sex chromosome onto another (*Schistosomatium douthitti* and *Heterobilharzia americana*, American schistosomes) with subsequent heterochromatinization of the W in *H. americana*.

Introduction

The digenetic trematodes (Subclass Digenea) are parasitic flatworms with adults in vertebrates and larval stages in various molluscan intermediate hosts. The life cycle generally follows this pattern: Adults reproduce bisexually. A zygote develops into a miracidium, which is a ciliated multicellular stage that enters the molluscan host (usually a snail) either by penetration or by being eaten. Within germinal sacs (sporocysts or rediae) in the mollusk, reproduction (usually assumed to be asexual) results in cercariae, which emerge from the host and – either with or without a second intermediate host – mature in vertebrates.

Most digenetic trematodes are hermaphrodites. Schistosomes (species in the family Schistosomatidae) are an exception; they are dioecious, with dimorphic

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adults, yet without sexual differentiation of miracidia, sporocysts, or cercariae. Males are homogametic and females heterogametic. Sex is determined during fertilization and all progeny of one miracidium have the same sex. Gonochorism of schistosomes is apparently secondary, and is believed to have evolved from the hermaphroditic condition prevailing in other trematodes.

As is the case with other hermaphrodites, paternal and maternal chromosome complexes in hermaphroditic trematodes are not distinguishable, and therefore no heteromorphic pairs of sex-related chromosomes would be expected in this group of parasites. However, a heteromorphic pair of chromosomes has been noted recently in two hermaphroditic trematodes: *Megalodiscus temperatus*, a paramphistomatid (Grossman and Cain, 1981), and a spirorchid (present report). The presence of such chromosomes in the spirorchid, which represents a group close to the ancestors of the dioecious schistosomes, could reflect the first stages of sex chromosome differentiation in digenetic trematodes, although the relation of heterochromosomes in hermaphroditic trematodes to the process of sex organ development or sex determination is not certain.

The present study compares the karyotypes of certain hermaphroditic and dioecious digenetic trematodes and their heterochromatin patterns in an effort to elucidate the possible pathways of karyotype evolution and cytogenetic mechanisms of sex chromosome differentiation in this group of parasites.

Our series of species, although limited, suggests a possible pathway by which the grossly heteromorphic sex chromosomes in gonochoristic schistosomes may have evolved from morphologically similar "pre-sex" chromosomes in hermaphroditic species. The forerunners of heteromorphic sex chromosomes in gonochoristic schistosomes seems to be traceable through intermediate stages in hermaphrodites and dioecious species.

It is further shown that the digenetic trematodes represent an interesting group of animals in terms of the manifestation of what appears to be a gradual evolutionary differentiation of sex chromosomes. Within the family Schistosomatidae, our study of 10 species in 3 genera suggests that sex chromosome differentiation was accompanied by increasing heterochromatinization of one sex chromosome (W).

Materials and Methods

Parasites (Table 1). Two *Helisoma duryi* and two *H. trivolvis* snails naturally infected with unknown strigeoid and spirorchid species, respectively, were collected locally near Tallahassee and Iowa City in 1979. These parasites have not been identified to species with certainty since only cercariae and molluscan stages were observed and studied.

However, it is reasonable to assume that the spirorchid species is *Spirorchis parvus* and this name will be used. The snail host was the same (*Helisoma trivolvis*) as reported in the life cycle study of *S. parvus* (Wall, 1941). Furthermore, similarity of cercarial morphology of the spirorchid studied here and that of *S. parvus* (Holliman et al. 1971) and geographical distribution of the latter (Brooks and Mayers, 1976) support our assumption.

The stock of *S. mansoni* was originally from Puerto Rico and had been maintained in Dr. Irving G. Kagan's laboratory (Center for Disease Control, Atlanta, Georgia, USA) and at Florida State University (Tallahassee, Florida, USA).

Schistosoma rodhaini and all the species in the *S. haematobium* complex (except *S. haematobium*) were supplied by Dr. Christopher A. Wright (Department of Zoology, British Museum, Natural

Table 1. Digenetic trematodes and snail intermediate hosts used

Trematode		Intermediate snail host	Principal definitive hosts	Source of parasite material; year isolated from nature
Species	Family			
Strigeoid[a]	probably Diplostomatidae	*Helisoma duryi*	Probably birds	Florida, U.S.A., 1979
probably *Spirorchis parvus*[a]	Spirorchiidae	*H. trivolvis*	Turtles	Iowa, U.S.A., 1979
African *Schistosoma mansoni* complex				
Schistosoma mansoni	Schistosomatidae	*Biomphalaria glabrata*	Man	Puerto Rico, more than 20 years ago
S. rodhaini		*B. sudanica*	Rodents	Kenya, 1968
African Schistoma haematobium complex				
S. haematobium	Schistosomatidae	*Bulinus truncatus*	Man	Egypt, 1950
S. bovis		*B. truncatus*	Sheep, goats, cattle	Sudan, 1980
S. mattheei		*B. globosus*	Cattle, sheep, goats	Transvaal, 1962
S. intercalatum		*B. cristallinus*	Man	Cameroon, 1969
S. margrebowiei		*B. natalensis*	Antelopes	Botswana, 1975
Asian schistosomes				
S. japonicum	Schistosomatidae	*Oncomelania hupensis*	Man and other mammals	Taiwan, 1962
North American schistomes				
Schistosomatium douthitti	Schistosomatidae	*Lymnaea catascopicum* (=*Stagnicola emarginata angulata*)	Rodents	Michigan, U.S.A. 1973 or earlier
Heterobilharzia americana		*L. cubensis*	Raccoon and other wild mammals (bobcat, rabbit, nutria)	Texas, U.S.A., 1980 or earlier

[a] Hermaphroditic. All others (schistosomes) dioecious

History, London), either directly or indirectly through Dr. Robert E. Kuntz (Parasitology Department, Southwest Foundation for Research and Education, San Antonio, Texas, USA).

Schistosoma haematobium and *S. japonicum* were supplied by Dr. Yung-San Liang (Center for Tropical Diseases, University of Lowell, Lowell, Massachusetts, USA). The *Schistosomatium douthitti* stock was obtained from Drs. Henry van der Schalie and Elmer G. Berry of the University

of Michigan and had been maintained in the Florida State University laboratory since 1973. Snails infected with *Heterobilharzia americana* were supplied by Dr. Norman C. Ronald in 1980 (Department of Veterinary, Microbiology and Parasitology, Texas A & M University, College Station, Texas, USA).

Karyotypes. Mitotic chromosome preparations of the trematodes were made from cells in sporocysts obtained from snails. More than 20 metaphase plates for each species were analyzed in addition to those investigated in detail in microphotographs. Sex of the schistosome material was conjectured on the basis of the heterochromatin constitution of sex chromosomes and was verified by mouse exposure to cercariae.

Air dried slides prepared from cell suspensions were used for all species except the spirorchid; for this, squashes were used. Methods used in slide preparation and C-banding were as described earlier (Grossman et al., 1980a; Short and Grossman, in press).

Results

Strigeoid (Fig. 1a). The chromosomes consisted of 10 pairs, including 6 pairs of subtelocentric chromosomes ranging from large to middle-sized and 4 pairs of small submetacentric chromosomes of approximately the same size. Heterochromatin, after C-banding treatment, was observed in the centromeric region of all chromosomes. One pair of chromosomes exhibited an additional terminal block of heterochromatin, which possibly is associated with the nucleolar organizing region.

Spirorchis sp. (Fig. 1b–d). Chromosomes consisted of 9 pairs, which include one pair of large metacentric chromosomes and 8 pairs of subtelocentric ones of varying lengths. Two kinds of karyotypes were observed in approximately equal proportions in sporocysts from both snails: in one snail, 6, each with one heteromorphic pair composed of a small and a medium sized chromosome (Fig. 1c), and 10 with corresponding pairs of small homomorphic chromosomes (Fig. 1d) in the other snail, 8 karyotypes had heteromorphic pairs and 7 had all homomorphic pairs. All subtelocentric chromosomes possessed darkly stained centromeric heterochromatin. The metacentric chromosomes showed a lightly stained gap in the centromere region which separated the heterochromatic darkly stained areas.

African Schistosoma. Karyotypes of all African schistosomes investigated here are very similar and the species cannot be distinguished easily from each other on the basis of chromosome morphology alone. Detailed analysis of two African schistosomes, *S. mansoni* and *S. rodhaini*, showed minor differences between these species involving centromere positions, including those of the Ws, apparently because of small inversions, duplications or deletions (Short and Grossman, in press).

Karyotypes of the African schistosomes presented here consist of 8 pairs of chromosomes, which are divided into three size groups: Two pairs of large chromosomes (including the sex chromosomes) and three pairs of middle-sized chromosomes are subtelocentric, and three pairs of small chromosomes are submetacentric or metacentric.

African schistosomes studied to date also are generally similar to each other in C-band patterns, with the exception of the W chromosomes. Except for the W, the C-banded pattern of *S. mansoni* (Fig. 1e; Short and Grossman, in press) is typical for the group. The heterochromatin composition of the

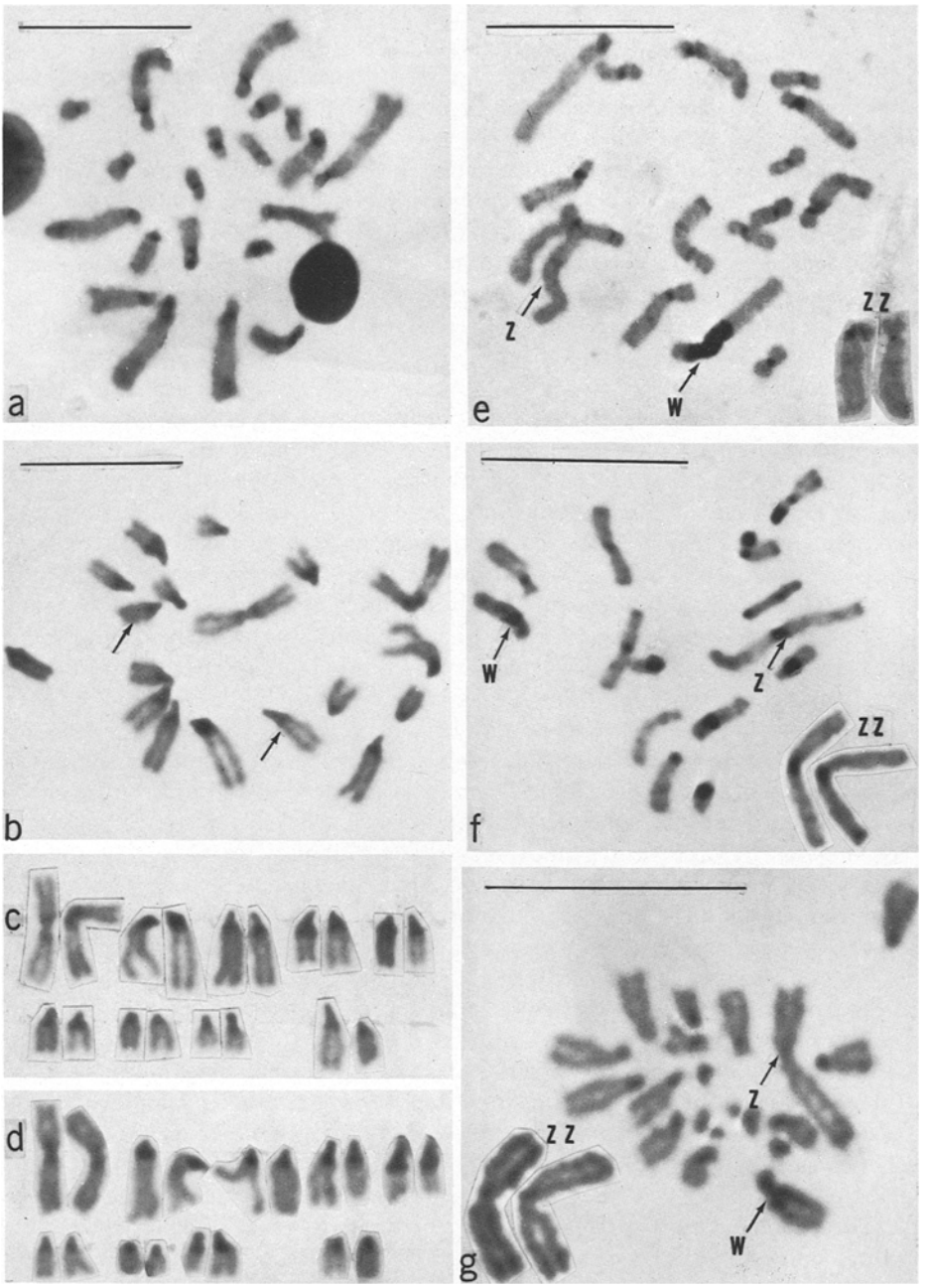

Fig. 1 a–g. C-banded karyotypes of digenetic trematodes. **a** Strigeoid (n = 10), **b** *Spirorchis parvus* (n = 9). Arrows indicate a heteromorphic pair of chromosomes. **c** Chromosomes of *S. parvus* from above. **d** Chromosomes of *S. parvus* from another metaphase plate with a pair of homomorphic chromosomes. **e** *Schistosoma mansoni* female karyotype. Arrows indicate the Z and W chromosomes. On the right – a pair of Z chromosomes from a male metaphase plate. **f** *Schistosomatium douthitti* female karyotype. Arrows indicate the Z and W chromosomes. On the right – a pair of the Z chromosomes from a male metaphase plate. **g** *Heterobilharzia americana* female karyotype. Arrows indicate the Z and W chromosomes. On the left – a pair of the Z chromosomes from a male metaphase plate. Length of scales 10 μm

W chromosomes of the various species studied here differs in certain easily noticeable ways. The centromeric area and proximal part of the long arms of the W chromosomes of *Schistosoma mansoni, S. rodhaini, S. intercalatum,* and *S. margrebowiei* are heterochromatic. In addition to the paracentromeric block of heterochromatin, the W chromosome in *S. haematobium* possesses 3–5 differentially staining heterochromatic bands along the long arm. *S. bovis* and *S. mattheei* are different from other species in the presence of a large (*S. bovis*) or small (*S. mattheei*) euchromatic gap between two blocks of heterochromatin (Fig. 3), with a proportionally shorter, terminally located, euchromatic region in *S. bovis*.

Asian Schistosoma. Karyotype descriptions of *S. japonicum* and *S. mekongi* were reported recently (Grossman et al. 1980a). Generally, Asian schistosomes are similar to African schistosomes in their chromosome numbers and morphologies. However, in Asian schistosomes the two largest pairs of chromosomes, one of which is the sex chromosomes, are metacentric or submetacentric, whereas in African species they are subtelocentric. Another, and more significant, difference between Asian and African schistosomes is in the comparative morphology of the sex chromosomes (Grossman et al., 1980a). The W chromosome in Asian schistosomes is noticeably smaller than the Z and has a more medial centromere, and the heterochromatic block of the W chromosome of *S. japonicum* is significantly smaller than in any of the African schistosomes (Fig. 3).

Schistosomatium douthitti (Fig. 1f). The karyotype of *S. douthitti* consists of 7 pairs, one pair of sex chromosomes and 6 pairs of autosomes which are composed of three pairs of middle-sized metacentric or submetacentric chromosomes, two pairs of subtelocentric and telocentric chromosomes, and one pair of small telocentric chromosomes (Puente et al., 1980). Darkly stained centromeric heterochromatin was observed in all chromosomes. The Z and W chromosomes differ in size and morphology: the Z chromosome is large and metacentric; the W is smaller and subtelocentric.

Heterobilharzia americana (Fig. 1g). The karyotype consists of 10 pairs of chromosomes, one pair of heteromorphic sex chromosomes, morphologically similar to that of *S. douthitti*, six pairs of middle-sized subtelocentric autosomes, a pair each of metacentric and telocentric small chromosomes and one pair of microchromosomes (Short and Grossman, in preparation). The heterochromatin constitution of *H. americana* autosomes is largely centromeric and thus generally similar to that of *S. douthitti*. The sex chromosomes differ not only in their morphology but also in their heterochromatin content, which makes them distinct from those of *S. douthitti*. There are two blocks of heterochromatin in the long arm of the W chromosome and the short arm apparently is heterochromatic also (Fig. 3).

Discussion

Karyotype Evolution in Digenetic Trematodes

Chromosome Numbers and Morphology

Comparison of evolutionary relationships of species of various animals and their karyotypes within a systematic group usually shows that less advanced

species possess a "primitive karyotype" with a higher number of telocentric chromosomes.

Further evolution of chromosome number and form often was accompanied by fusion of telocentric chromosomes to form karyotypes with a reduced number of larger, biarmed chromosomes. Such a general tendency of karyotype evolution was confirmed in the recent review of the evolution of chromosome numbers and chromosome morphology in vertebrates (Morescalchi, 1977).

Cytotaxonomy of trematodes, based mainly on chromosome numbers, has been discussed in reviews of Britt (1947), Saksena (1969), and Jha (1975). They indicated that aneuploidy and translocations, including Robertsonian, appear to be the most common mechanisms of chromosome number variations within digenetic trematodes. Polyploidy as a mechanism which changed chromosome numbers in this group was ruled out. The recent review by Benazzi and Benazzi Lentati (1976), dealing with chromosome morphology as well as numbers, added to our knowledge of karyosystematics of digenetic trematodes and basically confirmed the main conclusions of prior reviews.

According to Short and Menzel (1960) the fundamental similarity of the karyotypes of 9 species of 6 genera of family Schistosomatidae supports the hypothesis that the schistosomes represent a monophyletic group, and they further concluded that principal morphological changes in karyotypes, accompanying separation of genera in this group, seem to have been translocations, inversions, deletions and changes in number of chromosomes either by way of aneuploidy or of translocation. These authors mentioned that examination of karyotypes of spirorchids, hermaphroditic blood flukes of turtles, might furnish information on the type of hermaphroditic fluke that was ancestral to the gonochoristic schistosomes.

Comparison here of two hermaphroditic trematodes – one (probably a bird parasite) from the superfamily Strigeoidea, the other a turtle blood fluke from the family Spirorchiidae – with species of three known genera of Schistosomatidae (*Schistosoma, Schistosomatium* and *Heterobilharzia*) (Fig. 1) suggests that a strigeoid karyotype similar to the one presented here is the most primitive, because it contains a large number of chromosomes (N=10) and they are all telocentric. The spirorchid karyotype would be next most advanced because its haploid number of chromosomes is 9: 8 pairs of telocentric and one pair of large metacentric chromosomes. The origin of metacentric chromosomes in *Spirorchis magnitestis* from two small telocentric chromosomes was proposed by Jones and Mayer (1953), and this also could be true for *S. parvus*.

Eight pairs of chromosomes are characteristic for all eight species of *Schistosoma* studied here. This observation supports the hypothesis (Short and Menzel, 1960) that the ancestral schistosome karyotype consisted of 8 chromosome pairs. Comparison of chromosome morphology of the species of *Schistosoma* studied here leads to a suggestion that a karyotype of a hypothetical ancestral dioecious schistosome consisted of: a pair of large sex chromosomes, a pair of large metacentric (or submetacentric), three pairs of medium-sized and three pairs of small autosomes.

The origin of a new element in the karyotype of ancestral schistosomes – a pair of sex chromosomes – apparently resulted from a Robertsonian translocation, in which a pair of large telocentric chromosomes was involved, together

with another pair of small or medium-sized chromosomes. (The origin of sex chromosomes in schistosomes is considered below). A pair of large autosomes in the *Schistosoma* karyotypes is, evidently, homeologous with a pair of metacentric chromosomes in spirorchids. The origin of a third pair of medium-sized autosomes in schistosomes is uncertain and most likely resulted from the loss of genetic material by another pair of large telocentric "spirorchid" chromosomes.

Apparently, further speciation in the genus *Schistosoma* was accompanied by intrachromosomal aberrations especially in Ws, as demonstrated in a comparison of karyotypes of *S. mansoni* and *S. rodhaini* (Short and Grossman, in press). Detailed characterization and comparison of chromosome morphology of other schistosomes listed here will be a matter for forthcoming papers.

The North American schistosomes studied here (*S. douthitti* and *H. americana*) represent a group that is karyologically distinct from *Schistosoma* species in possessing grossly differentiated Z and W chromosomes (Figs. 1 and 3), the origin of which is considered below.

Although similar to each other in their sex chromosome morphology, *S. douthitti* and *H. americana* differ from one another in autosome complexes. The *S. douthitti* (n=7) autosomal complex is composed of two pairs of metacentric chromosomes and four pairs of various-sized telocentric or subtelocentric chromosomes. All nine pairs of autosomes of *H. americana* (N=10) are composed of different-sized telocentric chromosomes. Apparently, the differences in chromosome numbers and morphologies between these two American schistosomes have resulted from Robertsonian translocations (*S. douthitti*, N=7) and chromosome dissociations or duplication (*H. americana*, N=10), which occurred during speciation from hypothetical ancestral schistosomes.

Figure 2 represents possible evolutionary pathways of chromosome evolution in the trematode suborder Strigeata. The following reservations should be made in interpreting this scheme: 1) Karyotypes of only one strigeoid and one spirorchid species are used here and they may not be representative of their respective groups. The only other analysis of spirorchid chromosomes was done on *S. magnitestis* (Jones and Mayer, 1953) where chromosome number and morphology are very simlar to that described here. However, 16 chromosomes were reported for two other Strigeoidea species (Saksena, 1969) in contrast to the 10 in the strigeoid reported here. 2) Apparently, the karyotype of the strigeoid studied here (Fig. 1 a) is not a "primitive" one in the full sense of this word since it is composed of telocentric as well as biarmed subtelocentric chromosomes. Certainly, the fusion of biarmed chromosomes may be distinctly hazardous, since it would result in a permanent loss of the genetic material on the short arms. Therefore, assumptions should be made: either metacentric "spirorchid" chromosomes arose as a result of Robertsonian translocation before short arms appeared in corresponding chromosomes of strigeoids, or the short arms of chromosomes which were involved in fusion did not possess important loci. The same assumptions should be proposed for evolution of metacentric chromosomes in *S. douthitti*. 3) At the present time we have no exact information about what pairs of chromosomes were involved in centromeric fusions, chromosome dissociation, and possible chromosome translocations, since identification

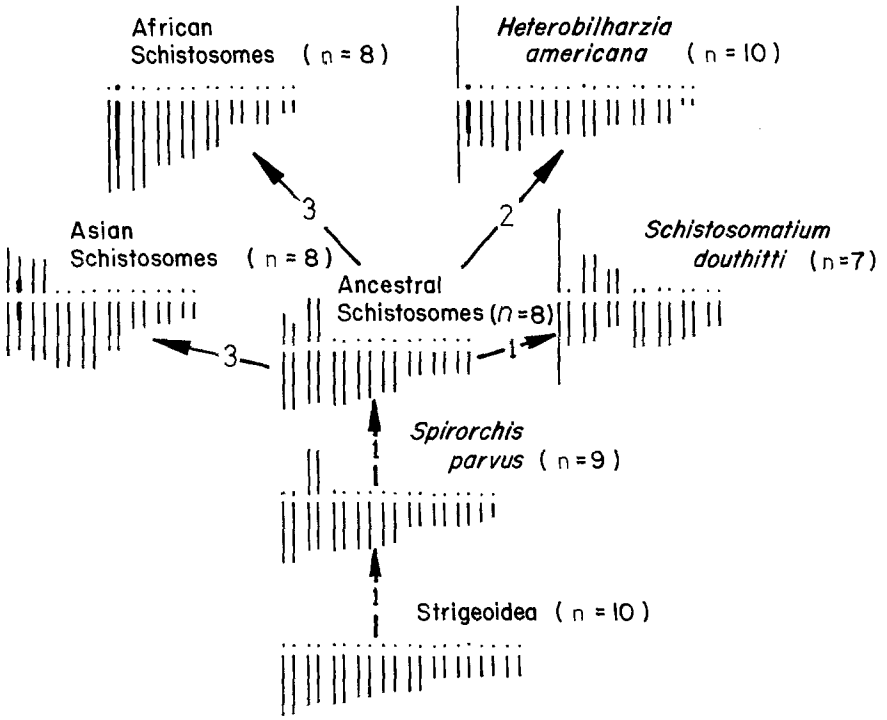

Fig. 2. A hypothetical scheme of the karyotype evolution in the trematode suborder Strigeata. The idiograms represent number of chromosomes, approximate centromere positions on chromosomes and heterochromatic segments on Ws. Numbers on arrows designate a possible main event occurred in autosomal complexes. 1 Robertsonian translocations. 2 Chromosomal dissociations. 3 Inversions. Apparently, various intrachromosomal micromutations (inversion, duplication, deletion) have also occurred at all stages of evolution of autosomal complex in this group of Trematoda

of chromosomes with G-bands was not successful. Hence, the proposed scheme is based only on differences in length and morphology of chromosomes and their heterochromatin composition. Further study of chromosome constitutions with the aid of G- and Q-banding techniques and calculation of centromeric indices and relative lengths of chromosomes should furnish this scheme with more details.

On the whole, the comparison of chromosome numbers and morphology and C-banding patterns of Strigeoid, *Spirorchis*, and schistosome species suggests that the spirorchid type of karyotype represented here (eight pairs of telocentric and one pair of biarmed chromosomes) is ancestral to the primitive schistosome karyotype (six pairs of telocentric and two pairs of biarmed chromosomes). The "Spirorchid" chromosomal complex presumably arose from a karyotype (ten pairs of telocentric chromosomes) which is characteristic for primitive trematodes (Benazzi and Benazzi Lentati, 1976; Grossman and Cain, 1981) and for the strigeoid studied here.

The scheme of karyotype evolution in the suborder Strigeata proposed here is in good agreement with the phylogeny of trematodes based on comparative embryology and morphology, and on complexity of life cycles (Cable, 1974).

Members of the Schistosomatidae, Spirorchiidae, and Strigeidae are considered commonly to be of the same evolutionary line (e.g., see Cable, 1974) with the schistosomes the most evolutionarily advanced and the strigeids the most primitive.

We do not propose independent evolution of two karyologically distinct groups of schistosomes: (1) with sex chromosomes similar in size in one group (African and Asian schistosomes) and (2) with sex chromosomes grossly differentiated in another group (American schistosomes of mammals) from two spirorchid ancestor-hermaphrodites. Such a suggestion seems unlikely because it would require two independent origins of dioecism.

Sex Chromosome Differentiation

Analysis of sex chromosomes of the schistosome species studied here, together with those studied earlier, viz. *S. mansoni* (Grossman et al., 1980b; Short and Grossman, in press), *S. rodhaini* (Grossman et al., 1981; Short and Grossman, in press), *S. japonicum*, and *S. mekongi* (Grossman et al., 1980a), are interesting from the point of view of evolutionary trends of morphological differentiation of sex chromosomes.

Two end points in the process of morphological differentiation of sex chromosomes are well documented. Groups of primitive lower vertebrates represent one extreme. In these the identity of male and female karyotypes indicates that sex chromosomes are morphologically similar, even though one of the sexes supposedly is heterogametic, with genetically differentiated sex chromosomes. At the other extreme, cytologically recognizable sex chromosomes have been described for many invertebrates and vertebrates, with the XX female/XY male system in mammals and Diptera, and the ZZ male/ZW female in reptiles, birds, and Lepidoptera (Ohno, 1974; White, 1977).

Apparently, grossly differentiated sex chromosomes (X and Y; Z and W) are different from each other genetically and morphologically. Genetic differentiation of potential sex chromosomes obviously may occur without any noticeable change in their gross morphology, as in differentiation of sex chromosomes in the lower vertebrates.

The process of accumulation of sex determinants on potential sex chromosomes precedes the process of morphological differentiation and leads to the development of sex determining and differentiating mechanisms, which include dosage compensation (Lucchesi, 1978).

For divergence of the sex-specialized chromosomes, the differential cluster of sex related genes has to be protected from crossing-over to reduce free recombination between heterologous segments of the potential sex chromosomes. Various chromosomal mutations (inversions, deletions, and translocations) or genes specifically supressing crossing-over have been considered as isolating mechanisms. This model of the evolution of the sex chromosome differentiation has been derived from a study of chromosome morphology in a number of snake species (Beçak et al., 1964; Ohno, 1967).

However, recent investigations (Ray Chaudhuri et al., 1970, 1971) demon-

strated that genetic differentiation of morphologically identical sex chromosomes in snakes is followed by the asynchronization of the DNA replication of Z and W chromosomes (facultative heterochromatinization of the W chromosome) rather than structural mutations as was supposed by Ohno and his colleagues.

Apparently, constitutive heterochromatinization (differentiation of Z and W chromosomes in their composition of satellite DNA) may precede allocycly of sex chromosome replication. The origin of highly repetitive (satellite) DNA in the W chromosome of snakes is perhaps the first step of a morphological differentiation of sex chromosomes in this group of animals (Singh et al., 1976).

The differentiation of sex chromosomes by heterochromatinization of one homolog, was recently demonstrated for other vertebrates as well, e.g., lizards (Bull, 1978) and newts (Schmid et al. 1979).

At the present time, it might be assumed that both of the processes described above, viz., constitutive heterochromatinization of the W chromosome and the asynchronization of DNA replication which is followed by facultative heterochromatinization of the W chromosome, simultaneously participated in a molecular differentiation of the sex chromosomes in snakes. The mechanism of such molecular differentiation of sex chromosomes is unknown, and it could be speculated that constitutive heterochromatinization and asynchronization of DNA replication of one homolog are manifestations of a common underlying process, such as DNA-methylation, repetition of species nucleotide sequences, etc. In this connection, it is of interest to mention here that Ray-Chaudhuri and coworkers pointed out that the facultatively heterochromatic W chromosome apparently contains constitutive heterochromatin because an association of the W-chromatin with the nucleolus was observed (Ray-Chaudhuri et al., 1971).

The described process of molecular differentiation initiates the genetic differentiation and genetic isolation of sex chromosomes – two divided processes which are separated in time according to Ohno's model (Ohno, 1967). Replacement of genetically active euchromatin by apparently genetically inert constitutive heterochromatin is an effective way of genetic differentiation of sex chromosomes. It is believed also that constitutive heterochromatin participates in the regulation of gene activity (Brown, 1966). Similarly, the process of facultative heterochromatinization leads to differential expression of gene activity of separate chromosomes (Lyon, 1966) or sets of chromosomes (Nur, 1967). At the same time, there is strong evidence that molecular differentiation of sex chromosomes affects the frequency and localization of chiasma formation. Extreme reduction of crossing-over between morphologically identical sex chromosomes was demonstrated for *Triturus helveticus* (Schmid et al., 1979). It was demonstrated also that crossing-over occurs away from the centromeric and telomeric heterochromatin, as in a number of Australian grasshopper species (Miklos and Nankivell, 1976).

Comparison of sex chromosome morphologies and their C-banding patterns in various species of schistosomes (Fig. 3) shows that both molecular (heterochromatinization) and morphological (translocation) differentiation were involved in their divergence. The differentiation of sex chromosomes in African schisto-

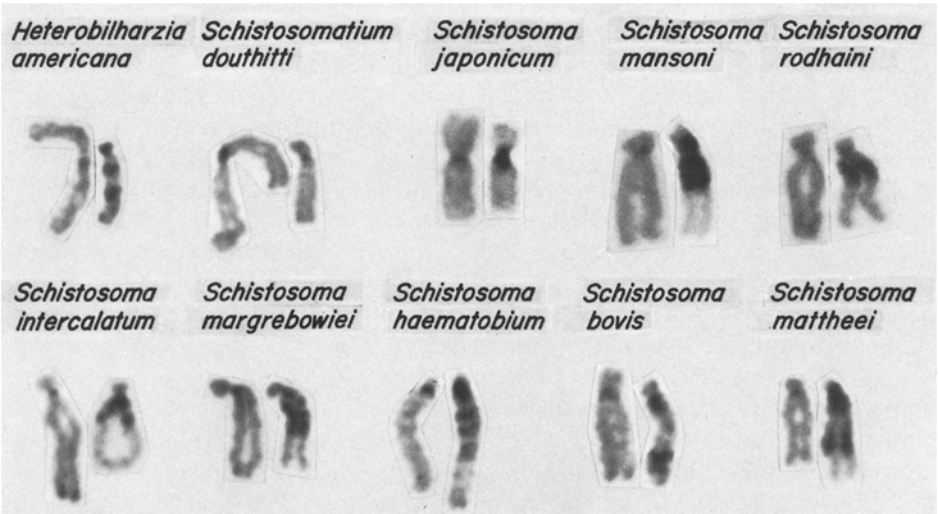

Fig. 3. Sex chromosomes of various schistosomes from female metaphase plates. Note differences in centromere position of the Z and W chromosomes of *S. douthitti* and *H. americana*. Apparent differences in size of the Z and W chromosomes of *S. intercalatum* and *S. margrebowiei* are not typical for these species and are due to differential contraction of sex chromosomes in the chosen metaphase plates

somes, in both *S. mansoni* and *S. haematobium* complexes, has apparently involved differential heterochromatinization of one chromosome, which became the W.

Various hypothetical steps in the evolutionary process can be seen when the Z and W chromosomes of species of the *S. haematobium* complex are compared. The long arm of the W chromosome of *S. intercalatum* possesses the smallest block of constitutive heterochromatin compared with those of other species of this complex. Further differentiation of Z and W chromosomes in *S. haematobium* appears as additional heterochromatic bands on the long arm of the W chromosome. No euchromatic gap has been found in the differentiated segment of the W chromosome in *S. margrebowiei* and the heterochromatic block in this species generally is larger than in *S. intercalatum* and in *S. haematobium*. A gap between two heterochromatic regions on the W chromosome of *S. bovis* is possibly the result of a paracentric inversion which divided a heterochromatic block into two regions, one of which is located subterminally, the other close to the centromere. Two separate heterochromatic regions also have been observed in the W chromosome of *S. mattheei*; however, the euchromatic gap between them was significantly smaller, with more extended heterochromatin in the W chromosome.

It is of interest that the Z chromosomes in all species of the *S. haematobium* complex demonstrated a great degree of similarity in size and centromere position, among themselves and with corresponding W's (numerical comparison of sex chromosomes of these species will be performed elsewhere). The same tendencies of sex chromosome differentiation (gradual differentiation of Z and W chromosomes by means of constitutive heterochromatinization of the W

chromosome with preservation of the Z chromosomes) was demonstrated in our karyological analysis of *S. mansoni* and *S. rodhaini*, members of the *S. mansoni* species-complex of African schistosomes (Short and Grossman, in press).

Apparently, speciation among African schistosomes was accompanied, and possibly even caused, by changing of the euchromatin/heterochromatin composition of the W chromosomes, while their Z chromosomes, as well as autosomal complexes, remained almost identical.

Sex chromosomes in Asian schistosomes – *S. japonicum* and *S. mekongi* – are different from those in African schistosomes in that they are metacentric or submetacentric, and it was demonstrated that the W chromosomes in both species are smaller than the corresponding Zs (Grossman et al., 1980a). Such differences in size of sex chromosomes are apparently due to a deficiency of part of the W chromosome. The C-banding technique showed that heterochromatinization of the W chromosome in *S. japonicum* participated in the morphological differentiation of W and Z chromosomes as well, since a region of constitutive heterochromatin is larger in the W than in the Z (Fig. 3).

Sex chromosomes in *Schistosomatium douthitti* and *Heterobilharzia americana* are different in their size and centromere position, in contrast to the similarity in morphology and size of Z and W chromosomes in *Schistosoma* species. Morphological differentiation of sex chromosomes in these mammalian schistosomes seems to be a result of a translocation of a chromosomal segment of the W onto the Z chromosome. A similar suggestion was made by Galton (1966) for Y and X mammalian chromosomes. In addition, differential constitutive heterochromatinization of the W chromosome occurred in *H. americana* (Fig. 3).

The Z chromosomes of these North American schistosomes are mediocentric and are the largest elements of the karyotypes with median centromeres; the W chromosome is significantly smaller than the Z with a subterminally located centromere. Preliminary (unpublished) analysis of six randomly selected metaphase plates of female *S. douthitti* showed that the relative lengths of Z and W chromosomes were 33.16 ± 1.47 and 12.33 ± 1.74, respectively. Comparison of sex chromosome fractions of total length of diploid complement (Z + W-length of diploid complement) of *S. douthitti* (45.49) with those of *S. mansoni* (41.21) and *S. rodhaini* (37.76) (Short and Grossman, in press) indicate that the amount of DNA situated on the total sex chromosomes did not change noticeably during speciation.

Figure 4 represents possible pathways of sex chromosome differentiation in schistosomes which are based on sex chromosome sizes and morphologies and on their constitutive heterochromatin contents. As follows from the scheme, inactivation of some chromosomal segments of one of the originally identical chromosomes by means of heterochromatinization, and/or translocation of these segments onto another homolog leads to ZZ male/ZW female sex chromosome heteromorphism in schistosomes.

Although, at the present time, we lack direct information about genetic mechanisms of sex determination in gonochoristic trematodes, there is good reason to believe that the Z chromosome in dioecious trematodes carries genes which determine maleness, since schistosome males are homogametic and fe-

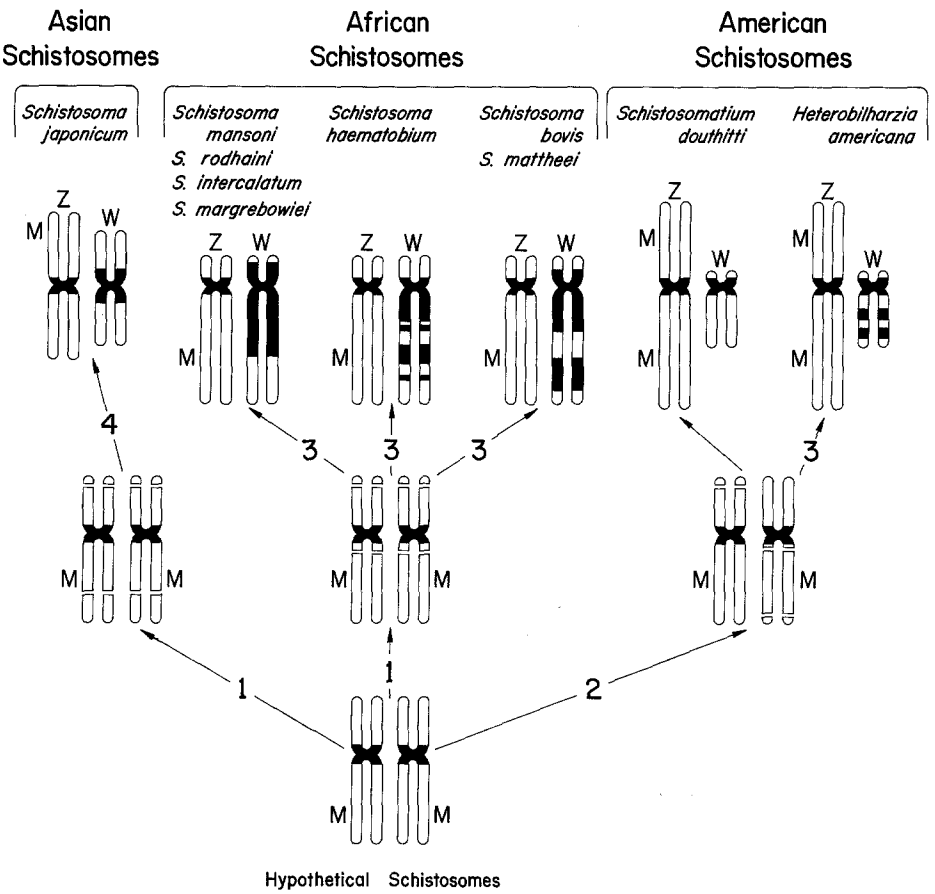

Fig. 4. Hypothetical scheme of evolution of sex chromosome differentiation in schistosomes. M male sex determinants located on the Z chromosome. Numbers on arrows designate a possible chromosomal mutation occurred during the Z and W differentiation. 1 pericentric inversion, 2 translocation, 3 differential heterochromatinization, 4 deletion

males are heterogametic. Genes determining femaleness could be located on autosomes as well as on the W chromosome.

Use of the G-banding technique in further investigations of the schistosomes studied here, as well as other species, should elucidate which specific segments of sex chromosomes and, possibly, which autosomes were involved in pathways of sex chromosome differentiation.

Heteromorphic Chromosomes in Hermaphroditic Trematoda

According to White (1977, Chapter 16), the finding of "vestiges of sex chromosomes in a few (*secondary*) hermaphroditic species which are closely allied to (*primary*) bisexual forms, and which may hence be presumed to have become hermaphroditic fairly recently" is quite possible (italics ours).

In contrast, "one must not expect animals such as flukes, snails and earthworms (*primary hermaphrodites*) to possess any special sex chromosomes" (ibid., italics ours). Thus, the finding of a heteromorphic pair of chromosomes in *primary* hermaphrodites should not necessarily indicate the presence of morphologically differentiated sex chromosomes, but rather intraspecific chromosomal polymorphism. Recently, a pair of heteromorphic small chromosomes was found in all metaphase plates in the hermaphroditic trematode *Megalodiscus temperatus* (Grossman and Cain, 1981). One chromosome in this pair was subtelocentric with a large region of constitutive heterochromatin; the other was telocentric and displayed only centromeric heterochromatin. During gametogenesis these chromosomes formed a bivalent despite their morphological and substructural differences. Two karyotypes, one with a homomorphic pair and another with a heteromorphic pair of satellite chromosomes are characteristic for the hermaphroditic snail *Bulinus natalensis* (Goldman et al., 1980). In these examples, there is no reason to believe that chromosomal heteromophism is related to sex.

However, in some instances, the conceivable continuous accumulation of genes which are related to sex organ development and differentiation on a pair of chromosomes of a *primary* hermaphrodite may under certain conditions differentiate those chromosomes from others in their sex specialization. At a certain level of such differentiation, these chromosomes might be considered to be potential sex chromosomes, which will transform into actual sex chromosomes in taxonomically related *secondary* gonochoristic organisms.

This process of "pre-sex" chromosome differentiation, apparently, might occur in some *primary* hermaphroditic trematodes which are generally assumed to be ancestral forms for *secondarily* dioecious trematodes with distinctly differentiated sex chromosomes. If the beginning of sex chromosome specialization and molecular differentiation actually did occur in such hermaphroditic trematodes, we might expect that possibly some presently living, hermaphroditic trematodes taxonomically related to dioecious species would possess a pair of identifiable sex-specialized chromosomes.

In the present study, a heteromorphic pair of chromosomes was found in a hermaphroditic trematode – *Spirorchis parvus*, which supposedly is related to a hypothetical ancestor of schistosomes. In 14 of 31 investigated karyotypes from both naturally infected snails, one pair of chromosomes was composed of small and medium-sized chromosomes (Fig. 1c). Four pairs of small chromosomes were observed in the rest of the karyotypes (Fig. 1d); no metaphase plate was observed with three pairs of medium-sized chromosomes. Apparently, more than one karyotypically different miracidium penetrated each of the *Helisoma trivolvis* snails studied here, since two karyotypes were observed in *S. parvus* sporocysts from both snails.

Comparison of spirorchid and schistosome karyotypes (Figs. 1 and 2) indicates that the sex chromosomes of the putative ancestor of schistosomes could be a result of centromeric fusion of a pair of large telocentric chromosomes with another pair of small chromosomes of the spirorchid. Furthermore, we can suggest that a long arm of the sex chromosome of hypothetical schistosomes is homeologous to a pair of large telocentric chromosomes of the spirorchid.

Following our scheme (Fig. 4) on location of male sex determinants, these large telocentric chromosomes would have accumulated maleness genes in spirorchids.

It would be an attractive hypothesis that heteromorphic chromosomes in *Spirorchis parvus* are homeologous to a pair of "pre-sex" chromosomes of a hypothetical ancestor of schistosomes, and, therefore, are related to development of the reproductive system in this species. At the same time, the medium-sized chromosomes may possess a regulatory gene(s) which suppresses the expression of male (M) genes on another pair of chromosomes during the development of the female reproductive system. A similar suppressor has been described for femaleness genes in *Melandrium* (Westergaard, 1948). In *S. parvus* the small chromosome of a heteromorphic pair apparently has lost such a suppressor gene(s) and, therefore, individuals with a pair of homomorphic small chromosomes should be expected to demonstrate a tendency toward maleness. By the same token, individuals with a pair of heteromorphic chromosomes will be true hermaphrodites, since the larger heterochromosome carries a set of regulatory gene(s).

It is of interest to mention here that great variability in size of ovaries has been observed in *S. parvus* (Holliman et al., 1971). Some of the investigated specimens possessed very small ovaries with as few as 7 cells on the long axis and 14 cells on the short axis, whereas in other specimens averages of the corresponding numbers have been 44 and 27 cells respectively. Significant reduction of ovary size in some individuals of *S. parvus* may be related to the presence of a homomorphic pair of "pre-sex" chromosomes in their karyotypes.

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