Chromosoma (Berl.) 75, 75–87 (1979)

CHROMOSOMA

Chromatin Diminution and a Chromosomal Mechanism of Sexual Differentiation in *Strongyloides papillosus*

Donna G. Albertson[1], Obioma C. Nwaorgu[2], and John E. Sulston[1]

[1] MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England;
[2] Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 OES, England

Abstract. Eggs obtained from feces of rabbits infected with *Strongyloides papillosus* were squashed and the karyotypes were determined. They contained cells with either two long and two medium sized chromosomes (2L2M), or one long, three medium and one short chromosome (L3MS). Two types of parasitic female gonad could be distinguished on the basis of oocyte chromosome morphology at prometaphase of the maturation division. All the oocytes in a gonad contained either two upaired long chromosomes and two unpaired medium sized chromosomes, or two unpaired medium sized chromosomes and two unpaired chromosomes segmented into beads in one region. At the maturation division in mitotic parthenogenesis the beads appear to be lost from one of the chromosomes. This generates a medium sized and a shorter chromosome, which together with the undiminished chromosomes make up the L3MS karyotype. Animals with beaded oocyte chromosomes lay eggs that develop into males. It is suggested that males are heteromorphic for the long homologue due to chromatin diminution, that occurs in the maturation division of mitotic parthenogenesis.

Introduction

The nematode *Strongyloides papillosus* displays both a parasitic and a free living life cycle (Fig. 6, p. 85). The parasitic female lives in the mucosa of the small intestine of sheep, cattle and rabbits. The eggs hatch in the feces of the host and develop into free living males, free living females and infective larvae. The development of the infective larvae is arrested until they re-enter a host animal, where they mature into parasitic females. Infection by this route is called the direct cycle. The free living animals must copulate to produce progeny which then may infect a host by the indirect cycle (Nigon, 1965). The choice of life cycle appears to be environmentally controlled (Moncol and Triantaphyllou, 1978).

Recently authors have reported that parasitic females of *S. papillosus* repro-

0009-5915/79/0075/0075/$02.60

duce by mitotic parthenogenesis (Zaffagnini, 1973; Triantaphyllou and Moncol, 1977), implying that eggs genotypically identical to the female parasite should be produced. In accord with this both parasitic and free living females have been shown to have identical karyotype comprising two pairs of chromosomes with one pair distinctly longer than the other (Zaffagnini, 1973; Triantaphyllou and Moncol, 1977). Triantaphyllou and Moncol (1977) have also reported that sex determination in *S. papillosus* does not depend on karyotypic differences between males and females, since no evidence of a distinct somatic male karyotype was found. However, these authors found that the karyotype was altered during spermatogenesis when a piece of the long chromosome detached.

In contrast to these observations, we report that eggs from feces of rabbits infected with *S. papillosus* have, in addition to the $2n=4$ karyotype, described above, a second karyotype present at nearly the same frequency. It appears that the alternative karyotype of $2n=5$ is derived from the $2n=4$ karyotype of the parthenogenic parasitic females by chromatin diminution. Eggs of this genetic constitution develop into free living males.

Materials and Methods

Nematode Strain. S. papillosus was transferred from a naturally infected sheep in the University of Cambridge Department of Clinical Veterinary Medicine flock to a rabbit. Subsequently the infection was transferred by indirect cycle infection to a second rabbit, which was used as the source of nematodes for this work.

Infection of Rabbits. For the *indirect cycle* infection fecal pellets were collected over a period of 24 h from the donor animal and mixed with sterile sawdust according to the method of Roberts and O'Sullivan (1950). The cultures were incubated at 25° C for 6 to 9 days in Kilner jars; nematodes were allowed to settle for 3 h in a modified Baermann apparatus (Soulsey, 1955). Rabbits were infected with 50,000 larvae by subcutaneous injection into the right flank.

In order to maintain a direct cycle infection, sawdust cultures were incubated for only 2 days at 25° C. These larvae were injected subcutaneously into a rabbit and the process was repeated every three weeks.

Enumerating Types of Larvae Hatching from Rabbit Feces. Fecal cultures in sawdust were incubated for 2 days at 25° C. Larvae were collected overnight by the modified Baermann procedure then sexed and counted.

Isolation of Eggs from Rabbit Feces. Freshly deposited rabbit feces or the contents of the large intestine of sacrificed rabbits were mixed with water with a mortar and pestle. The slurry was transferred to a 150 μm mesh sieve placed on top of a Buchner funnel with Whatman no. 1 filter paper and washed with water. Suction was applied briefly to the funnel before the supernatant was decanted and the eggs and fine debris were washed off the filter paper in a small volume (10 ml). An equal volume of 60% w/w sucrose was added, the tube was shaken vigorously and then centrifuged in a swinging bucket rotor at 2,500 rpm. for 2–3 min. The eggs at the meniscus were collected, diluted at least 1:5 with water and centrifuged to pellet the eggs. The eggs were washed several more times to reduce bacterial contamination.

Isolation of Parasites from the Small Intestine of Rabbits. Ten to twenty days post infection a rabbit was sacrificed by I.V. injection of 300 mg of Nembutal. The small intestine was removed and placed in a small volume of 0.8% saline, or PBS (0.15 M NaCl, 0.03 M KCl, 0.01 M phosphate, pH 7). The intestine was opened, and the mucosa scraped vigorously to loosen the worms. The worms were picked onto agar plates or into watchglasses containing water or tissue culture medium and then processed for cytology.

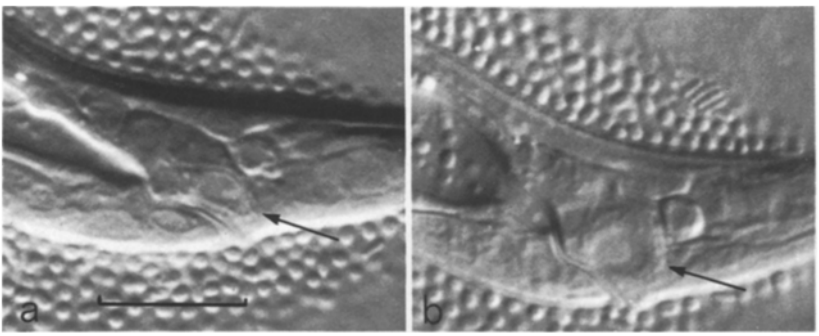

Fig. 1a and b. Light microscopy of newly hatched *S. papillosus* larvae. The region around the anus is shown. The arrows point to the B cell in the female (**a**) and the male (**b**) larva. The B cell in the male is swollen and has a larger nucleus and nucleolus than in the female

In order to recover larvae from individual animals, single worms wer picked from the intestines within 0.5 h of the death of the rabbit. It was necessary to take animals rapidly, because failures in reproduction lead to polyploid and otherwise aberrant eggs. Our observations with the light microscope suggest that only those eggs that had completed the maturation division at the time the host was sacrificed would continue to develop. The worms were freed of any eggs and placed in watchglasses with tissue culture medium or on 45 cm Petri dishes with NG agar (Brenner, 1974). Animals were left to lay eggs for 1–2 h. If no eggs were laid, the adult was cut in half approximately at the vulva and the eggs removed. Animals or pieces of animals were then processed for cytology. The eggs were placed within a ring of bacteria from an extract of rabbit fecal material seeded on NG plates. Plates were kept at 30° C for 5–7 h after which time the number of hatched animals was recorded. As many larvae as possible were recovered and mounted for sex determination by light microscopy. We have not developed a reliable culture method for maintaining individual larvae; incubation on NG plates invariably results in losses due in part to the larvae climbing the walls and dehydrating and in part to deaths. For this reason, sex determination was carried out immediately after hatching.

Cytology. Specimens were pipetted or transferred on an eyelash into a 10 µl drop of 45% acetic acid on a glass slide that had previously been dipped in 1 mg/ml bovine serum albumin and air dried. A 22 × 32 mm no. $1^1/_2$ coverslip was laid gently over the drop. If whole mounts were desired, the slide was placed immediately on dry ice. To prepare squashes, a tap on the coverslip was used to release the eggs from the animals. If isolated eggs were used, this step was omitted. Egg cells were squashed by pressing with the thumb on the inverted slide placed on a paper tissue. The longer the specimen had been in acetic acid, the more difficult it was to apply sufficient pressure for a good squash. Therefore it was important to squash the tissue as soon as possible after placing it in the acetic acid. After squashing, slides were transferred to dry ice for at least 10 min. The coverslip was pried off whole mount or squash slides with a razor blade. Slides were placed in methanol:acetic acid (3:1) for at least 20 min then stained with Hoechst 33258, as described previously (Albertson et al., 1978). Unless otherwise specified the bar in micrographs is 10 µm.

Light Microscopy. Larvae and free living worms were mounted and observed as described previously (Sulston and Horvitz, 1977). Newly hatched animals were sexed by the appearance of the precursor cell to the cloaca and other male specific structures. In newly hatched males of another nematode, *Caenorhabditis elegans* (Sulston and Horvitz, 1977) this cell, B, is swollen and its nucleus contains a prominent nucleolus. The same morphological differences in the B cell can be seen in *S. papillosus* larvae. The cell lies posterior to the anus and is relatively insignificant in appearance in the female (Fig. 1a), whereas it is a prominent feature of the male (Fig. 1b). In order to confirm the sexual difference, newly hatched larvae were picked, sexed at hatching according to the appearance of the B cell and allowed to mature. In all cases the sexing at hatching had been correct. At this early stage, infective larvae and free living female larvae are indistinguishable. It is not until the early second larval stage when the vulval precursor cells swell prior to cell division in the free living female larva that a distinction can be made.

Results

Karyotypes of Eggs from Feces

Infection of rabbits with 50,000 larvae (progeny of free living animals) typically results in the rabbits shedding 3,300 eggs per gram of feces within 16 days

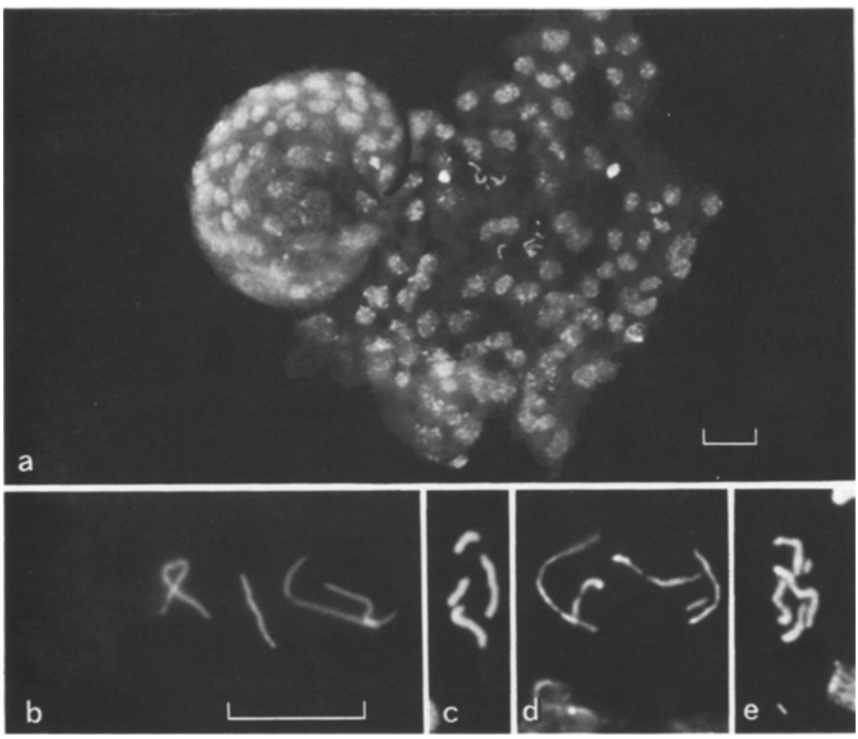

Fig. 2 a–e. Karyotypes of eggs isolated from infected rabbit feces. **a** Ruptured egg and the squashed cells from the egg contain nuclei in different stages of the cell cycle. In two cells prophase or prometaphyse figures are suitable for karyotyping. The karyotype of the egg is L3MS. **b** Prophase of first cleavage in 2L2M egg. The bar is the magnification for b–e. **c** 2L2M karyotype from an egg with at least 100 cells. **d** First cleavage prophase in L3MS egg. **e** L3MS karyotype in a cell from an egg with more than 100 cells

Table 1. Karyotypes from fecal egg squashes. For details see text

Experiment	Number of eggs embryonated or lacking suitable mitotic figures	Karyotype			
		2L2M	L3MS	2L2M+ L3MS	Other
1	232	24	29	3	5
2	not done	14	14	0	2
		42%	47%	3%	8%

after infection. When eggs from these infections are squashed and stained with a nuclear stain, only eggs containing less than 200 cells provide suitable prometaphases for determination of the karyotype. Figure 2a shows a typical squashed egg; two karyotypes can be seen. Since the eggs begin to develop in the intestine, this represents only 10–20% of them. In theses eggs two karyotypes were seen (Table 1). Forty-two percent of the eggs scored had two pairs of chromosomes, two long and two medium sized (2L2M) as shown in Fig. 2b and c. Such chromosomes have been reported previously (Zaffagnini, 1973; Triantaphyllou and Moncol, 1977). The karyotype of 47% of the scorable eggs was composed of one long chromosome, 3 nearly equal, medium sized chromosomes and a very small chromosome (L3MS) shown in Fig. 2d and e. A small percentage of eggs displayed both of the two major karyotypes or contained 5, 6, 7 or 8 chromosomes. In general such eggs were among the youngest in the population and so may have been developmentally abnormal. when the eggs were allowed to hatch three types of larvae were obtained in the cultures, and about one-half of these were males. It appears then that eggs recovered from feces of rabbits infected with *S. papillosus* are of two major karyotypes and can develop into three types of animals.

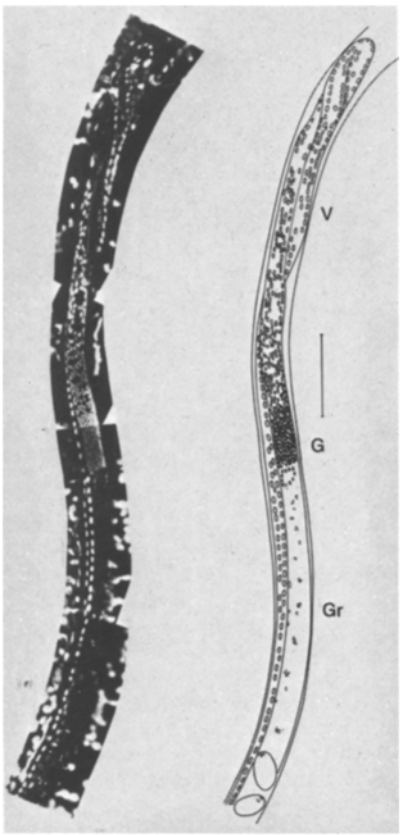

Fig. 3 a and b. Gonad of the parasitic female. **a** A parasitic female was mounted on a slide without squashing and one arm of the gonad was photographed. Vegetative zone, G, germinal zone, Gr, growth zone. The oocyte chromosomes of this animal are beaded. Scale as in b. **b** Drawing of the parasitic gonad in a. The bar is 100 μm

Oogenesis in Parasitic Females

Since two karyotypes were seen in the eggs isolated from rabbit feces, it was of interest to determine whether two types of parasitic females inhabited the small intestine of the host rabbit. Therefore worms were obtained from rabbit intestines and mounted for cytology as described in Materials and Methods. The structure of the gonad and oogenesis in the parasitic females have been described previously (Zaffagnini, 1973; Triantaphyllou and Moncol, 1977) and will only be summarized here. The nomenclature of Nigon and Roman (1952) is used.

The gonad of the parasitic female is composed of two reflexed arms, which open to the outside by a common centrally located vulva. Figure 3 shows one arm of the gonad in a whole mount of a parasitic female stained with the fluorescent nuclear stain Hoechst 33258. Near the reflex the gonad spirals about itself. The "vegetative zone" extends from the region indicated in Fig. 3 to the distal tip of the gonad lying beneath the plane of focus. The "germinal zone" (Fig. 3) contains compact nuclei and a few prometaphase figures com-

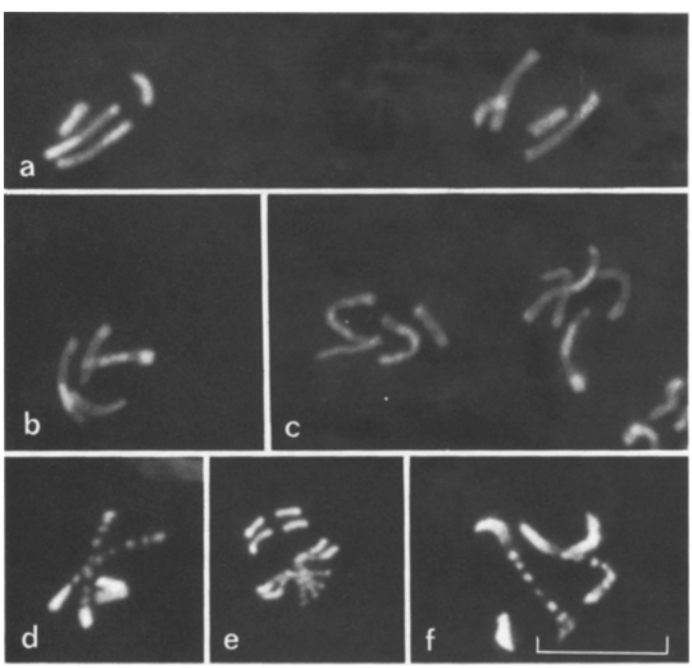

Fig. 4 a–f. Oocyte chromosomes in prometaphase of the maturation division. The growth zone of gonads was examined for the structure of the prometaphase chromosomes. **a** Two oocytes each containing 2 long and 2 medium chromosomes. **b and c** Unbeaded chromosomes showing Hoechst banding that is observed at some times. **d** Nucleus of oocyte with beaded chromosomes in the growth zone. **e** Oocyte nucleus in which the oocyte chromosomes have separated showing four beaded chromatids. **f** Beaded chromosomes with one chromosome showing six beads between the larger condensed region and the doubled terminal portion

posed of 4 chromosomes. From the germinal zone oocytes enlarge and form a single file in the growth zone. The chromosomes, near the germinal zone, are diffuse. As the oocyte matures the chromosomes condense to form two pairs of univalents in prometaphase of the maturation division. At this stage the oocyte chromosome morphology was studied. Usually the chromosomes in 5 to 6 oocyte nuclei can be scored in each arm of the gonad.

Parasitic females, isolated indirect cycle rabbits, could be divided into two classes on the basis of the structure of the oocyte chromosomes. In some gonads all the oocyte nuclei in the growth zone contained two long and two medium sized chromosomes (Fig. 4a–c; Zaffagini, 1973; Triantaphyllou and Moncol, 1977). Each chromosome was composed of two chromatids. Gonads were also seen in which two of the oocyte chromosomes were segmented into a region of six beads and a seventh slightly more oblong bead at one end (Fig. 4d–f). Each of these chromosomes also contained two chromatids. The two types of prometaphase figure were seen in more than 100 nematodes isolated from rabbits on three separate occasions. Both types of oocyte were never observed within one gonad, and both arms of the gonad were always the same.

In order to determine if these animals laid eggs of different karyotypes the nematodes isolated from one rabbit were mounted on slides with at most two worms to a slide so that gonad type might be correlated with eggs containing suitable figures for karyotyping. In only 17 cases was this successful, because reproduction fails almost immediately after the rabbit is sacrified. Therefore few normal eggs remain in the animals by the time they are mounted on slides (less than 1 h after death). As shown in Table 2 for each of two animals with unbeaded chromosomes in the oocytes, it was possible to determine that the

Table 2. Observations on gonads of individual parasitic females

Oocyte chromosome morphology	Karyotype of eggs in animal	Evidence of chromatin diminution seen in eggs[a]	Progeny[b]
unbeaded	2L2M	–	n.d.
unbeaded	–	–	n.d.
unbeaded	–	–	n.d.
unbeaded	2L2M	–	n.d.
unbeaded	–	–	n.d.
			n.d.
beaded	–	+	n.d.
beaded	L3MS	+	n.d.
beaded	L3MS	+	n.d.
beaded	–	+	n.d.
beaded	–	+	n.d.
beaded	L3MS	+	n.d.
beaded	–	+	n.d.
beaded	L3MS	–	n.d.
beaded	–	+	n.d.
beaded	–	–	n.d.
beaded	–	–	2♂
beaded	–	–	n.d.

[a] A + indicates that the process of chromatin diminution or the eliminated chromatin could be seen in some developing eggs in the animal

[b] n.d., not done. Not attempt was made to recover eggs from animals before fixation

karyotype of 1 to 2 eggs was 2L2M. In contrast, in each of four animals with beaded oocyte chromosomes, 1 to 2 eggs with the L3MS karyotype were seen. It appears then, that eggs of the L3MS karyotype arose from oocytes containing beaded chromosomes, while the 2L2M karyotype is associated with unbeaded oocyte chromosomes.

Generation of the L3MS Karyotype by Chromatin Diminution

Reproduction by mitotic parthenogenesis in *S. papillosus* involves a single maturation division (Zaffagnini, 1973; Triantaphyllou and Moncol, 1977). The divi-

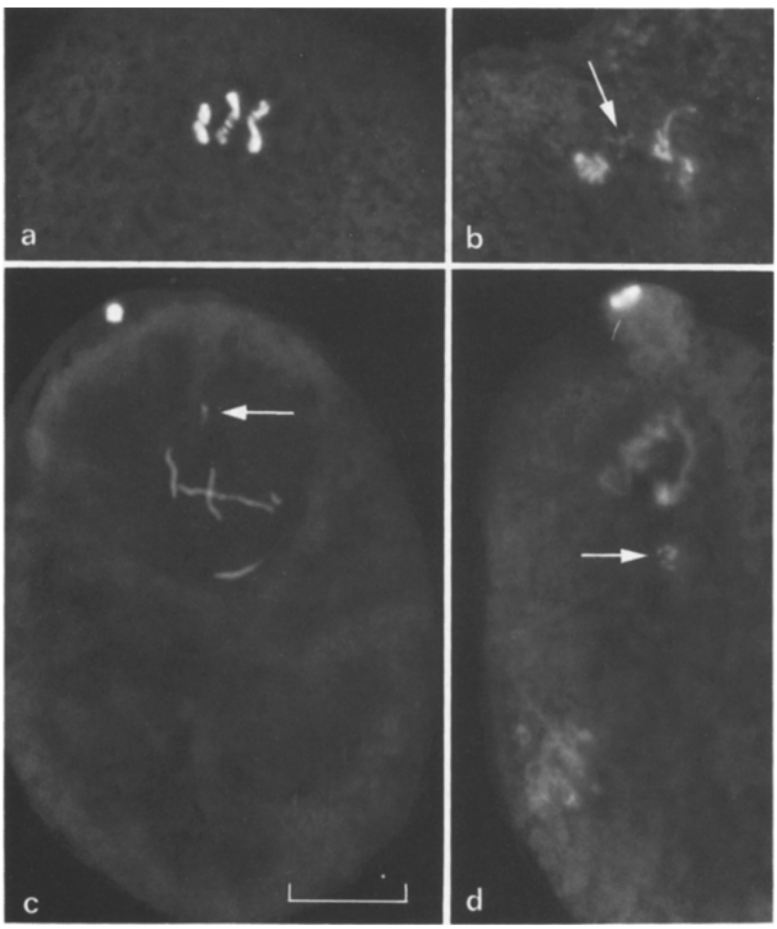

Fig. 5 a–d. Series of micrographs showing chromatin diminution at the maturation division. **a** An oocyte in prometaphase of the maturation division. **b** Late anaphase of the maturation division. Six beads (arrow) remain between the chromosomes that have migrated to the spindle poles. **c** An egg in prophase of the first cleavage, showing polar body, L3MS karyotype and eliminated chromatin (arrow) lying above the plane of focus of the nucleus. **d** Part of a squashed 6 cell egg in which eliminated chromatin (arrow) can still be seen. The polar body is a bright spot at the top of the egg

sion is mitotic and takes place at the end of the egg distal to the vulva; the polar body remains trapped within the egg shell. Figure 5a shows a beaded oocyte nucleus lying at one pole of the egg prior to polar body formation. The maturation division of oocytes with beaded chromosomes results in fragmentation of one of the beaded chromosomes. The details of this process are uclear. It appears, however that the beads do not migrate to the spindle poles with the chromosomes at anaphase (Fig. 5b) and remain in the cytoplasm as faintly staining dots (Fig. 5c and d). The oblong bead and the unbeaded part of the same chromosome are retained, however. These, together with the two medium sized chromosomes and a long chromosome make up the L3MS karyotype and can be seen along with the eliminated chromatin and polar body in an egg in Fig. 5c. The eliminated chromatin has been seen to persist in the egg cytoplasm up until at least the six cell stage (Fig. 5d).

The data in Table 2 show that in at least $^3/_4$ of the animals in which beaded chromosomes were observed the process of chromatin diminution or the eliminated chromatin could be seen in developing eggs. In three cases the karyotype of the egg was also found to be L3MS. Chromatin diminution was not observed in eggs from animals with unbeaded oocyte chromosomes. Thus, the L3MS karyotype seems to be derived from the parasitic female karyotype of 2L2M by eliminating a middle portion of one of the longer chromosomes. The ends are retained as the third medium sized chromosome and the short one.

Chromosomal Mechanism of Sex Determination

It seems likely that eggs with the L3MS karyotype develop into male larvae. Since animals with beaded oocyte chromosomes produce eggs with the L3MS karyotype, their progeny should be males. Table 3 shows that when eggs were isolated from the uteri of individual parasitic females and were allowed to hatch, only male larvae were obtained from the parasites with beaded oocyte chromosomes and only females from the parasites with unbeaded chromosomes.

A series of direct cycle infections provides further support for a sex linked chromosomal heteromorphism in *S. papillosus*. In this set of infections rabbits

Table 3. Comparison of oocyte chromosomes and sex of larvae hatching from eggs of individual parasitic females

Oocyte chromosome morphology	Progeny	
	♀	♂
unbeaded	2	0
unbeaded	1	0
unbeaded	1	0
unbeaded	1	0
unbeaded	1	0
beaded	0	2
beaded	0	1
beaded	0	1
beaded	0	1
beaded	0	1

Viable eggs were obtained from 21 out of a total of 43 adults picked. Each entry represents an animal for which it was possible to determine both cytology of the gonad and the sex of the progeny. Sex was determined at hatching by light microscopy

were infected with the first generation progeny of parasitic females, thereby eliminating the free living cycle. After a series of eight rabbits was infected by direct cycle, male progeny were no longer obtained from the fecal cultures of this, and subsequently infected rabbits in the series. Consequently, we expect that these parasitic females would no longer have oocytes with beaded chromosomes. To test this prediction nematodes were isolated the 14th rabbit in the series. The gonads of sixteen animals were examined and all of them contained oocytes with unbeaded chromosomes. From each of five of these animals a single female larva was recovered, but no males. The 2L2M karyotype was seen in one egg in the uterus of one animal and also in the eggs recovered from the large intestine of the rabbit. The L3MS karyotype was not observed. Larvae hatching from these fecal eggs were exclusively female. These results are consistent with the hypothesis that only eggs with the L2MS karyotype develop into males.

From these data on the direct cycle and from the data collected on the indirect cycle rabbit we conclude that males are derived from oocytes with beaded chromosomes. The oocytes undergo chromatin diminution at the maturation division giving rise to the L3MS karyotype.

Discussion

The life cycle and chromosome cycle of *S. papillosus* are summarized in Fig. 6. After entering a host, infective larvae mature into parasitic females in the intestine of the animal. Here the parasites reproduce by mitotic parthenogenesis. Two types of eggs are found in the feces of the host. Those with the 2L2M karyotype develop into free living females and infective larvae. Eggs with the L3MS karyotype, which are generated by chromatin diminution at the maturation division of the oocyte, develop into males. Chromatin diminution in *S. papillosus* results in an egg with a single karyotype for both somatic and germline cells, in contrast to chromatin diminution long known in *Ascaris* (Nigon, 1965), in which chromatin is eliminated from the soma, but retained in germline cells. Therefore, haploid sperms, which are formed by *S. papillosus* males (Triantaphyllou and Moncol, 1977) should carry either of two different chromosomal complements (Fig. 6), since a portion of one long chromosome was deleted. When free living males and females copulate only progeny of the 2L2M karyotype are found, because free living females reproduce by meiotic parthenogenesis (Zaffagnini, 1973; Triantaphyllou and Moncol, 1977) and in most instances, pseudogamy (Triantaphyllou and Moncol, 1977). Thus, although the sperm is required to initiate egg development, its genetic contribution is apparently lost.

If the male genome is not transmitted in the free living cycle, it raises the question how males are retained in these populations. In some instances fertilization may occur and further study may clarify this point (Triantaphyllou and Moncol, 1977). It seems, however, that the free living cycle may be necessary for the maintenance of males in the population, since male production can be selected against by favoring the direct cycle in *S. papillosus*. If pseudogamy

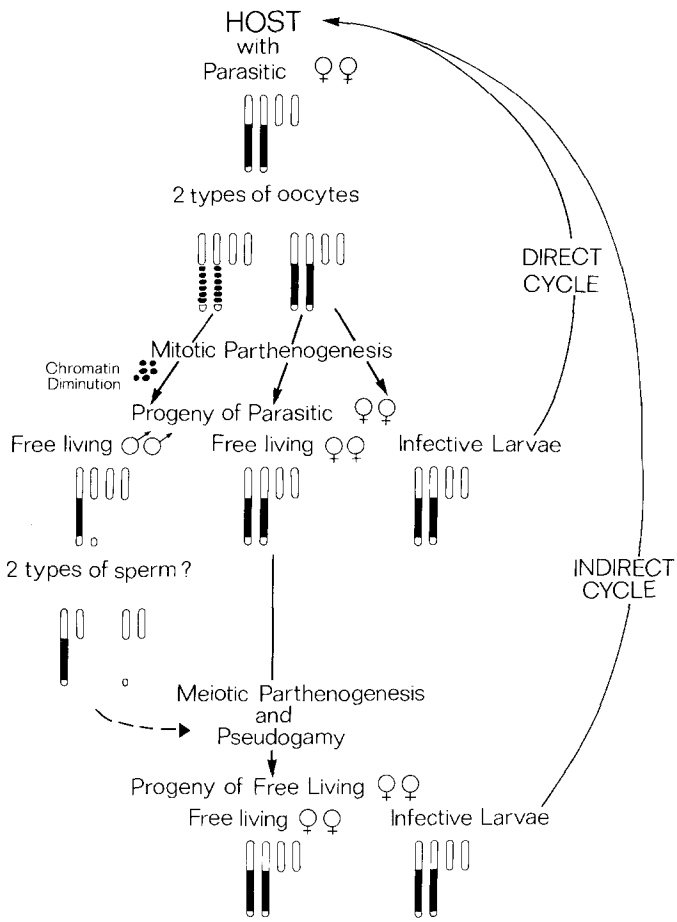

Fig. 6. The life cycle and chromosome cycle of *S. papillosus*. Parasitic females reproduce by mitotic parthenogenesis in the small intestine of the host animal. The oocyte chromosomes are either "beaded" or "unbeaded" as shown. The shaded region might represent the sex chromatin. Progeny of parasitic females develop in the feces and may become free living males, free living females or infective larvae. The karyotype of the males is derived from that of the parasitic female by chromatin diminution. Possibly, two types of haploid sperm are made by the males. Free living males and females must copulate to produce progeny of the 2L2M karyotype by meiotic parthenogenesis and pseudogamy. Infection by infective larvae, progeny of parasitic females, is called the "direct cycle", while infection by infective larvae from the free living cycle is the "indirect cycle"

is the more frequent mode of reproduction in the free living generation, then possibly, an epigenetic factor is transferred to the egg by the sperm which carry a large amount of cytoplasm (Nigon, 1965). Generally, the entire nematode sperm enters the posterior end of the egg which is destined to be the germline stem cell (Nigon, 1965). Thus, a factor favoring the production of oocytes with beaded chromosomes in the adult gonad might be transmitted to the germline of the egg at the time of activation. One might speculate further

that it is the sperm bearing the diminished chromosome that carries the epigenetic factor.

Such speculations suggest that there might be two types of parasitic females, those having 2L2M progeny and those with L3MS progeny. Our failure to find parasites with both beaded and unbeaded oocyte chromosomes in the gonad supports this suggestion. Environmental effects reported to influence male production (Moncol and Triantaphyllou, 1978) might favor growth or fecundity of one type of parasite over the other.

The interpretation presented here of the life cycle of *S. papillosus* is similar to that described for aphids (White, 1973). In some aphid species parthenogenic females produce both male and female eggs, while in other species females produce exclusively one type of egg. Male eggs are XO and are derived from the female XX by reduction at the single maturation division. It has been suggested previously (Triantaphyllou and Moncol, 1977) that the karyotype of *S. papillosus* of $2n=4$ evolved from one similar to that of *S. ratti* by translocation of an X to an autosome. In *S. ratti* females are $2n=6$ and males, $2n=5$, there being an XX, XO chromosome system of sex determination (Nigon and Roman, 1952; Bolla and Roberts, 1968). In *S. papillosus* then, the long chromosome could carry a quasiterminal X translocation. Elimination of this region from one long chromosome at the maturation division could generate a genetically XO egg that develops into a male.

In contrast to our observations, the only evidence of sex linked chromosomal heteromorphism that Triantaphyllou and Moncol (1977) reported was the behavior of the long chromosome in spermatogenesis in *S. papillosus* and *S. ransomi*. These authors observed a chromosomal satellite at a particular time in meiosis. Apparently it was derived from one homologue of the long bivalent which appeared asymmetric. Similar observations of chromosomal satellites have been reported by Herman et al. (1979) in another nematode, *Caenorhabditis elegans*. In this case genetically defined duplications that are translocated segments may be seen as satellites in oocyte nuclei in diakenesis. Thus, the cytological evidence presented by Triantaphyllou and Moncol (1977) and reported here support the suggestion of Triantaphyllou and Moncol (1977) that the long chromosome carries a translocation, possibly the X. Male differentation, as reported by Triantaphyllou and Moncol (1977) does not require the loss of a putative X segment such as we have described. Rather than being contradictory, these apparently conflicting observations on *S. papillosus* might suggest that two different races have been studied. This seems reasonable in light of an autosomal mutant in *C. elegans*. In this nematode hermaphrodites are normally XX and males XO. The mutant animals are fertile males although they are genotypically XX (Hodgkin and Brenner, 1977). Therefore, an XO chromosomal constitution may be sufficient, but is not necessarily required for male sexual differentiation.

In mitosis some quasiterminal duplications may be lost, breakage apparently occurring at or near the site of interchange of the translocation (Newmeyer and Galeazzi, 1977; Herman et al., 1979). A translocation carried by the long chromosome may behave similarly in *S. papillosus*. If so, then eggs with both the 2L2M and L3MS karyotypes or other variants might have been generated by mitotic loss of a translocation from one or both long chromosomes. The developmental fate of these eggs is unknown.

Acknowledgements. We should like to thank our colleagues Phil Anderson, Marty Chalfie, Marilyn Dew, Ed Hedgecock, Jonathan Hodgkin, Nichol Thomson and John White for skillfully picking individual worms from rabbit intestines. Hoechst 33258 was the gift of Dr. H. Loewe, Hoechst, AG, Frankfurt a.M., Germany, Roger Connan provided helpful discussion. D.G.A. was a fellow of the Muscular Dystrophy Association of America, Inc. O.C.N. Held a Junior Fellowship Award from the University of Nigeria.

References

Albertson, D.G., Sulston, J.E., White, J.G.: Cell cycling and DNA replication in a mutant blocked in cell division in the nematode Caenorhabditis elegans. Develop. Biol. **63**, 165–178 (1978)

Bolla, R.I., Roberts, L.S.: Gametogenesis and chromosomal complement in Strongyloides ratti (Nematoda Rhabdiasoidea). J. Parasitol. **54**, 849–855 (1963)

Brenner, S.: The genetics of Caenorhabditis elegans. Genetics **77**, 71–94 (1974)

Herman, R.K., Madl, J.E., Kari, C.K.: Duplications in Caenorhabditis elegans. Genetics **92**, 415–435 (1979)

Hodgkin, J.A., Brenner, S.: Mutations causing transformation of sexual phenotype in the nematode Caenorhabditis elegans. Genetics **86**, 275–287 (1977)

Moncol, D.J., Triantaphyllou, A.C.: Strongyloides ransomi: Factors influencing the in vitro development of the free-living generation. J. Parasitol. **64**, 220–225 (1978)

Newmeyer, D., Galeazzi, D.R.: The instability of Neurospora duplication Dp (IL→IR) H4250, and its genetic control. Genetics **85**, 461–487 (1977)

Nigon, V.: Développement et reproduction des nématodes. In: Traité de zoologie, anatomie, systématique, biologie, Tome IV (II). (P.P. Grassé, ed.), pp. 218–386. Paris: Masson et Cie 1965

Nigon, V., Roman, E.: Le déterminisme du sexe et le dévelopment cyclique de Strongyloides ratti. Bull Biol. Fr. Belg. **86**, 404–448 (1952)

Roberts, F.H.S., O'Sullivan, P.J.: Methods for egg counts and larval cultures for strongyles infesting the gastrointestinal tracts of cattle. Aust. J. agric. Res. **1**, 99–102 (1950)

Soulsey, E.J.L.: Textbook of veterinary clinical parasitology, Vol. I, p. 463. Oxford; Blackwell 1955

Sulston, J.E., Horvitz, H.R.: Post-embryonic cell lineages of the nematode Caenorhabditis elegans. Develop. Biol. **56**, 110–156 (1977)

Triantaphyllou, A.C., Moncol, D.J.: Cytology, reproduction, and sex determination of Strongyloides ransomi and S. papillosus. J. Parasitol. **63**, 961–973 (1977)

White, M.J.D.: Animal cytology and evolution, 3d edit., pp. 751–758. Cambridge: Cambridge University Press 1973

Zaffanini, F.: Parthenogenesis in the parasitic and free-living forms of Strongyloides papillosus (Nematoda, Rhabdiasoidea). Chromosoma (Berl.) **40**, 443–450 (1973)

Received March 2–May 22, 1979 / Accepted May 23, 1979 by Mary Lou Pardue
Ready for press June 4, 1979