REVIEW ARTICLE

How to become a parasite without sex chromosomes: a hypothesis for the evolution of *Strongyloides* spp. and related nematodes

ADRIAN STREIT*

Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Spemannstrasse 35, D-72076 Tübingen, Germany

(*Received 24 February 2014; revised 18 March 2014; accepted 20 March 2014; first published online 14 May 2014*)

SUMMARY

Parasitic lifestyles evolved many times independently. Just within the phylum Nematoda animal parasitism must have arisen at least four times. Switching to a parasitic lifestyle is expected to lead to changes in various life history traits including reproductive strategies. Parasitic nematode worms of the genus Strongyloides represent an interesting example to study these processes because they are still capable of forming facultative free-living generations in between parasitic ones. The parasitic generation consists of females only, which reproduce parthenogenetically. The sex in the progeny of the parasitic worms is determined by environmental cues, which control a, presumably ancestral, XX/XO chromosomal sex determining system. In some species the X chromosome is fused with an autosome and one copy of the X-derived sequences is removed by sex-specific chromatin diminution in males. Here I propose a hypothesis for how today's *Strongyloides* sp. might have evolved from a sexual free-living ancestor through dauer larvae forming free-living and facultative parasitic intermediate stages.

Key words: Evolution of parasitism, *Strongyloides* spp., sex determination, mode of reproduction, chromosome evolution.

INTRODUCTION

Parasitism is a highly successful strategy for organisms of various taxa. Parasitic species are found in all animal phyla, indicating that this lifestyle has arisen numerous times in evolution (Poulin, 2007; Poulin and Randhawa, 2014). The phylum Nematoda (Roundworms), in addition to many free-living species occupying virtually all terrestrial and aquatic habitats, contains a high number of parasitic species. Many of them cause diseases in humans and companion animals or reduce productivity in crops and livestock (Anderson, 2000; Lee, 2002). Even within this one phylum, parasitism must have arisen multiple times independently (Blaxter *et al*. 1998).

In most animal species, among them the vast majority of parasitic nematodes, there exist individuals of different sexes, normally males and females. The original cues that determine the sex and the regulatory machineries that interpret these cues are highly diverse (Haag and Doty, 2005; Haag, 2007). Sex can be determined genetically (genetic sex determination, GSD). In species employing GSD the sex is fixed at the moment the individual is formed, usually by the fusion of an egg and a sperm cell. The sex an individual adopts can also depend on environmental inputs, like temperature (environmental sex determination, ESD). In this case, the very early individual is sexually indifferent and its gender is specified only later in ontogeny. Various genetic and environmental sex-determining systems have been found within the Nematoda (Pires-daSilva, 2007). A genetic mechanism with two X chromosomes in females but only one X chromosome in males along with two sets of autosomes (XX/XO GSD) appears to be the most common and possibly ancestral sex determining system in nematodes (Pires-daSilva, 2007). Evolutionary transitions between GSD and ESD must be relatively frequent, at least in some taxa (Sarre *et al*. 2004; Valenzuela, 2008; Chandler *et al*. 2009). Within the nematodes ESD has presumably arisen from GSD ancestors multiple times independently (Pires-daSilva, 2007). One example for a parasite nematode taxon, that most likely switched from GSD to ESD fairly recently, is the genus *Strongyloides* (Streit, 2008).

The adult parasite worms of the about 50 known species of *Strongyloides* live in the small intestines of

* Corresponding author: Max-Planck-Institut für Entwicklungsbiologie, Abteilung IV, Evolutionsbiologie, Spemannstrasse 35, D-72076 Tübingen, Germany. E-mail: adrian.streit@tuebingen.mpg.de

Parasitology (2014), **141**, 1244–1254.
doi:10.1017/S003118201400064X

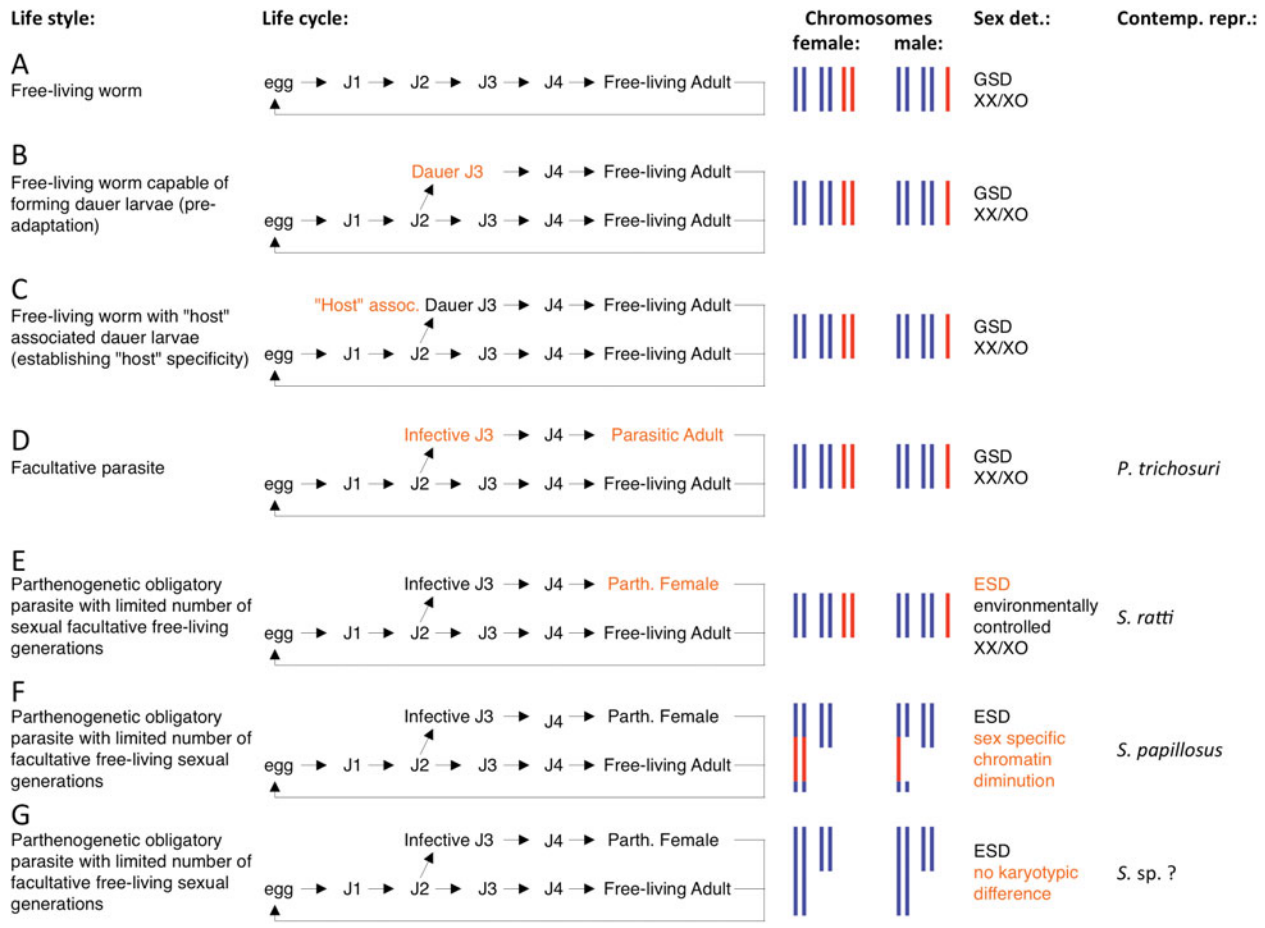

Fig. 1. Hypothesis for the evolutionary history of *Strongyloides* sp. For explanations see text. Evolutionary novelties are highlighted in orange (bold grey in the print version). Chromosomes and parts of chromosomes present in two copies in males and in females are represented in blue (black in the print version). Chromosomes and parts of chromosomes present in only one copy in males but two copies in females are represented in red (grey in the print version).

various vertebrates (Speare, 1989; Dorris *et al*. 2002; Viney and Lok, 2007). The biology and the life cycle of *Strongyloides* spp. (Fig. 1E–G) have been reviewed recently (Viney and Lok, 2007; Streit, 2008). In brief: the parasitic worms are all female and reproduce by mitotic parthenogenesis. They produce male and female progeny, which leave the host with the faeces at various stages of development, depending on the species. The female progeny can develop either into infective third-stage larvae (L3i) (homogonic or direct development) or into free-living adults (heterogonic or indirect development). The male progeny invariably become free-living. The free-living worms reproduce sexually and, with very few exceptions (Yamada *et al*. 1991; Streit, 2008), produce only female progeny that develop into L3i.

The sex of all species of *Strongyloides* investigated thus far is influenced by the immune status of the host. An increasing host immune response, for example over the duration of an infection, leads to a higher proportion of males (Streit, 2008). In spite of having an ESD system, in *Strongyloides ratti* (a parasite of rats) and *Strongyloides stercoralis* (a parasite of humans and dogs), X chromosomes exist (Nigon and Roman, 1952; Hammond and Robinson, 1994; Harvey and Viney, 2001). In these species females have two X chromosome, males only have one. Both sexes have two pairs of autosomes. Other species, i.e. *Strongyloides papillosus* (a parasite of sheep) and *S. vituli* (in cattle) have only two pairs of chromosomes (Albertson *et al*. 1979; Nemetschke *et al*. 2010*a*; Kulkarni *et al*. 2013). For *S. papillosus* it was shown that the genetic material homologous to the X chromosome and chromosome I of *S. ratti* is combined in one chromosome (Nemetschke *et al*. 2010*a*). In males of these species sex-specific chromatin diminution removes one copy of the genetic material related to the *S. ratti* X chromosome (Albertson *et al*. 1979; Nemetschke *et al*. 2010*a*; Kulkarni *et al*. 2013). There may even exist species of *Strongyloides* with two chromosomes and no chromosomal differences between the sexes (Triantaphyllou and Moncol, 1977).

Many species belonging to the Strongyloididae and related taxa can be isolated from the wild and cultured in the laboratory relatively easily. For many of them the phylogenetic relationship is resolved (Dorris *et al*. 2002; Hasegawa *et al*. 2009) and for some a set

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

of tools and techniques has been developed to the extent that they can be considered emerging satellite model organisms (Nolan *et al*. 1988; Nolan and Schad, 1992; Viney, 1999, 2006; Dorris *et al*. 2002; Viney *et al*. 2002; Grant *et al*. 2006*a*, *b*; Eberhardt *et al*. 2007; Hasegawa *et al*. 2009; Nemetschke *et al*. 2010*b*; Shao *et al*. 2012). Therefore this group of nematodes is excellently suited to serve as a test case to study the evolutionary transitions between different lifestyles, life histories and reproductive strategies.

Based on theoretical and experimental work from numerous authors (mentioned at the corresponding places in the text), I propose a hypothesis for the evolutionary path from an ancestral free-living nematode with a simple life cycle and XX/XO GSD to a species of *Strongyloides* with its alternative life cycles, no genetic differences between the sexes and ESD. The hypothesis has to account for four major transitions: (i) from free-living to parasitic life style; (ii) from sexual to parthenogenetic reproduction; (iii) from GSD to ESD; and (iv) from three pairs of chromosomes, one of which is a sex chromosome, to two pairs of chromosomes with no sex chromosome. The hypothesis is summarized without references in the next paragraph and in Fig. 1. After that I discuss the individual steps in more detail.

THE HYPOTHESIS IN SUMMARY

The hypothetical evolutionary path starts with a sexually reproducing free-living ancestor. It had a simple life cycle with four juvenile stages (Fig. 1A). Further, it employed XX/XO genetic sex determination with two pairs of autosomes and one pair of X chromosomes in females. Next this ancestor evolved the ability to form dauer larvae, an alternative third juvenile stage specialized for surviving adverse conditions (Fig. 1B). The dauer larvae then established increasingly species-specific non-parasitic, e.g. phoretic or necromenic, interactions with other organisms (putative future hosts, Fig. 1C). In the next step the dauer branch of the life cycle became parasitic, resulting in a facultative parasite (Fig. 1D). Next, four changes in unresolved order occurred (Fig. 1E). (i) The number of consecutive free-living generations was limited, eventually to one. (ii) The free-living generation stopped producing males. (iii) The reproductive mode in the parasitic form changed from sexual to parthenogenetic. (iv) Sex determination became dependent on the host's immune status transforming the different numbers of X chromosomes from sex-determining signal to part of the machinery that interprets the now sex-determining environmental cue. Next the X chromosome fused with an autosome. However, the originally X chromosomal genomic regions were still present in only one copy in males due to sex-specific chromatin diminution (Fig. 1F). As the final step, physical elimination of genetic material from males was abandoned, completing the transition from XX/XO GSD to ESD without a genetic difference between the sexes (Fig. 1G).

THE INDIVIDUAL STEPS OF THE HYPOTHESIS

Initial remarks

One of the problems with evolutionary history is that we can never go back and observe directly how and when something happened. We can only draw conclusions from what we observe today either in living organisms or in fossils. The latter are virtually non-existent for nematodes. It is also important never to forget that every organism present today is a modern organism, which is separated from a common ancestor for exactly the same time as any other organism it is compared with. However, if a lineage breaks up into multiple species after a certain evolutionary novelty arose, in some branches this particular trait will undergo further change while in others it will remain virtually unaltered. If we now look at the evolutionary history of a specific taxon, as with *Strongyloides* spp. here, features that were intermediate steps on the way to the taxon of interest may still be present in related taxa, because these taxa did not change this particular trait, not because they are generally closer to the common ancestor. For the purpose of this article I will refer to species that show a trait proposed as an intermediate step towards *Strongyloides* spp. as 'contemporary representatives' of this intermediate state.

The ancestor (Fig. 1A)

Based on phylogenetic analyses it is most parsimonious that modern nematode parasites are descendants of multiple different non-parasitic nematode ancestors (Blaxter *et al*. 1998; Holterman *et al*. 2006; Holterman *et al*. 2008). The typical nematode life cycle contains four juvenile stages (J1–J4, synonymously also called larval stages L1–L4), which are separated from each other and from the adult by moults (Lee, 2002). As four juvenile stages are found in most nematodes of all clades (Lee, 2002) and *Strongyloides* spp. develops through four juvenile stages today, there is no reason to postulate that the ancestor was different in this respect.

XX/XO GSD is very common among nematodes including several relatives of *Strongyloides* spp. and might well be ancestral for all nematodes (Pires-daSilva, 2007). XX/XO GSD is therefore a likely scenario in the free-living ancestor. By parsimony two autosomes are postulated because this requires the least number of changes in order to arrive at *Strongyloides* spp.

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

Dauer larvae as pre-adaptation for parasitism (Fig. 1B and C)

It is hard to imagine that the transition from a free-living to a parasitic lifestyle happened in one step. It had long been proposed that for this evolutionary step, as for other major changes, features that evolved for a completely different reason but later facilitated the transition to parasitism must have existed (Osche, 1962; Poulin, 2007). Osche introduced the somewhat controversial term 'pre-adaptations' for such features. I will use the term here but I would like to stress that it has no teleological meaning. A pre-adaptation for parasitism is not a form of adaptation to parasitism but something that existed prior to the transition and the adaptation to the parasitic lifestyle. It happened to be present and coincidentally facilitate the transition to parasitism, which was in no way 'intended' or inevitable at the time when the pre-adaptation arose for reasons completely unrelated to parasitism. For a recent discussion of the evolution of parasitism in general see Poulin and Randhawa (2014).

Many free-living nematodes can form dauer larvae to survive adverse conditions like starvation (Lee, 2002). At least in clades IV and V (c.f. Blaxter *et al*. 1998) there are many parallels between dauer larvae of free-living nematodes and infective larvae (L3i) of animal parasitic nematodes. Both are non-feeding but motile third-stage larvae. They show morphological similarities and there are conserved aspects in the genetic cascade that controls their formation (Dieterich and Sommer, 2009; Ogawa *et al*. 2009; Wang *et al*. 2009; Sommer and Ogawa, 2011). For these reasons, dauer larvae and L3i very likely are evolutionarily related (homologous). In many cases dauer larvae adhere to other organisms (Lee, 2002; Sommer and Ogawa, 2011). These interactions range from relatively unspecific, purely phoretic interactions for dispersal to highly species-specific associations. Examples for the latter are the so-called necromenic associations between species of the genus *Pristionchus* and particular scarab beetles (Herrmann *et al*. 2006; Sommer and Ogawa, 2011). The dauer larvae adhere to their 'host' beetle and resume development after the death of the beetle, feeding and reproducing on the emerging microbial population. Once the food is exhausted the worms again form dauer larvae, which populate a new beetle. These interactions, in spite of being specific, are neither parasitic (the larvae do not harm the beetle) nor obligatory. The apparent homology of dauer larvae and infective larvae and the capability of many dauer larvae to form species-specific interactions with other organisms make the capability to form dauer larvae a prime candidate for a pre-adaptation for parasitism (Dieterich and Sommer, 2009; Sommer and Ogawa, 2011). For a very recent, more detailed discussion and review of this topic see Crook (2014).

Facultative parasitism (Fig. 1D)

Once dauer larvae have established interactions with particular 'hosts' it is conceivable that they evolve strategies to exploit the host. At least two strategies are imaginable. Either, the worms start reproducing on/in the host without killing it rapidly (parasitic) or they acquire the capability of actively killing the host to speed up the necromenic cycle. With the entomopathogenic family Steinernematidae there exists a likely example for the second strategy among the relatives of *Strongyloides* spp. (Dorris *et al*. 2002). For the purpose of this article the genus *Parastrongyloides* is more important. It is very closely related with *Strongyloides* spp. and its best-studied representative, *Parastrongyloides trichosuri*, is a facultative parasite (Dorris *et al*. 2002; Grant *et al*. 2006*b*). *Parastrongyloides trichosuri*, when parasitic, lives in the small intestines of Australian possums (Mackerras, 1959; Grant *et al*. 2006*b*). Individuals of this species appear to have a free 'choice' between a free-living and a parasitic life style. *Parastrongyloides trichosuri* consists of males and females in the parasitic and in the free-living generation (Mackerras, 1959; Grant *et al*. 2006*b*) and reproduces sexually in both generations (Mackerras, 1959; Grant *et al*. 2006*b*). It has three chromosomes, employs XX/XO sex determination ($2n = 6$ in females and $2n = 5$ in males) and its X chromosome is homologous to the X chromosome of *S. ratti* (Kulkarni *et al*. 2013). With this, *P. trichosuri* is an excellent candidate for a contemporary representative of a facultative parasitic, fully sexual ancestor stage of *Strongyloides* spp.

Intermediate remarks

The procedure proposed so far allowed the gradual and sequential evolution of strategies for host finding and for survival within the host without depending upon the host. Up to this point, evolutionary reversal to a situation as depicted in Fig. 1A is straightforward. It simply takes the loss of the dauer/parasitic cycle. Among the close relatives of *Strongyloides* spp. there is indeed a taxon that was proposed to have reverted to a simple non-parasitic lifestyle, namely *Rhabditophanes* spp. (Dorris *et al*. 2002). This conclusion should, however, be taken with caution because it is partially based on the erroneous phylogenetic placing of *Rhabdias bufonis* by Dorris *et al*. (2002) (Blaxter *et al*. 2014). From the facultative parasitic stage, as described in Fig. 1D, the transition to an obligatory parasite with no free-living adults is easy to imagine, simply by losing the free-living cycle. Losing one of the two life cycles is presumably genetically rather easy. From work in the model nematode *Caenorhabditis elegans* we know that strains which cannot form dauer larvae, and strains which

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

must develop through the dauer stage, can arise after single point mutations (Riddle and Albert, 1997).

Obligate parthenogenetic parasite with a limited number of facultative sexual free-living generations and environmentally controlled XX/XO sex determination (Fig. 1E)

In the case of *Strongyloides* spp. the transition to obligate parasitism did not occur through a simple loss of the free-living cycle but rather through the limitation of the number of consecutive free-living generations. The fundamental differences between the life cycles in Fig. 1D (represented by *P. trichosuri*) and Fig. 1E (represented by *S. ratti*) are (i) the limitation of the number of consecutive free-living generations to one, (ii) the absence of males in the progeny of the free-living generation, (iii) the non-sexual (parthenogenetic) reproduction in the parasitic generation and (iv) the switch to environmental sex determination in the progeny of the parasitic females.

To (i) and (ii): the well-studied species *S. ratti* and *S. stercoralis* appear to be completely incapable of forming consecutive free-living generations and no males are formed in the progeny of the free-living generation (Yamada *et al*. 1991; Viney, 1999; Harvey and Viney, 2001). There is some indication that the limitation of consecutive generations to only one and the complete loss of males from the progeny of the free-living worms evolved gradually. For some species of *Strongyloides* small numbers of second-and-more-generation free-living animals have been observed under very specific culture conditions (reviewed in Streit, 2008). Most notably, Yamada *et al*. (1991) were able to raise up to 11 consecutive free-living generations in *Strongyloides planiceps*, although with dramatically declining fecundity after generation seven. In this study, as well as in two earlier publications by Beg (1968) on *Strongyloides fuelleborni* and by Beach (1936) on *Strongyloides simiae*, both sexes were present in the consecutive free-living generations in numbers that permitted the propagation of the cultures. Other authors described either a shortage (Augustine, 1940) or complete absence (Hansen *et al*. 1969) of males in second-and-more-generation free-living *Strongyloides* spp.; in the study by Yamada *et al*. (1991) the proportion of males also was lower than the 50% that would be expected in an XX/XO genetic sex determining system. The mechanism for how the formation of males is reduced or entirely prevented is not known. In *S. papillosus* it was shown that genetically male-determining mature sperm is never formed (Nemetschke *et al*. 2010*a*). On the other hand, recombination between the full-length chromosome and the two remnants of chromatin diminution (which when incorporated into sperm would lead to the male karyotype after fertilization, see below) were observed. This places the exclusion of the male-determining chromosomes between prophase of meiosis I and mature sperm. Therefore, a mechanism comparable to the one in the unrelated nematode *Rhabditis* sp. SB347, where predominantly female-determining sperm is produced due to asymmetric spermatogenic meiosis is a likely scenario (Shakes *et al*. 2011). Such a mechanism would also provide the flexibility for very different degrees of bias. To (iii): although sexual reproduction is the predominant mode of reproduction in multicellular organisms, transitions to parthenogenetic reproduction are not uncommon throughout the metazoans (Schön *et al*. 2009). Within the nematodes, transitions from reproductive systems with males and females and obligatory outcrossing to systems with obligatory or facultative self-reproducing females, either by means of self-fertilization (hermaphroditism) or by parthenogenesis occurred rather frequently (Denver *et al*. 2011). Further, there is experimental evidence demonstrating that such transitions can be achieved with as little as two mutations (Baldi *et al*. 2009). Therefore, it is reasonable to assume that in *Strongyloides* spp. the transition from sexual to parthenogenetic reproduction also could occur relatively easily (for a general discussion of conditions favouring or constraining the evolution of parthenogenesis see Engelstadter, 2008 and Schwander *et al*. 2010). One prerequisite, however, was that the mechanisms controlling oocyte maturation had diverged sufficiently between the parasitic and the free-living females that it was mechanistically possible to switch to parthenogenesis in the parasitic but not in the free-living generation. In this context, comparative studies of oogenesis in parasitic and free-living *P. trichosuri* will be highly interesting. Upon the transition to parthenogenesis, males became obsolete in the parasitic generation and they lost their ability to develop into the parasitic form (see below and Box 1).

Box 1. Speculative order of events between Fig. 1D and E.

First the parasitic female switched to parthenogenetic reproduction. This automatically led to an all-female progeny, with the exception of rare males formed as the result of X chromosome mis-segregation, similar to what is seen in *C. elegans* (Brenner, 1974). The resulting population had a female bias (compared with whatever the optimal sex ratio might have been before the change (cf. Hamilton, 1967) in both generations because all individuals contributed by parasitic mothers to the free-living and the parasitic populations were now females and the proportion of males produced by free-living parents had not changed. Since males were obsolete in the parasitic generation and underrepresented in the free-living generation, in

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

the progeny of the free-living worms there was a strong selection pressure for males to stay free-living and for females to become parasitic. Under these circumstances males lost their capability of forming infective larvae and females only rarely underwent multiple consecutive free-living generations. The result was a species where among the progeny of free-living adults virtually all the females developed into L3i and all the males into free-living males. In populations newly founded by parasitic females, a cohort of males could still be initiated by the rare males occurring in the progeny of the parasitic generation as the result of X chromosome mis-segregation. In the next step, the parasitic worms regained the capability of producing large numbers of males by increasing and controlling the number of X losses. The males produced all developed into free-living males because they had lost their capability of forming L3i in the previous step. The addition of males to the free-living population through the progeny of the parasitic worms and the smaller number of females, because a considerable fraction of their siblings now developed into males, resulted in a change in the gender balance such that males were now overrepresented. This may have led to a selective pressure for a reduction of the proportion of males in the progeny of the free-living generation, ultimately to zero.

Currently we do not have well-characterized contemporary representatives for any of the intermediate steps that could support or reject the order of events presented above. However, at least for some steps such contemporary representatives are likely to exist in the form of the species capable of forming small numbers of second-generation free-living animals described in the main text. Be that as it may, at the moment the analysis of these x-generation free-living animals is insufficient. For example, in no case is it known if male offspring of free-living worms can also develop into infective larvae. For most of these publications the isolates studied are no longer available. Due to the difficulties in *Strongyloides* taxonomy in some cases it is unclear to which species the *Strongyloides* under investigation really belonged (c.f. Augustine, 1940; Speare, 1989; Eberhardt *et al*. 2008; Streit, 2008) and their phylogenetic positions are not known.

To (iv): one problem that arose with the transition to parthenogenesis was how males, which are still required in the free-living generation, can be generated. The ancestral genetic sex-determining mechanism, which depended on the existence of two types of genetically different (X bearing and non-X bearing) sperm was no longer available in the context of parthenogenetically reproducing females. Based on findings in multiple, very different taxa it has been proposed that genetic regulatory cascades that control sex determination have a tendency to evolve bottom up such that new elements are added at the top (Wilkins, 1995). In this way the decisive sex-determining signal can change but the underlying regulatory components used for the interpretation of the signal and the execution of sex determination remain the same. In the case of *Strongyloides* spp. this means that a host immune response dependent mechanism to remove one of the X chromosomes from a portion of the parthenogenetically produced embryos arose. This rendered the sex-determining system environmental. Hermaphrodites of the model nematode *C. elegans*, which are essentially females, produce a virtually all-hermaphrodite progeny. However, a small number of males are formed as the result of X chromosome non-disjunction events. It is known that environmental conditions, such as high temperature, can influence the frequency of X chromosome non-disjunction. Further, mutations in single genes (*him-5* and *him-8*) can dramatically and X-chromosome-specifically increase this frequency (Brenner, 1974; Hodgkin *et al*. 1979; Hodgkin, 1983). Although the situations in *C. elegans* and in *Strongyloides* spp. are not directly comparable because in the former the reduction of the number of X-chromosomes occurs during a meiotic division and in *Strongyloides* an X is lost during a mitotic division, the example of *C. elegans* illustrates that the type of genetic change required can occur relatively easily requiring only very few mutations (see also Box 2). As a result of this evolutionary step, in the progeny of the parasitic *Strongyloides* spp. females the number of X chromosomes was no longer the sex-determining signal but became part of the cascade that interprets the new signal. The underlying ancestral sex determination machinery did not have to change because the number of X chromosomes still differed between the sexes. At the same time, in the progeny of the free-living population the system could still be used for chromosomal GSD, if at the time males still existed among the progeny of the free-living generation.

Box 2. Speculation on the recognition of the X chromosome.

All processes leading to the controlled production of males by parasitic females, as they are described in the main text, require the specific recognition and elimination or silencing of the X chromosome or the X-derived sequences. Among the molecular machineries that recognize specifically the X chromosomes in different organisms, the dosage compensation (DC) machineries, which equalize the amount of X chromosomal gene products between sexes with different numbers of X chromosomes, are the best-studied ones

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

(Lucchesi *et al*. 2005; Ferrari *et al*. 2014). However, others also exist. In *C*. *elegans* it is known that in the germ line the X chromosome is very different from the autosomes with respect to various chromatin modifications and transcriptional activity (Schaner and Kelly, 2006). Further, there exist a family of four homologous genes, that code for the so-called ZIM proteins, which are C2H2 zinc-finger proteins that are chromosome-specific components of the chromosomal pairing centres, which initiate the pairing of homologous chromosomes during meiosis (Phillips and Dernburg, 2006). Mutations in *him-8*, which encodes the X-specific ZIM, lead to the X chromosome specific increase in chromosome non-disjunctions mentioned above (Hodgkin *et al*. 1979; Phillips and Dernburg, 2006). It is very likely that such X-recognizing molecular machineries existed also in the ancestor of *Strongyloides* spp. At the example of the X chromosome DC mechanism I speculate below how this machinery might have been recruited for sex determination. Characterized X chromosome DC mechanisms, in a first approximation, work according to one of three principles (Lucchesi *et al*. 2005; Ferrari *et al*. 2014): (i) increasing the transcriptional activity of the single X chromosome in males, as in *Drosophila melanogaster*; (ii) inactivating one of the two X chromosomes in females, as in mice and humans or (iii) reducing the expression levels of X-linked genes from both female X chromosomes, as in *C*. *elegans*. In (i) the DC machinery is active in males, in (ii) and (iii) in females. If the expression level of X-linked genes is to be used for sex determination, as a measure for the number of X chromosomes present, dosage compensation must be off at the time the signal is read. If the ancestor of *Strongyloides* spp. as depicted in Fig. 1G had a DC operating according to principle (iii), like *C*. *elegans*, one would expect that it was on in the parasitic female but switched off during oogenesis before the one-cell embryo was formed. If, due to a new mutation the resetting fails in some cases, the result is an embryo with X-linked genes expressed at half the level of normal. This is equivalent to having only one X chromosome and the affected individual embryo might develop as a male. This could have been a mechanism to render the sex-determining system independent of the copy number (but not the expression level) of X-linked or derived genes.

It is noteworthy that there was no need for the transition to parthenogenesis and the rise of the new sex-determining mechanism to occur simultaneously. If parthenogenetic reproduction in the parasitic generation pre-dated the restriction of the number of consecutive free-living generations, it is conceivable that there was an intermediate state where the parasitic females, similar to *C*. *elegans* hermaphrodites, produced essentially only female progeny and the males were all the offspring of free-living mothers (see also Box 1).

It is clear that the capability of the parasitic females to self-reproduce must have preceded the loss of the ability of males to form infective larvae. But other than that, based on current knowledge, it is very difficult to propose an order of the events that led from Fig. 1D–E. Also, some of the steps proposed are rather large leaps. In order to illustrate that it is possible that the proposed transition occurred through a cascade of relatively small, conceivable steps, in Box 1 I present a highly speculative order of events. However, other than for the hypotheses discussed in the main body of this article, which are supported (although far from being proven) by observational and experimental evidence, for the scenario in Box 1 such evidence is highly limited or absent and other orders of events are equally plausible.

Chromosome fusion and chromatin diminution (Fig. 1F)

Whereas in the last step the X chromosome lost its function as part of the sex-determining signal, in this step leading to a state today represented by *S*. *papillosus* it lost its physical independence through fusion with an autosome. For *S*. *papillosus* it was shown that the equivalents of the *S*. *ratti* chromosomes I and X are combined into one chromosome such that the X-related regions are now flanked by chromosomal regions related to chromosome I (Nemetschke *et al*. 2010*a*). Nevertheless, males and females differ genetically. In males one copy of the genetic material that is evolutionarily related to the X chromosome of *S*. *ratti* is removed from one-cell embryos destined to become males (sex-specific chromatin diminution, Albertson *et al*. 1979; Nemetschke *et al*. 2010*a*). The fact that *P*. *trichosuri* has three chromosomes, employs XX/XO sex determination and its X chromosome is homologous to the one of *S*. *ratti* strongly suggest that two autosomes and an X chromosome are the ancestral state (Kulkarni *et al*. 2013) and thus supports the order of Fig. 1D–F. The situation with four chromosomes in *S*. *papillosus* is derived and arose through the integration (or terminal fusion followed by a chromosomal rearrangement) of the X chromosome into the autosome number I. Chromatin diminution serves to functionally reconstitute the ancestral environmentally controlled XX/XO sex-determining system. It is an open question how big the mechanistic obstacles for the switch from disposing of an entire chromosome (chromosome elimination) to the

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

removal of only a portion of a chromosome (chromatin diminution) actually were. At first, it appears much more complicated to remove a portion of a chromosome and retain both ends rather than getting rid of the entire chromosome. However, we do not know how the elimination of one X chromosome is achieved in *S. ratti*. For *S. papillosus* we know at least that the chromatin to be eliminated undergoes fragmentation (Albertson *et al*. 1979). This is reminiscent of the evolutionarily independent, much better understood case of chromatin diminution in giant roundworms (*Ascaris* spp. and relatives), which creates a genetic difference between the soma and the germ line (Tobler and Müller, 2001; Streit, 2012; Wang *et al*. 2012). In *Ascaris* spp. new telomeric sequences are added in an apparently unspecific manner to the free ends of the fragments to be retained and of the ones bound for destruction (Müller *et al*. 1991; Tobler and Müller, 2001). This indicates that at least this nematode has a general repair mechanism for chromosome breaks, which is used in the process of chromatin diminution. If similar mechanisms are at work in *Strongyloides* spp. it is conceivable that in *S. papillosus* the mechanism already in place for the elimination of one X chromosome in combination with the general repair mechanism for chromosome breaks was sufficient to achieve chromatin diminution after the chromosomal fusion occurred. Should this be the case, in *Strongyloides* spp. the conceptually different chromosome elimination and chromatin diminution (cf. Tobler and Müller, 2001) would be achieved essentially by the same mechanism.

Environmental sex determination without genetic difference between the sexes (Fig. 1G)

The last step to complete the transition from chromosomal GSD to ESD without genetic differences between the sexes could be achieved by abandoning chromatin diminution and, for example, replacing it with silencing of one copy of the X-derived sequences or reducing their average expression to half in males. Mechanisms to achieve such effects are well known from various X chromosome dosage compensation (DC) systems which recruit different types of regulatory chromatin modifications (Lucchesi *et al*. 2005; Ferrari *et al*. 2014) (see also Box 2).

Alternatively, the loss of chromatin diminution may have been preceded by a change in the sex-determining genetic cascade removing the dose of X-derived gene products from the mechanism thereby making physical elimination or differential transcriptional activity of the corresponding DNA unnecessary. The experiments by Hodgkin (2002) in *C. elegans* suggest that such changes require only very few mutations. In natural populations this type of change has, for example, been well characterized in the house fly *Musca domestica* where in some populations new sex-determining loci arose on autosomes (Dubendorfer *et al*. 2002; Hediger *et al*. 2010). They feed into the regulatory cascade downstream of the original sex-determining locus on the Y chromosome. As a consequence populations of *M. domestica* without Y chromosomes appeared. In *Drosophila melanogaster*, another dipteran, there is evidence that the dot chromosome is evolutionarily derived from an X chromosome, providing an example of an X chromosome that reverted to being an autosome (Vicoso and Bachtrog, 2013).

Currently, it is an open question if species of *Strongyloides* without karyotypic differences between the sexes exist. Triantaphyllou and Moncol (1977) looked for but failed to detect indication for chromatin diminution in their isolates of *S. papillosus* and *Strongyloides ransomi* and proposed that in these species males and females do not differ with respect to their chromosomes. This view was later challenged, at least for *S. papillosus* (Albertson *et al*. 1979; Nemetschke *et al*. 2010*a*). However, Triantaphyllou and Moncol (1977) studied a different isolate of *S. papillosus* than the later authors. From the *M. domestica* example and the findings by Hodgkin (2002) we know that different sex-determining systems are possible within one species. Alternatively, given the difficulties with *S. papillosus* taxonomy (Eberhardt *et al*. 2008), it is also possible that Triantaphyllou and Moncol (1977) studied a different species. Interestingly, they described fragmentation of the larger chromosome followed by the loss of a portion (in *S. ransomi*) or by recovery (in *S. papillosus*) during male spermatogenesis and interpreted this finding as a hint for the X chromosomal evolutionary origin of one half of the long chromosome. Future genetic and cytological analysis of further species of *Strongyloides*, i.e. *S. ransomi*, will tell if species as depicted in Fig. 1G exist in nature.

CONCLUDING REMARKS

The model presented here describes the case of one specific group of species. There is no reason to assume that the way to parasitism was the same in all the independent cases of animal parasitic lifestyle within the nematodes. Nevertheless, some of the thoughts presented about individual steps are also valid for other parasitic nematodes. For example, dauer larvae are also prime suspects for representing pre-adaptations toward parasitism in clade V, which contains many gastrointestinal nematodes, among them the human hookworms (Blaxter *et al*. 1998). Also in this group of nematodes there is an example of a species with parasitic and free-living generations. *Rhabdias bufonis*, a parasite of toads, is able to form protandrous hermaphroditic parasitic

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

and gonochoristic free-living adults (Anderson, 2000). Note that the phylogenetic placement of *R. bufonis* as a close relative of *Strongyloides* spp. by Dorris *et al*. (2002) was in the meantime found to be wrong by the authors of this paper (Blaxter *et al*. 2014). Instead it should be placed in clade V (M. Blaxter, personal communication).

In this article, I intentionally concentrate on the question of what happened during evolution and do not discuss why it happened. The evolutionary path to *Strongyloides* spp. is a singular historical succession of events, which was driven by a combination of selective forces and stochastic events. For some changes it is rather easy to speculate what the selective advantage might have been. For example, for becoming capable of clonal self-reproduction several advantages are imaginable: (i) a single animal can found a new population; (ii) since all infective larvae are females and with this productive, the parasitic generation avoids sexual conflict and paying the two-fold cost of sex (Chapman *et al*. 2003), essentially without suffering from negative effects of asexuality because occasional sexual reproduction is still possible through the free-living generation; (iii) having the opportunity to 'choose' clonal reproduction when the conditions for parasitic stages are favourable and thereby prevent breaking up beneficial gene combinations and to opt for sexual reproduction and the creation of new genotypes once conditions deteriorate.

For other evolutionary changes there are no putative selective advantages easily recognizable. This does, of course, not mean that no such advantages could exist. However, nematodes, in particular the ones with females capable of self-reproduction, are very efficient colonizers (Herrmann *et al*. 2010). It is very likely that for such species new small, relatively isolated populations are founded frequently. Therefore stochastic small population effects may play a major role in the evolution of such taxa and there is no need to postulate a direct selective advantage for every evolutionary step. For example, the fusion of the X chromosome with an autosome happened at a specific time point. There is no slow transition from one state to the other. It is hard to imagine that the fusion was of immediate and direct benefit to the carrier. It is more likely that it was slightly detrimental or, at best, neutral and spread by nearly neutral evolution (Ohta, 1996) or was hitch-hiking with a beneficial mutation. The latter would be facilitated by clonal reproduction during which the two loci in question are not separated. The chances of the novelty to persist and become fixed at least in a local population might have been increased because it occurred in a very small population or even in an individual that founded a new population. The chromosomal rearrangement may even have contributed to the genetic isolation of this population, and with this to speciation.

The hypothesis presented here is speculative and contains some rather large steps. Many observations invoked to support certain claims could probably be interpreted differently or merely show that a postulated evolutionary transition is plausible but do not really indicate that in the specific case it happened as suggested. This hypothesis was devised bearing in mind the best studied (*S. ratti*, *S. stercoralis* and *S. papillosus*) and a hypothetical species of *Strongyloides* with no genetic difference between the sexes as endpoints. However, this hypothesis can be tested, refined and amended with additional branches to related free-living, (entomo)pathogenic and parasitic nematodes. In order to do so, on one hand additional species need to be characterized with respect to their life histories, chromosome numbers and sex determination. At first, these analyses can be relatively superficial and serve to identify new putative contemporary representatives of intermediate steps and of new endpoints of interest. These candidates can then be analysed more thoroughly. On the other hand, more detailed analysis of the 'model' *Strongyloides* and *Parastrongyloides* species, in particular of the mechanisms of chromosome elimination/chromatin diminution and male meiosis and spermatogenesis will provide additional hints. Eventually, one would like to gain insights into the molecular/genetic changes at the base of the evolutionary changes. Modern deep genome sequencing approaches will in the near future produce a wealth of information. However, they will only provide very limited new insight into the what, how and why of the evolution of nematodes unless they are paralleled and followed up by functional genetic studies and very old-fashioned analyses of the natural history, morphology, cytology and, very crucially, the phylogeny of the species in question. While sequencing and molecular phylogeny can these days be applied easily to many species, functional studies are more laborious and require a certain investment into the system in order to make it workable. Therefore these studies will have to be restricted to a relatively small number of appropriately selected species (cf. Sommer, 2009).

ACKNOWLEDGEMENTS

I thank Drs Matthias Herrmann, James Lightfoot and Ralf J. Sommer for critically reading the manuscript. Work in my laboratory was funded by the Max Planck Society.

REFERENCES

Albertson, D. G., Nwaorgu, O. C. and Sulston, J. E. (1979). Chromatin diminution and a chromosomal mechanism of sexual differentiation in *Strongyloides papillosus*. *Chromosoma* **75**, 75–87.

Anderson, R. C. (2000). *Nematode Parasites of Vertebrates. Their Development and Transmission*, 2nd Edn. CAB International, Wallingford, UK.

Augustine, D. L. (1940). Experimental studies on the validity of species in the genus *Strongyloides*. *American Journal of Hygiene* **32**, 24–32.

https://doi.org/10.1017/S003118201400064X Published online by Cambridge University Press

Baldi, C., Cho, S. and Ellis, R. E. (2009). Mutations in two independent pathways are sufficient to create hermaphroditic nematodes. *Science* **326**, 1002–1005.

Beach, T. D. (1936). Experimental studies on human and primate species of *Strongyloides* – V. The free-living phase of the life cycle. *American Journal of Hygiene* **23**, 243–277.

Beg, M. K. (1968). Studies on the life-cycle of *Strongyloides fulleborni* von Linstow, 1905. *Annals of Tropical Medicine and Parasitology* **62**, 502–505.

Blaxter, M., Koutsovoulos, G., Jones, M., Kumar, S. and Elsworth, B. (2014). Phylogenomics of nematoda. In *Systematics Association Special Volume on Next Generation Systematics* (ed. Cotton, J., Hughes, J. and Olsen, P.). Cambridge University Press, Cambridge, UK.

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T. and Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**, 71–75.

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

Chandler, C. H., Phillips, P. C. and Janzen, F. J. (2009). The evolution of sex-determining mechanisms: lessons from temperature-sensitive mutations in sex determination genes in *Caenorhabditis elegans*. *Journal of Evolutionary Biology* **22**, 192–200.

Chapman, T., Arnquist, G., Bangham, J. and Rowe, L. (2003). Sexual conflict. *Trends in Ecology and Evolution* **18**, 41–47.

Crook, M. (2014). The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong. *International Journal for Parasitology* **44**, 1–8. doi: 10.1016/j.ijpara.2013.08.004.

Denver, D. R., Clark, K. A. and Raboin, M. J. (2011). Reproductive mode evolution in nematodes: insights from molecular phylogenies and recently discovered species. *Molecular Phylogenetics and Evolution* **61**, 584–592.

Dieterich, C. and Sommer, R. J. (2009). How to become a parasite – lessons from the genomes of nematodes. *Trends in Genetics* **25**, 203–209.

Dorris, M., Viney, M. E. and Blaxter, M. L. (2002). Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal for Parasitology* **32**, 1507–1517.

Dubendorfer, A., Hediger, M., Burghardt, G. and Bopp, D. (2002). *Musca domestica*, a window on the evolution of sex-determining mechanisms in insects. *International Journal of Developmental Biology* **46**, 75–79.

Eberhardt, A. G., Mayer, W. E. and Streit, A. (2007). The free-living generation of the nematode *Strongyloides papillosus* undergoes sexual reproduction. *International Journal for Parasitology* **37**, 989–1000.

Eberhardt, A. G., Mayer, W. E., Bonfoh, B. and Streit, A. (2008). The Strongyloides (Nematoda) of sheep and the predominant Strongyloides of cattle form at least two different, genetically isolated populations. *Veterinary Parasitology*, **157**, 89–99. doi: 10.1016/j.vetpar.2008.07.019.

Engelstadter, J. (2008). Constraints on the evolution of asexual reproduction. *Bioessays*, **30**, 1138–1150.

Ferrari, F., Alekseyenko, A. A., Park, P. J. and Kuroda, M. I. (2014). Transcriptional control of a whole chromosome: emerging models for dosage compensation. *Nature Structural and Molecular Biology* **21**, 118–125. doi: 10.1038/nsmb.2763.

Grant, W. N., Skinner, S. J., Howes, J. N., Grant, K., Shuttleworth, G., Heath, D. D. and Shoemaker, C. B. (2006*a*). Heritable transgenesis of *Parastrongyloides trichosuri*: a nematode parasite of mammals. *International Journal for Parasitology* **36**, 475–483.

Grant, W. N., Stasiuk, S., Newton-Howes, J., Ralston, M., Bisset, S. A., Heath, D. D. and Shoemaker, C. B. (2006*b*). *Parastrongyloides trichosuri*, a nematode parasite of mammals that is uniquely suited to genetic analysis. *International Journal for Parasitology*, **36**, 453–466.

Haag, E. S. (2007). Why two sexes? Sex determination in multicellular organisms and protistan mating types. *Seminars in Cell and Developmental Biology* **18**, 348–349.

Haag, E. S. and Doty, A. V. (2005). Sex determination across evolution: connecting the dots. *PLoS Biology* **3**, e21.

Hamilton, W. D. (1967). Extraordinary sex ratios. A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. *Science* **156**, 477–488.

Hammond, M. P. and Robinson, R. D. (1994). Chromosome complement, gametogenesis, and development of *Strongyloides stercoralis*. *Journal of Parasitology* **80**, 689–695.

Hansen, E. L., Buecher, E. J. and Cryan, W. S. (1969). *Strongyloides fulleborni*: environmental factors and free-living generations. *Experimental Parasitology* **26**, 336–343.

Harvey, S. C. and Viney, M. E. (2001). Sex determination in the parasitic nematode *Strongyloides ratti*. *Genetics* **158**, 1527–1533.

Hasegawa, H., Hayashida, S., Ikeda, Y. and Sato, H. (2009). Hypervariable regions in 18S rDNA of *Strongyloides* spp. as markers for species-specific diagnosis. *Parasitology Research* **104**, 869–874.

Hediger, M., Henggeler, C., Meier, N., Perez, R., Saccone, G. and Bopp, D. (2010). Molecular characterization of the key switch F provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly. *Genetics* **184**, 155–170.

Herrmann, M., Mayer, W. E. and Sommer, R. J. (2006). Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. *Zoology (Jena)* **109**, 96–108.

Herrmann, M., Kienle, S., Rochat, J., Mayer, W. E. and Sommer, R. J. (2010). Haplotype diversity of the nematode *Pristionchus pacificus* on Réunion in the Indian Ocean suggests multiple independent invasions. *Biological Journal of the Linnean Society* **100**, 170–179.

Hodgkin, J. (1983). Male phenotypes and mating efficiency in *Caenorhabditis elegans*. *Genetics* **103**, 43–64.

Hodgkin, J. (2002). Exploring the envelope. Systematic alteration in the sex-determination system of the nematode *Caenorhabditis elegans*. *Genetics* **162**, 767–780.

Hodgkin, J., Horvitz, H. R. and Brenner, S. (1979). Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* **91**, 67–94.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J. and Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* **23**, 1792–1800.

Holterman, M., Rybarczyk, K., van den Elsen, S., van Megen, H., Mooyman, P., Santiago, R. P., Bongers, T., Bakker, J. and Helder, J. (2008). A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* **8**, 23–34. doi: 10.1111/j.1471–8286.2007.01963.x.

Kulkarni, A., Dyka, A., Nemetschke, L., Grant, W. N. and Streit, A. (2013). *Parastrongyloides trichosuri* suggests that XX/XO sex determination is ancestral in Strongyloididae (Nematoda). *Parasitology* **140**, 1822–1830. doi: 10.1017/S0031182013001315.

Lee, D. L. (2002). *The Biology of Nematodes*. Taylor & Francis, London, UK.

Lucchesi, J. C., Kelly, W. G. and Panning, B. (2005). Chromatin remodeling in dosage compensation. *Annual Review of Genetics* **39**, 615–651.

Mackerras, M. J. (1959). *Strongyloides* and *Parastrongyloides* (Nematoda: Rhabdiasoidea) in Australian marsupials. *Australian Journal of Zoology* **7**, 87–104.

Müller, F., Wicky, C., Spicher, A. and Tobler, H. (1991). New telomere formation after developmentally regulated chromosomal breakage during the process of chromatin diminution in *Ascaris lumbricoides*. *Cell* **67**, 815–822.

Nemetschke, L., Eberhardt, A. G., Hertzberg, H. and Streit, A. (2010*a*). Genetics, chromatin diminution, and sex chromosome evolution in the parasitic nematode genus *Strongyloides*. *Current Biology* **20**, 1687–1696.

Nemetschke, L., Eberhardt, A. G., Viney, M. E. and Streit, A. (2010*b*). A genetic map of the animal-parasitic nematode *Strongyloides ratti*. *Molecular and Biochemical Parasitology* **169**, 124–127.

Nigon, V. and Roman, E. (1952). Le déterminisme du sexe et le development cyclique de *Strongyloides ratti*. *Bulletin biologique de la France et de la Belgique* **86**, 404–448.

Nolan, T. J. and Schad, G. A. (1992). Cryopreservation of infective third-stage larvae of *Strongyloides ratti*. *Journal of the Helminthological Society of Washington* **59**, 135–140.

Nolan, T. J., Aikens, L. M. and Schad, G. A. (1988). Cryopreservation of first-stage and infective third-stage larvae of *Strongyloides stercoralis*. *Journal of Parasitology* **74**, 387–391.

Ogawa, A., Streit, A., Antebi, A. and Sommer, R. J. (2009). A conserved endocrine mechanism controls the formation of dauer and infective larvae in nematodes. *Current Biology* **19**, 67–71.

Ohta, T. (1996). The current significance and standing of neutral and neutral theories. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* **18**, 673–677; discussion 683. doi: 10.1002/bies.950180811.

Osche, G. (1962). Das Praeadaptationsphänomen und seine Bedeutung für die Evolution. *Zoologischer Anzeiger* **169**, 14–49.

Phillips, C. M. and Dernburg, A. F. (2006). A family of zinc-finger proteins is required for chromosome-specific pairing and synapsis during meiosis in *C. elegans*. *Developmental Cell* **11**, 817–829. doi: 10.1016/j.devcel.2006.09.020.

Pires-daSilva, A. (2007). Evolution of the control of sexual identity in nematodes. *Seminars in Cell and Developmental Biology* **18**, 362–370.

Poulin, R. (2007). *Evolutionary Ecology of Parasites*, 2nd Edn. Princeton University Press, Princeton, NJ, USA.

Poulin, R. and Randhawa, H. S. (2014). Evolution of parasitism along convergent lines: from ecology to genomics. *Parasitology*, in press. doi: 10.1017/S0031182013001674.

Riddle, D. L. and Albert, P. S. (1997). Genetic and environmental regulation of dauer larva development. In *C. Elegans II* (ed. Riddle, D. L., Blumenthal, T., Meyer, B. J. and Priess, J. R.), pp. 739–768. Cold Spring Harbor Laboratory Press, New York, NY, USA.

Sarre, S. D., Georges, A. and Quinn, A. (2004). The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. *Bioessays* **26**, 639–645.

Schaner, C. E. and Kelly, W. G. (2006). Germline chromatin (24 January 2006). In *WormBook*. doi: 10.1895/wormbook.1.73.1. www.wormbook.org.

Schön, I., Martens, K. and Van Dijk, P. J. (2009). *Lost Sex: The Evolutionary Biology of Parthenogensis*. Springer, Berlin, Germany.

Schwander, T., Vuilleumier, S., Dubman, J. and Crespi, B. J. (2010). Positive feedback in the transition from sexual reproduction to parthenogenesis. *Proceedings of the Royal Society B: Biological Sciences* **277**, 1435–1442.

Shakes, D. C., Neva, B. J., Huynh, H., Chaudhuri, J. and Pires-daSilva, A. (2011). Asymmetric spermatocyte division as a mechanism for controlling sex ratios. *Nature Communications* **2**, 157. doi: 10.1038/ncomms1160.

Shao, H., Li, X., Nolan, T. J., Massey, H. C., Jr., Pearce, E. J. and Lok, J. B. (2012). Transposon-mediated chromosomal integration of transgenes in the parasitic nematode *Strongyloides ratti* and establishment of stable transgenic lines. *PLoS Pathogens* **8**, e1002871. doi: 10.1371/journal.ppat.1002871.

Sommer, R. J. (2009). The future of evo-devo: model systems and evolutionary theory. *Nature Reviews Genetics* **10**, 416–422.

Sommer, R. J. and Ogawa, A. (2011). Hormone signaling and phenotypic plasticity in nematode development and evolution. *Current Biology* **21**, R758–766. doi: 10.1016/j.cub.2011.06.034.

Speare, R. (1989). Identification of species of Strongyloides. In *Strongyloidiasis: A Major Roundworm Infection of Man* (ed. Grove, D. I.), pp. 11–83. Taylor & Francis, London, UK.

Streit, A. (2008). Reproduction in *Strongyloides* (Nematoda): a life between sex and parthenogenesis. *Parasitology* **135**, 285–294.

Streit, A. (2012). Silencing by throwing away: a role for chromatin diminutioning. *Developmental Cell* **23**, 918–919.

Tobler, H. and Müller, F. (2001). Chromatin diminution. In *Encyclopedia of Life Sciences*, John Wiley & Sons, Chichester, UK. doi: 10.1038/npg.els.0001181. www.els.net/.

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

Valenzuela, N. (2008). Sexual development and the evolution of sex determination. *Sexual Development* **2**, 64–72.

Vicoso, B. and Bachtrog, D. (2013). Reversal of an ancient sex chromosome to an autosome in *Drosophila*. *Nature* **499**, 332–335. doi: 10.1038/nature12235.

Viney, M. E. (1999). Exploiting the life cycle of *Strongyloides ratti*. *Parasitology Today* **15**, 231–235.

Viney, M. E. (2006). The biology and genomics of Strongyloides. *Medical Microbiology and Immunology* **195**, 49–54.

Viney, M. E. and Lok, J. B. (2007). *Strongyloides* spp. (23 May 2007). In *WormBook*. doi: 10.1895/wormbook.1.141.1. www.wormbook.org.

Viney, M. E., Green, L. D., Brooks, J. A. and Grant, W. N. (2002). Chemical mutagenesis of the parasitic nematode *Strongyloides ratti* to isolate ivermectin resistant mutants. *International Journal for Parasitology* **32**, 1677–1682.

Wang, J., Mitreva, M., Berriman, M., Thorne, A., Magrini, V., Koutsovoulos, G., Kumar, S., Blaxter, M. L. and Davis, R. E. (2012). Silencing of germline-expressed genes by DNA elimination in somatic cells. *Developmental Cell* **23**, 1072–1080. doi: 10.1016/j.devcel.2012.09.020.

Wang, Z., Zhou, X. E., Motola, D. L., Gao, X., Suino-Powell, K., Conneely, A., Ogata, C., Sharma, K. K., Auchus, R. J., Lok, J. B., Hawdon, J. M., Kliewer, S. A., Xu, H. E. and Mangelsdorf, D. J. (2009). Identification of the nuclear receptor DAF-12 as a therapeutic target in parasitic nematodes. *Proceedings of the National Academy of Sciences USA* **106**, 9138–9143.

Wilkins, A. S. (1995). Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway. *Bioessays* **17**, 71–77.

Yamada, M., Matsuda, S., Nakazawa, M. and Arizono, N. (1991). Species-specific differences in heterogonic development of serially transferred free-living generations of *Strongyloides planiceps* and *Strongyloides stercoralis*. *Journal of Parasitology* **77**, 592–594.