Dispatches

Sex Determination: A Worm Does It by Elimination

Parasitic nematode worms of the genus *Strongyloides* have an alternation of many asexual, all-female generations with a sexual generation composed of males and females. Males of *S. papillosus* have now been shown to be produced by elimination of chromosomal material that constitutes the X chromosome in its close relatives.

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The amazing variety of different sex determination systems among animals and plants presents a large field for investigation by both developmental and evolutionary geneticists [1–3]. The advent of methods for generating dense genetic maps in non-model organisms by the use of naturally occurring molecular markers, combined with the use of information from their (often distant) relatives with complete genome sequences, is leading to rapid advances in this area [4]. This will increase our knowledge of both the nature of the evolutionary transitions between different modes of sex determination, and the genes responsible for the primary decisions concerning sexual identity in different systems.

Some systems of sex determination, however, almost defy understanding in both evolutionary and genetic terms, notably the rodent species in which both males and females are apparently X0 [3,5]. One of the most interesting cases of such a baffling sex chromosome system is that of the nematode *Strongyloides papillosus*, a parasite of sheep. The cytogenetics of this system has recently been elucidated by the work of Linda Nemetschke and colleagues [6], as reported in this issue of *Current Biology*. Species of *Strongyloides* live in the intestines of their host as females. They all seem to have environmental sex determination (ESD), in which free-living males and females are produced when the immune response of the host increases sufficiently, in response to an unknown signal. These reproduce sexually, giving rise to female larvae that can reinfect the host, and then reproduce asexually, producing successive generations of females.

In itself, this is not especially puzzling, as ESD is a well-known mechanism of sex determination [1]. But in some *Strongyloides* species, such as the rat and human parasites *S. ratti* and *S. stercoralis*, there is a sex chromosome system such that females are XX and males are X0 [7]. This is a common mode of sex determination in nematodes, and may well be basal to the phylum [1,7]. Males are formed as a result of the loss of an X chromosome early in life, and the offspring of males and sexual females are all XX, with one paternal and maternal X chromosome, so that sperm that lack X chromosomes are either lacking, dysfunctional, or produce offspring that fail to develop [7]. This type of chromosomal sex determination is likely to mean that sex is determined by the ratio of the number of X chromosomes to the autosome (X/autosome balance), a mechanism first discovered in *Drosophila* [8]; the molecular basis for this has been worked out in great detail in the nematode *Caenorhabditis elegans* [9].

But males and females of *S. papillosus* do not have an obvious genetic difference that is related to the presence or absence of an X chromosome. Instead, females have two pairs of chromosomes, one pair (L) being much longer than the other (M) [10]. Classical cytogenetic observations suggest that an internal portion of one of the L chromosomes is eliminated in the (asexually generated) oocytes that are destined to develop as males [11]. As a result, males have one large, two medium and one small chromosome (i.e., their karyotype is 1L 2M 1S, whereas the females are 2L 2M; Figure 1).

These observations have generated the hypothesis that the L chromosome corresponds to a fusion between the X chromosome present in species such as *S. ratti* and an autosome [10], and that the material that is eliminated to produce males is part or whole of this ancestral X chromosome [6,11], as shown in Figure 1. X-chromosome–autosome fusions are well-known evolutionary events [12], and the X chromosome of eutherian mammals is a fusion between the ancestral X, shared with marsupials, and an autosome [3]. Furthermore, elimination of chromosomal material during embryonic development (diminution) has long been known to occur in some other parasitic nematodes, such as *Ascaris* [12]. This hypothesis thus has some intrinsic plausibility in the light of the properties of nematode genomes.

Nemetschke *et al.* [6] have performed a set of elegant genetic experiments that confirm this hypothesis and add many details to our understanding of this unique system of sex determination. First, they used an expressed-sequence tag (EST) library from *S. papillosus* to identify 65 ESTs with clear homology to counterparts in the *S. ratti* genome sequence, and bridged gaps using inverse PCR. The results gave evidence for extensive synteny, with only six cases of failure to bridge gaps between sequences that are neighbours in *S. ratti.* They could thus identify *S. papillosus* genes that were distributed over all three chromosomes (X, I, and II) of *S. ratti*. Twenty-two molecular markers with variants within *S. papillosus* were developed from among this set and used to genotype males and females. Out of nine loci that are X-linked in *S. ratti*, eight were always hemizygous in males but could be found as heterozygotes in females, whereas none of the loci that are autosomal in *S. ratti* were always hemizygous in males. This suggests strongly that the majority of ancestrally X-linked loci are eliminated from the genomes of individuals destined to be males.

They followed this up by genotyping individual females isolated from parasitised rabbits, which they allowed to reproduce outside the host. Only a small fraction of progeny survived long enough for their sex to be

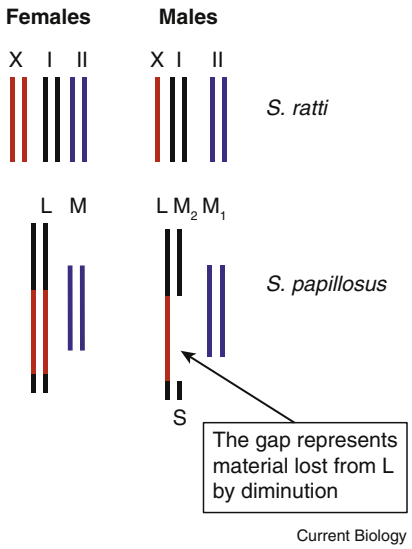

Figure 1. The chromosomes of *S. ratti* (top) and *S. papillosus* (bottom).

In *S. ratti*, females have two X chromosomes, whereas males have only one. There are two pairs of autosomes (chromosomes I and II) in both sexes. In *S. papillosus*, females have two chromosomes, L and M. The L chromosome contains a section homologous to the X of *S. ratti* (red), inserted into material homologous to chromosome I of *S. ratti* (black). Chromosome M (blue) appears to be largely homologous to chromosome II of *S. ratti*. In oocytes that give rise to males by a mitotic division, the X-homologous material is eliminated from one of the two L chromosomes. Males thus receive a haploid complement of an L and two M chromosomes (M_1) equivalent to those of females, as well as a small (S) chromosome and another medium-sized chromosome (M_2) derived from the diminution of the other L chromosome.

determined, but they were able to show that all surviving larvae were genetically identical to their mother with respect to at least one heterozygous locus that was classified as immune to diminution in the previous experiment. But for loci that were inferred to be subject to diminution, all 5 females that they scored were identical in genotype to the mother, whereas the 25 males were hemizygous, apparently randomly receiving one of the two alleles from a heterozygous locus in their mother.

Analysis of the crosses showed that loci that are located on chromosome I in *S. ratti* are genetically linked in *S. papillosus* and are divided into two groups that surround the loci that are subject to diminution. This entire linkage group must therefore constitute chromosome L. The arrangement of the marker loci suggests that this was created either by an insertion of the ancestral X into the homologue of the *S. ratti* chromosome I, or a fusion followed by a chromosome rearrangement (Figure 1). Furthermore, all markers that correspond to chromosome II in *S. ratti* are on another, independent linkage group in *S. papillosus*, presumably representing chromosome M. The one marker that is X-linked in *S. Ratti*, but is not subject to diminution, maps to this linkage group in *S. papillosus*, implying a transposition between chromosomes at some point in the evolutionary history of these two species.

A further set of experiments shed light on the paradox that the males and sexual females produce all-female offspring. Males heterozygous at loci immune to diminution tend to transmit an excess of one of their two alleles at these loci to their progeny. The tighter the linkage of a locus to the eliminated group of genes, the higher the bias, with complete bias in the case of loci that are very closely linked to the eliminated loci (which show little or no recombination among themselves in females). Furthermore, the alleles in deficiency are located on the L chromosome that was broken up in the father. Genotyping of individual sperm showed that the bias must be caused by exclusion of alleles before or during sperm development, since the excluded alleles are missing from sperm. These results imply that only sperm that carry an intact L chromosome are functional, and that the transmission bias reflects the loss of L chromosome markers located on the chromosome that is broken up.

These fascinating observations raise the question of why this bizarre system has evolved. It is straightforward to see why a system of asexual propagation of females would be favoured in a parasite, whose lifestyle means that it would be common for a host to be infected by one or a very small number of individuals, so that males available for fertilising females might often be absent. This is the principle of 'reproductive assurance', which is known to play an important role in modulating transitions from outcrossing sexual systems to self-fertilising or asexual derived systems [13,14]. It is also in principle straightforward to see why the production of sexual males and females should be favoured when the parasite is under stress by the immune system, since a variety of population genetic processes can favour sexual reproduction more strongly in situations where selection is unusually intense; indeed, species with an alternation of sexual and asexual generations generally reproduce sexually at times of environmental stress [15,16]. One can thus conjecture that, in the ancestors of the species we have been discussing, selection favoured a process in which loss of an X chromosome promotes male development, presumably by an X/autosome balance pathway of the type that operates in *C. elegans*. X0 males will be viable despite the lack of an entire large chromosome if an X chromosome dosage compensation mechanism operates, as in *C. elegans* [17].

But why should sexual males and females produce only female offspring, presumably as a result of the failure of sperm lacking the X chromosome to develop? The answer may be that males from these crosses are of no reproductive value, since they cannot fertilise the asexual females. Selection would favour a mechanism that avoids the production of males, since a higher number of successful offspring are then produced. Alternatively, there might be a form of X-linked segregation distortion, in which a gene or gene complex on the X chromosome destroys developing sperm that lack an X chromosome, as has been found in many *Drosophila* species [18].

This scenario may explain what is seen in *S. ratti,* and the *S. papillosus* system can be understood as its evolutionary derivative. The X–autosome fusion that generated the L chromosome must have been established either by selection or genetic drift, as has happened in many other groups of organisms [12]. In the absence of elimination of the portion of the L corresponding to the X, no males would be produced. Elimination of the entire L chromosome would presumably be lethal, since autosomal genes are not compensated for the lack of a large portion of the genome in the same way as happens with the X chromosome. Either the diminution mechanism was retained as a carryover from a segregation

distortion system present in the ancestor, or it was evolved *de novo* to allow the production of males, which would be favoured by selection when sexual reproduction is advantageous. It would be interesting to determine the mechanism by which *S. ratti* produce all-female offspring from matings between males and females, as this scenario predicts that sperm that lack an X should be dysfunctional or lacking, just as in *S. papillosus*, perhaps as a result of chromatin diminution. In addition, we would expect to see evidence for dosage compensation of X-linked genes in males, possibly using the same mechanisms as in *C. elegans* [17].

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DOI: 10.1016/j.cub.2010.08.032

Plant Development: Early Events in Lateral Root Initiation

How are the lateral root founder cells specified in the pericycle to initiate lateral root development? An Aux/IAA28 signaling module activates transcription factor *GATA23* to control founder cell identity.

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Higher plants have a branched root system that anchors them in the soil, allowing the uptake of essential nutrients and water. This root system consists of the primary root, which exhibits several branching mechanisms, including the formation of lateral roots. In *Arabidopsis,* lateral roots originate after embryogenesis from the root pericycle layer and emerge in the differentiation zone [1]. The pericycle layer consists of quiescent cells at the phloem pole and cells competent to initiate cell division at the xylem pole [2]. Genetic analysis has shown that the pericycle heterogeneity and diarch vascular organization are set up early in the root meristem and are regulated by the same genetic pathway [3,4]. Primary root growth is driven by a group of stem cells at the root apex. New daughter cells are continuously produced and displaced further away from the root tip. Therefore, there is a chronology of cells where the youngest occupy the meristematic zone and older cells pass through the elongation zone where they attain their final size before they differentiate at the differentiation zone [5]. Although the earliest cellular events in lateral root initiation are only detected several millimeters distal to the root meristem [2,6], the decision by xylem pole pericycle (XPP) cells to develop lateral roots is taken in the 'basal meristem', the region at the transition between the root meristem and the elongation zone (Figures 1A,B) [7,8].

The role of the phytohormone auxin as an important factor controlling lateral root development is well established. The Aux/IAA family of auxin signaling inhibitors represses the activity of a group of transcription factors called auxin response factors (ARFs), which initiate transcription of auxin-responsive genes. Auxin regulation is achieved by rapidly modulating levels of Aux/IAAs throughout development. Auxin binds to the F-box protein TIR1, which forms part of the SCF^{TIR1} ubiquitin ligase complex. When bound to auxin, the SCF^{TIR1} complex targets Aux/IAA proteins for proteolytic degradation, which releases the ARFs from Aux/IAA-mediated repression [9]. In *Arabidopsis*, Aux/IAA and ARF proteins are represented by large gene families and specific responses between co-expressed ARFs and IAAs can mediate different developmental responses [10,11].

During lateral root development auxin functions through successively acting regulatory modules: the SOLITARY ROOT (SLR/IAA14)–AUXIN REPONSE FACTOR (ARF7–ARF19)–LATERAL ORGAN BOUNDERIES DOMAINS (LBD/ASL) module regulates the division of XPP cells during lateral root initiation and the successive BODENLOS (BDL/IAA12)–ARF5 module regulates lateral root organogenesis (Figure 1B) [12–14]. Which factor(s) decides the founder cell