

Quick guide

Ascaris

Jianbin Wang^{1,2,3}
and Richard E. Davis^{1,2,*}

What is *Ascaris*? *Ascaris* is a parasitic nematode (roundworm) of the small intestine. *Ascaris* is estimated to infect upward of 800,000 people, and ascariasis (infection with *Ascaris*) is a neglected disease of poverty. Light infections may be asymptomatic, but moderate to heavy infections with these large worms (mature females ~30 x 0.5 x 0.5 cm; Figure 1A,B) can lead to abdominal symptoms and even lethal obstruction of the small intestine. In young children, infection can lead to malnutrition, growth retardation and inhibition of cognitive development. Two main species of *Ascaris*, *Ascaris suum* and *Ascaris lumbricoides*, have historically been recognized and thought to infect pigs and humans, respectively; however, they are known to cross-infect. Recent genomic and other studies suggest the two may not be distinct species. Relatives of *Ascaris* include an important parasite of horses (for example, *Parascaris univalens*; originally known as *Ascaris megaloccephala*) and parasites of dogs and cats (*Toxocara*

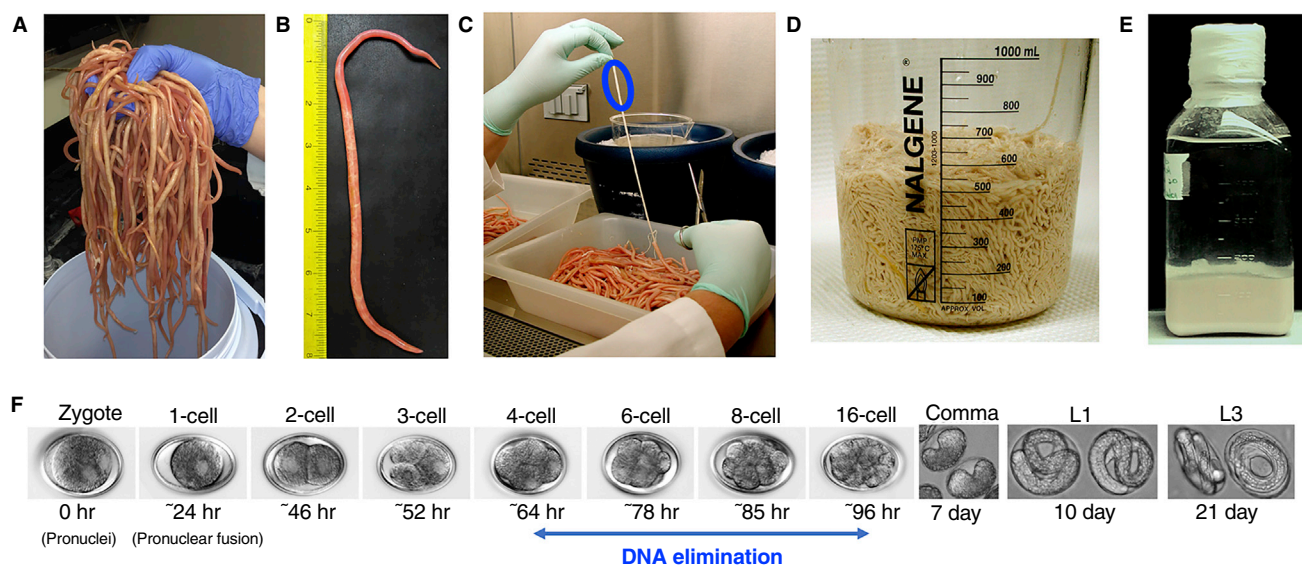
spp.) whose larval stages are pathogenic in humans. These nematodes are commonly known as ascarids and are members of Clade III, one of the five defined clades of nematodes. Nematodes represent a highly divergent phylum, including the well-known nematode model *Caenorhabditis elegans* (Clade I). *C. elegans* and *Ascaris* are estimated to have diverged from each other over 400 million years ago.

Ascaris is sexually dimorphic (Figure 2) and undergoes sexual reproduction in the small intestine of its host, producing large numbers of eggs. The eggs do not undergo cellular development until they leave the host in the feces. In the soil or water, infective larvae (L3) develop within the eggs. In spite of periodic mass drug treatments, continued re-infections readily occur in endemic areas as *Ascaris* eggs can remain viable for many years. When the infective eggs are ingested by humans in contaminated water, food, or other sources, the larvae hatch in the small intestine, invade the intestinal mucosa, and then enter the vasculature. The larvae are carried to the liver by the portal circulation and then by the vascular system to the lungs where they further mature (10 to 14 days). In the capillaries of the lungs, the larvae penetrate the alveolar walls, and then migrate up the bronchial tree to the throat where they are then

swallowed. Heavy human infections can lead to symptoms similar to pneumonia (Loeffler's syndrome). Once back in the small intestine the larvae develop into the large, sexually reproducing adults. Two to three months are required from ingestion of infective eggs to oviposition of females. Adult worms live 1 to 2 years in the human host.

Why is *Ascaris* considered a reproductive machine? *Ascaris* females are estimated to release 200,000 to 1,000,000 eggs per day and the female uterus (~20–25 cm in length) may hold up to 27,000,000 eggs. Following fertilization in the seminal receptacle (Figure 2C), a chitinous shell and an impermeable inner membrane are synthesized surrounding the zygote. The male and female pronuclei do not fuse and remain separate as the zygote undergoes an important maturation as it traverses the uterus over an ~18 hr period (Figure 2C,F; zygotes 1–4). Following release of the eggs from the uterus and their passage out of the host, the environmental change (oxygen, redox potential, and other factors) activates the eggs leading to pronuclear fusion and their subsequent slow and synchronous development that is temperature and moisture dependent.

To obtain *Ascaris* eggs for analysis of their developmental stages,

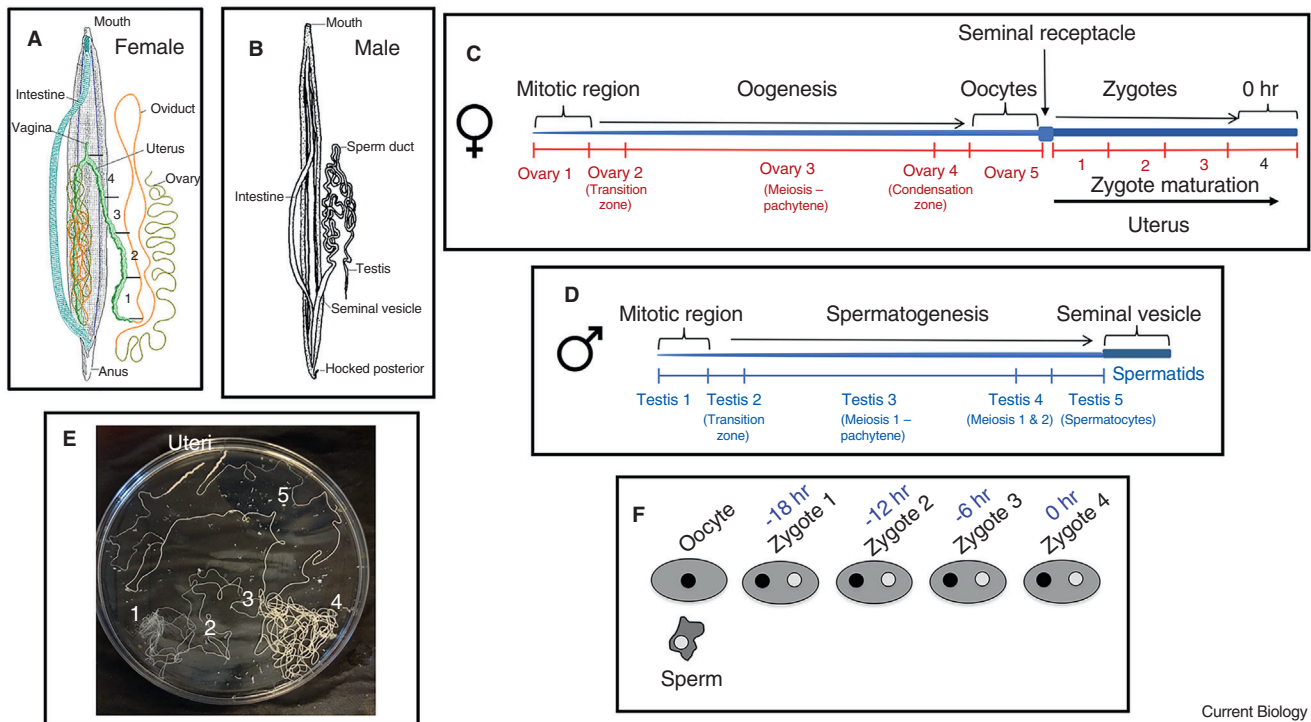


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Figure 1. Adult *Ascaris*, egg isolation, and development within the egg.

(A) *Ascaris* collected from pigs. (B) Female *Ascaris*. (C) *Ascaris* uterus dissected from a female. The blue circle illustrates the terminal 3–6 cm of the uterus collected for isolation of mature eggs. (D) Collection of *Ascaris* uteri. (E) *Ascaris* eggs following their digestion from uteri, isolation, and cleaning for storage. (F) *Ascaris* slow and synchronous development within the egg at 30°C *in vitro*.





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Figure 2. *Ascaris* male and female, reproductive systems, and zygote maturation within the uterus.

(A,B) Anatomy of female and male *Ascaris*, respectively. Adult females and males (with a curved posterior) are on average 30 and 15 cm in length, respectively. Note the long, extended reproductive systems. In the female, one of the two uteri and lobes of the reproductive system are extended for illustration. The numbers 1–4 mark the four regions of the uterus where zygotes prior to pronuclear fusion are undergoing maturation and can be isolated (see also C,F). (C) Illustration of the female reproductive system (~2 meters in length extended; not to scale for the region of uterus) showing the different regions of the ovary and where oocytes can be isolated, the seminal receptacle (where sperm is stored and where fertilization is initiated), and the uterus containing zygotes at different stages of maturation (zygotes 1–4) as they move through the uterus (see also F). (D) Illustration of the male reproduction system (~1 meter in length extended). About 20–40 μ l of packed spermatids can be isolated from a single seminal vesicle. (E) Isolated female reproductive system illustrating the length in a 150 mm petri dish. The uterus has been largely removed (a short, thickened region remains; see Figure 1C for uterus). (F) Illustration of *Ascaris* oocytes, amoeboid sperm, and zygote maturation and time to traverse the uterus. Zygote 4 (0 hr) is the oviposited form which leaves the host in feces.

adult females are collected at a pig slaughterhouse, dissected, and the mature eggs (zygote 4 or 0 hr) are harvested from the terminal quarter of the uterus (Figure 1C). The collected uteri are digested in 0.5 N NaOH to remove the uterine tissue and the remaining eggs washed extensively in water and then stored at pH 2.0 at 4°C (Figure 1E). From 500 females, ~25 ml of eggs (~250,000,000 eggs) can be obtained. The eggs can be stored at 4°C for many years. Incubation of the eggs at 30°C in liquid with gentle shaking leads to relatively synchronous development. Pronuclear fusion occurs after ~24 hr and development to a motile L1 larvae within the egg occurs in 10 days (Figure 1F). Note that eggs collected from distal stages of the uteri (zygotes 1–3) have not undergone the requisite maturation and thus do not develop well. The early *Ascaris*

cell cycles are ~15 hr in length. Large quantities of specific developmental stages can be readily obtained (Figure 1F). For comparison, *C. elegans* development initiates immediately after fertilization and the worms lay one egg at a time with ~60 eggs/24 hr under typical laboratory growth conditions.

What is unique about *Ascaris* early development? Theodore Boveri spent many years observing early *Ascaris* development and in 1910 described the early development and cell lineages that derive from the first 16 cells. The early cell lineages Boveri described in *Ascaris* were subsequently found to be identical in the nematode *C. elegans*. Further characterization of these lineages was meticulously defined by John Sulston in his seminal lineage studies of *C. elegans* published in 1983. Early development of *C. elegans* is driven largely from the

deposition of maternal mRNAs/proteins into oocytes and their regulated, post-transcriptional expression following fertilization. Immediately following fertilization in *Ascaris*, the maternal to zygotic transition is initiated with the decay of many mRNAs and the onset of transcription of over 2,000 genes. This represents one of earliest examples of the onset of zygotic transcription in development and one of the only examples where transcription in early development occurs prior to pronuclei fusion. The long early cell cycles (~15 hours, compared to ~15 minutes in *C. elegans*) and maturation of the zygote in the uterus enables early *Ascaris* development to be driven by transcription. In general, the same set of genes that are used post-transcriptionally during *C. elegans* early development are also used in *Ascaris*, but are produced by transcription as

needed. Thus, conserved and key groups of genes required for early development in these two nematodes have been rewired to be regulated either by transcriptional or post-transcriptional mechanisms to carry out the same developmental program. This represents a novel example of divergent gene regulatory mechanisms that result in similar gene expression patterns.

What research tools are available in

Ascaris? An *Ascaris* genome with 24 assembled chromosomes is available. Extensive RNA-seq are available for different tissues of male and female adult *Ascaris*, regions of the ovary and testes, stages of development, and larval stages (Figures 1F and 2). In addition, extensive small RNA transcriptome data and histone ChIP-seq analysis are available for *Ascaris*. Thus, comprehensive genome, transcriptome, and epigenome resources are available for *Ascaris* (see https://parasite.wormbase.org/Ascaris_suum_prjna62057/Info/Index).

A variety of molecular tools are also available in *Ascaris*. One of the most significant is the ability to make cell-free extracts from early embryos which have been used for analysis of *in vitro* transcription, cis- and trans-splicing, and mRNA translation and decay. A distinct advantage of the *Ascaris* system is that large numbers of embryos can be obtained and developed synchronously to specific stages for extract preparation. Methods for introduction of DNA and RNA using particle bombardment (biolistics) are available for embryos and have been primarily used (as have the cell-free extracts) to study the molecular details of spliced leader RNA trans-splicing. In addition, methods for RNAi are available in embryos, larvae and adults. The large size of *Ascaris* and the ability to dissect specific tissues and regions of the reproductive systems (Figure 2) have facilitated a variety of studies.

What are *Ascaris*' major scientific contributions? *Ascaris* and its relatives have been the subject of key scientific investigations since the late 19th century. Edouard van Beneden identified and used the ascarid of horses, *Parascaris*, since he could obtain and observe stages of gametogenesis in the long reproductive organs, large sperm and

oocytes, clear eggs, synchronous and slow early development, and the very large chromosomes (genome size of ~2.5 Gb in *P. univalens* with 2N = 2). van Beneden's key studies on *Parascaris* described the processes of gametogenesis, including the reductive divisions of meiosis, and re-establishment of the diploid number following the fusion of the egg and sperm. He was also the first to describe the constancy of chromosomes in cells and within a species using *Parascaris*. Theodor Boveri found the attributes of *Parascaris* chromosomes from van Beneden's studies to be an exceptional model and used the *Parascaris* system to develop the theory of chromosome inheritance, the chromosome cycle, and the key role of centrosomes in chromosome segregation. The chromosomal basis of sex determination was also proposed by Boveri. These were fundamental observations that changed our view of chromosomes and inheritance and provided the foundation for modern genetic and chromosome studies.

Boveri, using *Parascaris univalens*, made the surprising discovery that in early somatic cells of the developing embryos the single, large chromosome underwent numerous DNA breaks and DNA fragments were eliminated. Large chromosome fragments were not segregated at anaphase of mitosis; they localized to the cytoplasm following cell division, and eventually were degraded and lost. The remaining retained chromosome fragments formed 29 new autosomes and 6 sex chromosomes. Chromosome breaks and subsequent retention and loss of chromosome fragments occurred only in somatic cells, resulting in distinct somatic and germline genomes, with a reduced genome in somatic cell lineages. More recent studies indicate that 90% of the *Parascaris* germline genome is eliminated in somatic cells, including the loss of repetitive sequences and 1,000 genes. DNA elimination occurs in all ascarids. Programmed DNA elimination or programmed genome rearrangements have subsequently been identified in a broad range of organisms including ciliates and some other invertebrates (insects, mites, and copepod crustaceans), elasmobranchs, lampreys/hagfish, and in vertebrates. The function(s) and mechanism(s) of this

novel process remain the focus of many current studies in *Ascaris* and other organisms.

The large size of *Ascaris* has also enabled key studies on the anatomy and functional aspects of the nematode nervous system. Early studies on energy metabolism under anaerobic or hypoxic conditions and the discovery of a metazoan glyoxylate cycle were carried out in *Ascaris*. The ability to obtain large numbers of spermatids and their subsequent *in vitro* activation led to a variety of studies that provided key insights into the cell biology of amoeboid sperm in nematodes.

What does the future hold for research on *Ascaris*?

While *Ascaris* and its relatives are best known as parasites that lead to morbidity in humans and domestic animals, studies on these parasites have provided key scientific insights into a variety of biological processes. The large size of *Ascaris*, ability to dissect specific tissues and regions of the male or female reproductive tissues, ability to obtain large numbers of eggs and develop them to discrete stages, and a variety of molecular tools and genomic and transcriptomic resources are available in *Ascaris*. The biological attributes of *Ascaris* and molecular tools have provided and continue to provide a system for fundamental and interesting studies.

Where can I find out more?

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¹Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO 80045, USA. ²RNA Bioscience Initiative, University of Colorado School of Medicine, Aurora, CO 80045, USA. ³Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996, USA.

*E-mail: Richard.davis@cuanschutz.edu