



The Parasite's Way of Life

Only kings, presidents, editors, and people with tapeworms have the right to use the editorial “we.”

MARK TWAIN

CIRCADIAN RHYTHMS ARE PHYSIOLOGICAL OSCILLATIONS THAT OCCUR ON A ROUGHLY 24 HOUR CYCLE. A familiar example is the sleep/wake cycle observed in animals. These cycles are primarily driven by the rhythmic binding of transcription factors to the promoters of specific genes, resulting in a predictable pattern of gene expression and repression. Circadian rhythms are common in all living things, including bacteria, plants and animals. And parasites are certainly no exception. Consider, for instance, flatworms in the genus *Schistosoma*, which reproduce asexually in specific species of freshwater snails. Asexually-produced larvae ultimately give rise to motile, swimming stages called **cercariae** (singular: cercaria), which seek out a mammal serving as the next host for the parasite. In the case of one species, *S. bovis*, that host is a cow or other ungulate. In *S. rodhaini*, alternatively, rodents fill this role. *S. bovis* cercariae emerge in the morning, coinciding with the time when ungulates are most likely to visit a water source. Rats and other rodents, on the other hand, are nocturnal. *S. rodhaini* cercariae capitalize on this behavior with peak emergence shortly before dawn or soon after dusk.

The selective advantage of these different circadian rhythms for schistosomes is obvious; cercarial emergence is timed to increase the likelihood that the parasite will encounter its next host. But finding an appropriate host, while essential, is only the beginning: the first of many obstacles that any parasite must surmount if it is to ultimately survive and reproduce. Once it has arrived and gained entry, the parasite must navigate the labyrinth of the host's anatomy, locate its required site within the host, find a mate and possibly outcompete other parasites, all while coping with often hostile conditions as well as active host immune defenses.

The manner in which a given parasite species overcomes these hurdles is reflected in its life cycle. In this chapter, we provide an overview of basic life cycle patterns. In doing so, we will consider some of the colorful history of life cycle elucidation. We will then tease apart the various components of the life cycle, considering it as a series of problems that a parasite must solve. The astonishing diversity that we observe among parasitic organisms, described in Chapter 2, is in many ways a consequence of the ingenious, devious and sometimes perplexing solutions, that different parasitic taxa have evolved.

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Species names highlighted in red are included in the Rogues' Gallery, starting on page 505.

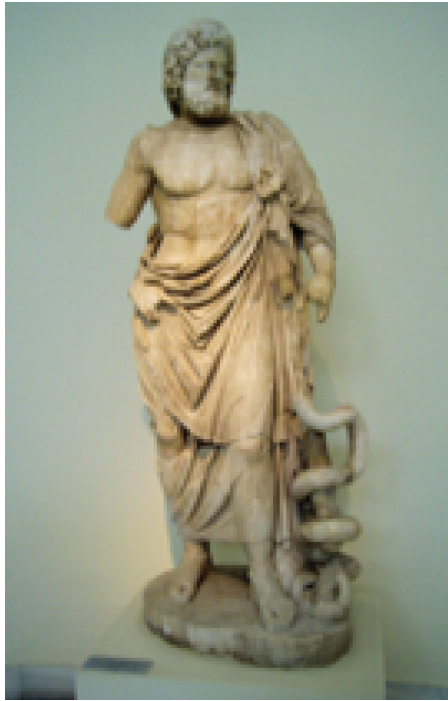


Figure 3.1 A historical reference to *Dracunculus medinensis*, the Guinea worm. Asclepius, the Greek god of medicine and healing, holds his staff (the staff of Asclepius). The snake-entwined staff is believed to come from the age-old practice of guinea worm removal, in which the long female worm is slowly wrapped on a stick. The staff of Asclepius (or sometimes the staff of Caduceus, entwined with two snakes) has come to symbolize modern medicine. (Courtesy of Her Dax, published under CC BY-SA 3.0.)

For an interesting look at the parasites that infected ancient humans go to <https://www.sciencehistory.org/distillations/the-parasites-in-our-past>

3.1 A HISTORICAL PERSPECTIVE OF THE PARASITE LIFE CYCLE

A particular parasite's **life cycle** is one of its most characteristic biological features. The life cycle can be defined as the sequence of developmental events that occurs over the course of an organism's lifetime. For parasites, at least some of these events occur in or on the host. Many parasites require different hosts for different developmental events. In such cases, these hosts are colonized in a specific sequence.

As we will see, given the way parasite life cycles are usually depicted, completion of the life cycle might involve progeny of the initiating parasite and involvement of another individual as a mate.

Early medical and natural history studies gave rise to an understanding of parasite life cycles

Because they are relatively large, certain helminths were the earliest infectious agents recognized in humans and animals (**Figure 3.1**). Ancient Egyptian medical chronicles, including the Ebers papyrus dated at about 1500 BC, describe nematodes, possibly *Ascaris* and the hookworm *Ancylostoma duodenale*, that commonly infected humans. Around 400 BC, Hippocrates described seed-like structures in human feces that were most likely tapeworm reproductive segments. In one of the earliest discussions of habitat site selection in the host, in approximately 180 AD, the Greek physician Galen described nematodes in the human intestine that inhabited the distal end of the colon near the anus (probably pinworms). Other larger worms preferred the upper portion of the small intestine (probably *Ascaris*).

The fall of Rome, starting in approximately 400 AD, ushered in the Dark Ages, during which parasitology, like other sciences, largely regressed. Although Chinese and Arabic texts from the Middle Ages contain some references to helminths, real advancement in our understanding of parasites would not occur until the Early Modern period of the Renaissance. In the 15th century, for instance, Francesco Redi described dozens of parasites from a variety of animal hosts (**Figure 3.2**).

In 1735, Carl Linnaeus published his *Systema Naturae*. In it he described various parasitic helminths, including pinworms, guinea worm, liver flukes and the pork tapeworm. Yet even as the list of worms known to inhabit animals steadily grew, there was little or no understanding of the complex life cycles or developmental changes that are typical of many parasites.

In the 1830s, a Danish biologist, Johann Steenstrup, suggested that all the various organisms that he found inhabiting freshwater snails and sheep livers might be different developmental stages of the same organism that somehow moved from sheep to snails and back to sheep. These stages are now recognized as the sporocysts, rediae, cercariae and adults of the liver fluke *Fasciola hepatica*. Steenstrup also suspected that the cyst-like structures that were commonly found in pork were a developmental stage of a parasitic worm of some sort.

Acting on Steenstrup's hunch, a German physician, Friedrich Kuchenmeister, decided to investigate. He had already observed that these cysts, which he called bladder worms, looked something like small tapeworms. Furthermore, similar bladder worms were typically found in pigs and other animals like rodents that might be described as prey. Tapeworms, on the other hand, were usually seen in dogs, cats and other predators. Might the

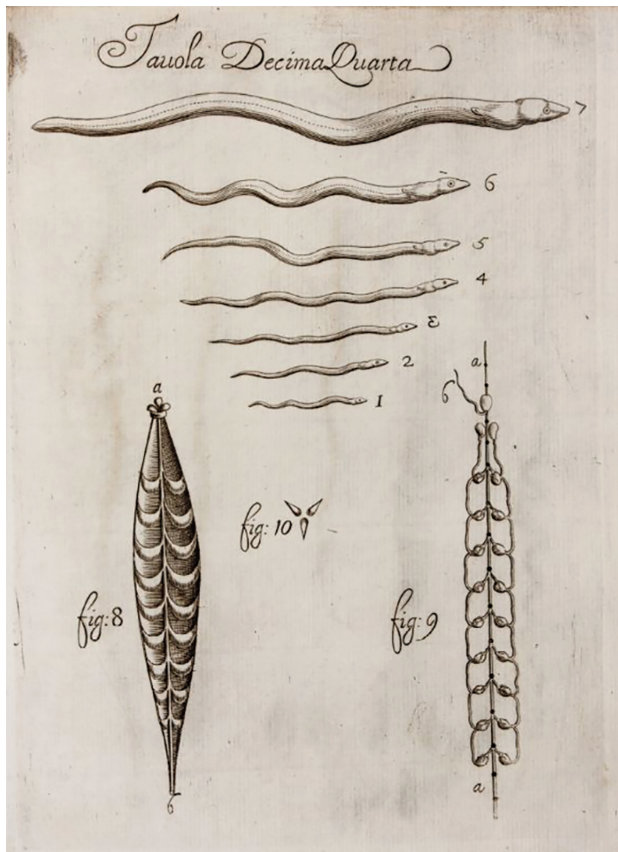


Figure 3.2 An early description of parasites. In his 1684 book, *Osservazioni intorno agli animali viventi che si trovano negli animali viventi* (*Observations on Living Animals found in Living Animals*), Francesco Redi described over 100 animal parasites, including the *Ascaris* nematodes infecting humans. Because of his early contributions, Redi has been called “the father of modern parasitology.”

bladder worms be immature tapeworms that develop into adults when a predator eats an infected prey animal? In 1851, he decided to find out by feeding bladder worms extracted from rabbit muscle tissue to foxes. After he found adult tapeworms in the foxes, he conducted other experiments in which he fed tapeworm segments released in the feces of a dog to a sheep. When he dissected the sheep several weeks later, Kuchenmeister found cysts in the animal’s brain. Finally, he showed that his human patients, whom he knew were often infected with tapeworms called *Taenia solium*, became infected after eating pork that contained cysts. In a particularly illustrative (some might say gruesome) experiment, Kuchenmeister obtained cysts from raw pork. He then mixed these cysts into food that was destined to be eaten by a condemned murderer. Several days later the prisoner was executed. When Kuchenmeister inspected the dead man’s intestine, he found young adult *T. solium*.

As the idea that specific parasites could appear radically different at different times of their development gained acceptance, other insights into parasite transmission followed, setting the stage for the elucidation of complete parasite life cycles. In 1860, Friedrich Zenker showed that humans also became infected with *Trichinella spiralis* when they consumed infected, undercooked pork. Shortly thereafter, the Italian Giovanni Grassi demonstrated that transmission could occur for some parasites via the ingestion of eggs. Specifically, he consumed *Ascaris lumbricoides* eggs and later found eggs in his own feces.

Such self-infection was instrumental in demonstrating that certain other helminth parasites could gain access to their vertebrate host by a different route—directly through the skin. Since 1845, for example, hookworms have been recognized as a causative agent of human disease. The

manner by which they infect humans, however, was not discovered until 1896. When the German Arthur Looss attempted to infect guinea pigs by feeding them hookworm larvae, he accidentally spilled some of these larvae onto his hand. When his skin became inflamed, Looss speculated that the hookworm larvae had penetrated directly through the skin on his hand. A few weeks later, he found hookworm eggs in his feces. He described the species as *Ancylostoma duodenale*. Looss evidently thought highly of his self-infection technique. Fresh from his discovery regarding hookworms, Looss purposely put *Strongyloides stercoralis* on his skin. Seven weeks later he found larvae in his feces, demonstrating that this nematode also gained access to humans via dermal penetration. See **Box 3.1** for other parasitologists who went this extra mile in pursuit of their science.

BOX 3.1

The Grand Tradition

Parasitologists often display a lack of squeamishness when it comes to infecting themselves in pursuit of knowledge. Indeed, according to the famed parasitologist and author Asa Chandler, the “good parasitologist” has been infected with one or more of the organisms he or she studies. To many parasitologists, such infection represents a badge of honor, a way to earn their stripes, so to speak. A surprising number have gone as far as to participate in the grand tradition of self-infection.

The list of parasite life cycles that have been clarified at least in part by parasitologists willing to be part of their own experiments is long, and our few examples only scratch the surface. Self-infection or the infection of others has also been carried out for several other reasons. Some were no doubt motivated by simple curiosity or perhaps other more obscure motives. Others may have felt like Clark Read, who, after attempting to infect himself with the rat tapeworm, *Hymenolepis diminuta*, explained, “It seemed like the thing to do in light of all the rats I have infected with this parasite.”

In other instances, the act may have been purely practical. In 1922, for example, Claude Barlow consumed over 100 metacercariae of the digenetic trematode *Fasciolopsis buski* to better understand how clinical symptoms developed in humans. Asa Chandler, quoted above, clearly did not simply talk the talk. Chandler wanted to know if the various species of tapeworms in the genus *Diphyllobothrium* might actually

be all the same species. *Diphyllobothrium* spp. use mammals as the definitive host. A wide variety of mammals become infected when they consume fish, which serve as the second intermediate host. Species designations were based on structural differences in the various cestodes, but Chandler suspected that the differing morphologies were simply a consequence of which mammal the parasites infected. He predicted that if these so-called species all went through the same host (himself) the morphological difference would vanish. His willingness to serve as his own experimental subject allowed him to show that he was correct. In 1968 Dale Little swallowed infective eggs of the nematode *Trichuris trichiura*. The eggs were especially large, and Little wished to know if egg size was a genetic trait in these helminths. When the eggs he eventually passed all came out just as large as the ones that he consumed, he got his answer. Egg size was indeed an inheritable trait.

Other scientists have infected themselves for more pedestrian reasons: to avoid bureaucratic red tape or difficulty of obtaining permits to import parasites into the country. In the 1920s, for instance, Hans Vogel infected himself with *F. buski*, just to import these intestinal trematodes quietly into Germany. Likewise, Claude Barlow, infected himself with *Schistosoma haematobium* cercariae in Egypt, so that there would be no trouble with customs officials. Barlow wanted to see if *S. haematobium* was capable of

infecting species of North American snails. As a bonus, his infection provided a continuous source of material for teaching, and he made a number of new observations, such as the ability of *S. haematobium* to release its eggs in seminal fluid. More recently, in the 1980s, Robin Overstreet imported *Diphyllobothrium latum* from Finland by carrying the tapeworms in his intestine. Harvey Blankespoor wanted to know what happened to avian schistosomes when they penetrated human rather than bird skin. As he explained it, he carried out his experiment on himself to avoid any possibility of a lawsuit.

Then there was the Canadian student who evidently was not fond of his roommates. He allegedly slipped infective *Ascaris lumbricoides* eggs into their food. Two of the apparent victims almost died due to an allergic reaction. Although the district attorney contemplated a charge of attempted murder, charges were eventually dropped as all the evidence was circumstantial. None of the roommates passed an adult worm, meaning that no “murder weapon” could be found.

References

- Grove DI** (1990) *A History of Human Helminthology*. CAB International, Wallingford, UK.
- Kean BH, Mott KE & Russel AJ** (1978) *Tropical Medicine and Parasitology Classic Investigations*, vols I and II. Cornell University Press, Ithaca, New York.
- Mayberry LF** (1996) The infectious nature of parasitology. *J Parasitol* **82**:855–864.

Mosquito transmission was first demonstrated for filarial worms

Few diseases are as disfiguring as elephantiasis, which is caused by infection with nematodes in the genera *Wuchereria* and *Brugia*. They are commonly known as **filarial worms** (from the Latin for threadlike). Because of the obvious and often extensive deformity observed in limbs and genitals (see **Figure 1.1A**), even ancient civilizations were familiar with the condition (**Figure 3.3**). The term elephantiasis comes from the ancient Greeks, who described those suffering from the condition as elephant-like. Elephantiasis is notable for another important reason; it is the first parasitic condition for which the causal pathogen was shown to be transmitted by infected mosquitos.

Adult worms inhabit lymphatic vessels. The characteristic symptoms of elephantiasis result from obstruction of the vessels and subsequent inflammation. Following mating, females release large numbers of larvae called **microfilariae**. In 1863, French surgeon Jean-Nicolas Demarquay was the first to observe microfilariae in accumulated lymphatic fluid. Several years passed, however, before the Scottish physician Timothy Lewis made the link between microfilariae and elephantiasis. In 1876, the Australian Joseph Bancroft first described the adult worm, which is now known as *Wuchereria bancrofti* in his honor.

Shortly thereafter, Patrick Manson, another Scottish physician, made a critical discovery regarding the life cycle of filarial worms when he investigated their mode of transmission. Manson already knew that arthropod transmission of parasitic worms was a possibility as he was aware of earlier work demonstrating that humans became infected with Guinea worm when they inadvertently consumed infected copepods in drinking water. He suspected that filarial worms were also transmitted through the consumption of an arthropod vector, but when he found microfilariae in the blood of dogs and humans, he hypothesized that blood-feeding insects might be involved. To find out, he allowed mosquitoes to feed on the blood of a human volunteer who was afflicted with elephantiasis. He subsequently found larval worms in these mosquitoes. Yet Manson believed that larval parasites passed from the infected mosquito into water, where they might be accidentally consumed by a human. It was Manson's assistant, George Low, who, in 1900, finally identified the actual mode of transmission, the bite of an infected mosquito, when he found larval filarial worms in the insects' mouthparts.

Arthropod transmission for filarial worms suggested that other diseases may be similarly transmitted

The discovery that mosquitoes were involved in the transmission of filarial worms had enormous implications for parasitology and microbiology. As it became apparent that parasites might be spread by a previously unsuspected means, the mode of transmission for many infectious agents became clear. In 1892, for example, two Americans, Theobald Smith and Frederick Kilbourne, found that redwater fever, a serious disease of cattle, was transmitted by ticks. An apicomplexan in the family Babesiidae, *Babesia bigemina*, causes red-water fever. Kilbourne and Smith's study was the first to demonstrate that a unicellular eukaryotic pathogen (a "protozoan" or a "protist"; recall that these terms have no taxonomic validity and are often used interchangeably) could develop in and be transmitted by an arthropod. Moreover, once the mode of transmission was revealed, means of control became apparent. One simply had to kill the involved tick, *Rhipicephalus*

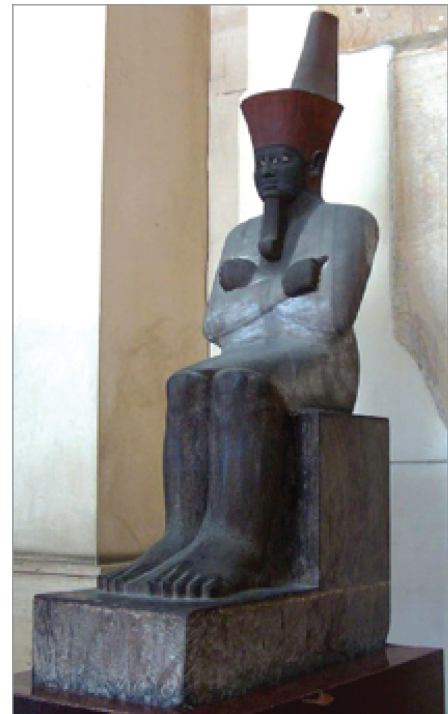


Figure 3.3 Elephantiasis in an Egyptian pharaoh. Mentuhotep II was Pharaoh for over 50 years, from approximately 2046 BC to 1995 BC. The swollen legs carved in this statue suggest elephantiasis, caused by filarial worm infection. Blockage of the lymphatic vessels by adult worms causes fluid buildup and tissue distention. (Courtesy of Anastasia Grishchenko.)

Figure 3.4 Walter Reed and the Yellow Fever Commission. In 1881 the Cuban physician Carlos Finley proposed that yellow fever might be transmitted by mosquitoes. In 1898, with the outbreak of the Spanish-American War, the US Army became especially interested in yellow fever. The Yellow Fever Commission, headed by Walter Reed (seated), was established in Cuba to determine the exact source of the disease. As part of the study, mosquitoes reared in the laboratory were allowed to feed on infected patients. These same mosquitoes were later permitted to feed on human volunteers, including Commission members James Carroll and Jesse Lazear. Both of these men developed yellow fever. Lazear died from his infection. (Painting by Robert Thom, *The Conquest of Yellow Fever*. University of Michigan Museum of Art, Collection of the University of Michigan Health System, Gift of Pfizer Inc.)



annulatus, or prevent them from feeding on cattle. Similarly, in 1898, once the Yellow Fever Commission in Cuba established that *Aedes aegypti* mosquitoes transmitted yellow fever, the incidence of the disease fell rapidly as mosquito control measures were introduced (Figure 3.4).

No historical discussion of arthropod-borne parasites, of course, would be complete without considering malaria, at or near the top of anyone's list of important human diseases. Unlike some of the helminths previously discussed, whose large size made them obvious even to ancient health practitioners, there was no real understanding of diseases like malaria or bacterial pathogenesis until the acceptance of the germ theory of disease in the late nineteenth century.

Through much of recorded history, at least as far back as ancient Rome in 200 BC, the severe fevers induced by malaria were thought to be due to the unhealthy vapors that emanated from wetlands. Indeed the very name malaria is a literal translation of “bad air” in Italian. Our understanding of this disease began in 1880 when Charles Laveran, a physician with the French Foreign Legion in Algeria, found the parasite in a blood sample taken from a soldier suffering from malaria. He quickly concluded that the organisms he had found were the cause of the disease. Following his groundbreaking work on mosquito transmission of filarial worms, Patrick Manson first hypothesized mosquito transmission for malaria as well. Manson shared his suspicion with Ronald Ross, a young English physician based in India who had returned to England on leave in 1894. He convinced Ross to study mosquito transmission upon his return to India in 1895. Ross painstakingly dissected thousands of mosquitoes, searching for the telltale developmental stages of the parasite. Finally, in 1897, he demonstrated that avian malaria, caused by *Plasmodium relictum*, was transmitted by mosquitoes in the genus *Culex* (Figure 3.5). A year later, in 1898, Italian scientists showed conclusively that the important human pathogen, *Plasmodium falciparum*, was transmitted by mosquitoes in the genus *Anopheles*. These researchers included Giovanni Grassi, the same individual who intentionally consumed *Ascaris* eggs, as described above. The discovery that mosquitoes were involved in the transmission of two

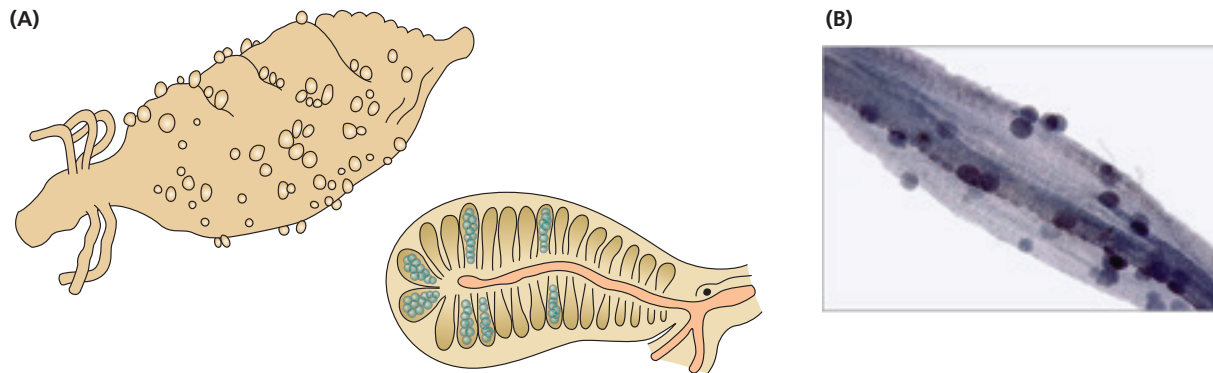


Figure 3.5 Mosquito transmission of malaria. (A) Ronald Ross's drawings of *Plasmodium* oocysts in the digestive tract of a mosquito. (B) A microscopic image of *Plasmodium* oocysts, also on the mosquito gut wall. (B, Courtesy of SJ Upton, Kansas State University.)

other important causative agents of human malaria, *P. malariae* and *P. vivax*, soon followed.

It was not until 1947 that the complete *Plasmodium* life cycle, including those stages that occur in the human host, was fully elucidated. By that time, it was assumed that the **sporozoite**, the developmental stage found in the salivary glands and mouthparts of infected mosquitoes, was infective to humans. Oddly, sporozoites could not be detected in the blood of recently infected people, and no blood stages were observed for over a week. Where did the sporozoites go? English scientists Henry Shortt and Cyril Garnham got the answer when they infected rhesus monkeys with sporozoites taken from the salivary glands of infected mosquitoes. One week later, they found what they called preerythrocytic stages in the monkey's liver cells. It then became evident that the sporozoites first infected liver cells, where they underwent asexual reproduction, before leaving the liver and entering red blood cells.

3.2 AN OVERVIEW OF PARASITE LIFE CYCLES

Having described a few of the historical breakthroughs that sparked our understanding of parasitic life cycles, we turn to a closer look at the life cycles themselves. All living things have a life cycle, defined as the developmental events, including growth and reproduction that occur in the course of an organism's life. Parasitic life cycles are distinguished by the need for hosts in which to accomplish at least some of these events. Perhaps the simplest way to consider the various forms the parasitic life cycle can take is to distinguish between direct and indirect life cycles.

Parasites with direct life cycles use only a single host

Parasites with **direct life cycles** require only a single host to complete their development and reproduce. Such life cycles are also described as **monoxenous**. Several of the parasites discussed in the historical overview in Section 3.1 have direct life cycles. These include the nematode *Ascaris lumbricoides*, utilizing only humans to complete its life cycle. Other examples are the pinworm (*Enterobius vermicularis*), and the hookworm *Ancylostoma duodenale*. Hookworms are noteworthy in that they also have obligatory, free-living larval stages. Many protozoa also have direct life cycles. *Giardia lamblia* and *Entamoeba histolytica* are two examples.

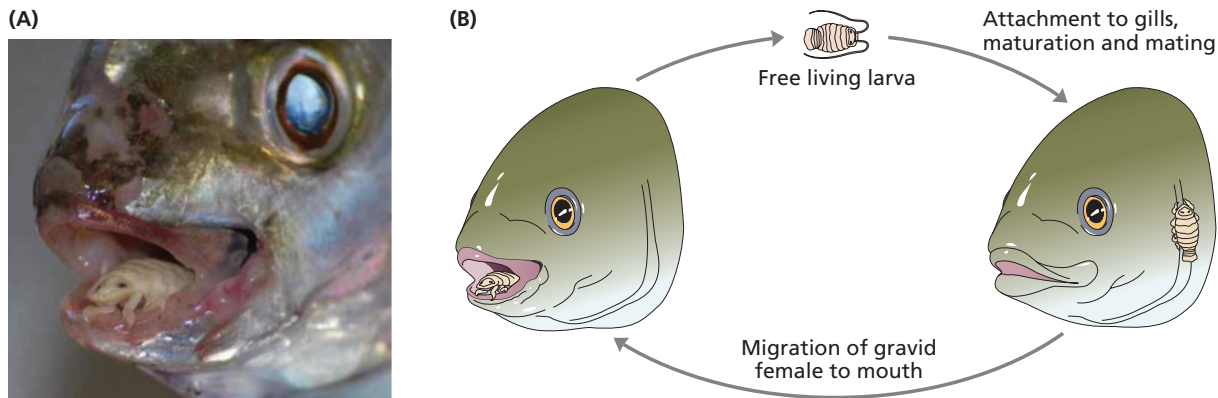


Figure 3.6 A direct life cycle. Parasites that use only a single host have a direct or monoxenous life cycle. (A) *Cymothoa exigua* is a parasitic crustacean in the order Isopoda. An adult female is seen here in the host's mouth. (B) The *C. exigua* life cycle. Motile larvae are released into the environment. Larvae develop to the point where they can attach to the gills of an appropriate host. Here they undergo a process known as protandrous hermaphroditism. They first develop into males and upon further maturation into females. Mating is believed to take place on the gills of the host. The now gravid female makes her way to the fish's mouth, where she attaches to the base of the tongue and begins to feed on blood in the tongue. The female will remain in this position for the rest of her life. As the tongue atrophies from a lack of blood, the isopod actually takes the place of the tongue, remaining firmly attached via her rear thoracic appendages. Oddly, the fish can use the isopod as if it were a normal tongue. This is the only recognized example of a parasite actually replacing a host's body part. As the female's eggs hatch, giving rise to a new generation of larvae, the life cycle is completed.

To learn about other parasites with highly unusual life cycles you may wish to visit:
<http://dailyparasite.blogspot.com/>

Parasites with direct life cycles are notable because their propagules can reinfect the same host in which sexual reproduction occurred. For example, individuals infected with adult *A. lumbricoides* who pass eggs in their feces may inadvertently consume these eggs, resulting in multiple generations of worms within the same individual host. Although direct cycles are relatively simple, they are not necessarily boring or conventional. **Figure 3.6** reminds us that when it comes to the bizarre and curious, parasites can set the bar high.

Two or more hosts are necessary for those parasites with indirect life cycles

An **indirect life cycle**, also called a **heteroxenous** life cycle, implies that two or more different hosts are required. Sexual reproduction occurs in the **definitive host**, whereas larval development takes place in the **intermediate host**. Depending on the parasite, larval development may be accompanied by asexual reproduction and in many cases, there is more than one intermediate host. Some heteroxenous parasites may also employ **paratenic hosts**—nonessential hosts that nevertheless increase the likelihood of transmission to the definitive host. Little or no obvious development or reproduction occurs in such hosts.

Several of the organisms discussed in the historical overview, including *Fasciola hepatica*, *Taenia solium*, *Wuchereria bancrofti*, and *Plasmodium falciparum*, have indirect life cycles. Members of the phylum **Acanthocephala** provide an additional example of a relatively straightforward two-host indirect life cycle. Acanthocephalans inhabit and sexually reproduce in the gut of vertebrates such as fish, birds and reptiles (see **Figure 2.21B**, which shows the characteristic proboscis, used to penetrate and attach to the host's gut wall). The vertebrate consequently serves as the definitive host (**Figure 3.7**).

The digenetic trematode *Alaria americana* provides us with a more complex (possibly the *most* complex) multi-host life cycle. The term “digenetic”

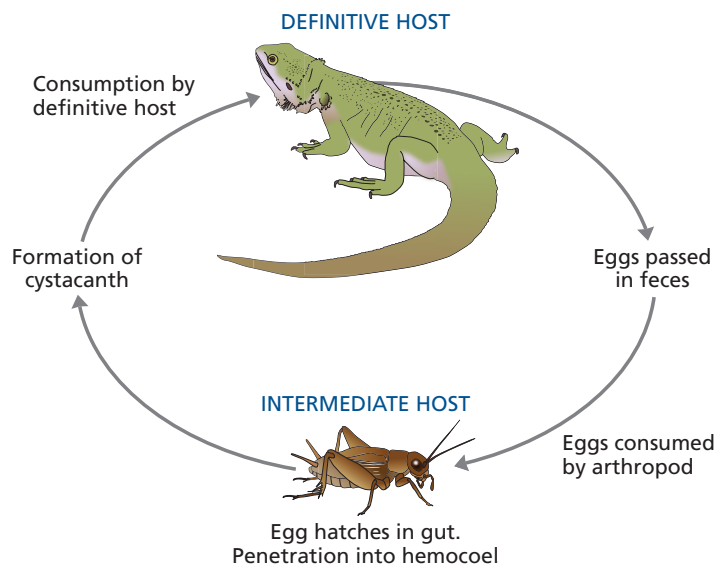


Figure 3.7 A generalized acanthocephalan life cycle. Certain details of the life cycle vary according to parasite species. In all cases, however, the definitive host is a vertebrate. Following fertilization, the female releases eggs, which are passed in the feces. The eggs may then be consumed by the intermediate host, almost always a specific type of arthropod. The egg hatches in the gut of the arthropod, and the acanthor that emerges penetrates the gut, entering the body cavity, or hemocoel. Within the hemocoel, the acanthor develops into a second larval stage called the acanthella, which ultimately develops into a cyst-like stage called a cystacanth. If the infected arthropod is subsequently eaten by the definitive host, the life cycle is completed, as the cystacanth excysts in the digestive system, where it develops into the adult. In many cases, the infected arthropod is first consumed by a paratenic host, in which the cystacanth persists. The paratenic host must later be eaten by the definitive host to complete the life cycle.

refers to its placement in the subclass Digenea, along with many other trematodes of medical or veterinary interest. *A. americana* is most common in North America, typically in temperate regions near water. Carnivorous mammals including foxes, coyotes or skunks serve as definitive hosts. Adult worms are **monoecious**—they possess both male and female reproductive organs and are capable of producing both eggs and sperm. Adults mature in the small intestine and fertilized eggs exit with the feces. These eggs must reach fresh water, where they hatch in roughly two weeks, releasing a ciliated **miracidium** (plural **miracidia**). This initial, usually free-swimming larval stage is typical of digenetic trematodes. In the case of *Alaria*, as in most other trematodes, a miracidium seeks specific types of aquatic snails, which it penetrates upon contact. It then sheds its ciliated epithelium, enters the snail's renal veins and develops into a sac-like form called a **mother sporocyst**. The sporocyst reproduces asexually to produce numerous **daughter sporocysts**, which migrate to the snail's digestive gland. After a period of prolonged development of as long as several months, daughter sporocysts give rise to the next life cycle stage, called **cercariae** (singular **cercaria**). This juvenile, tail-bearing stage exits the snail and swims to the surface. It may hang upside down, periodically sinking, whereupon it again swims to the surface. If nearby water currents mechanically stimulate the cercaria, the larval trematode will swim in the direction of the current, seeking its second intermediate host, a tadpole. Using protease-containing penetration glands in its body, the cercaria penetrates through the skin of the tadpole, loses its tail, and enters the juvenile amphibian's body cavity. It then develops into a **mesocercaria**, the infective stage for the next host in the life cycle. Should the tadpole undergo metamorphosis, mesocercariae will remain infective in the now-adult amphibian (Figure 3.8).

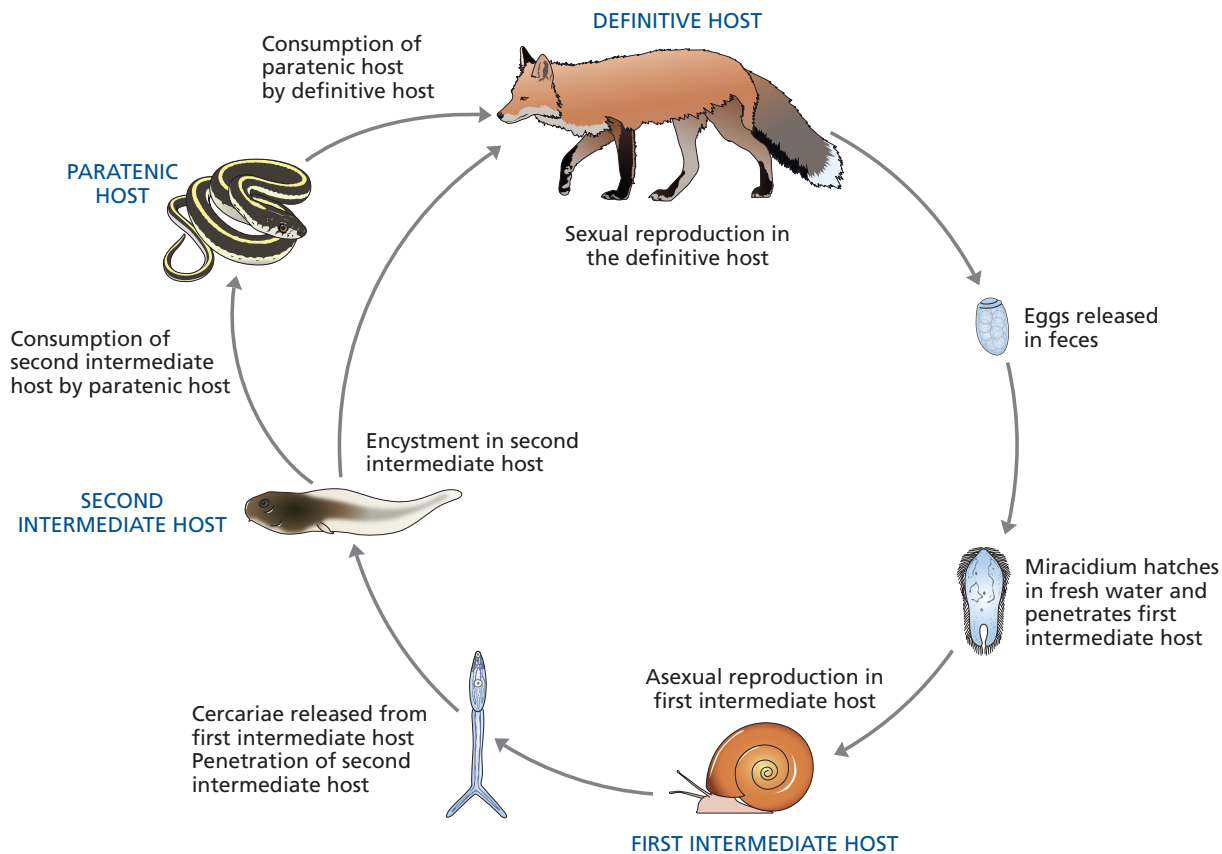


Figure 3.8 The life cycle of *Alaria americana*. The complex indirect life cycle of this digenetic trematode may include up to four hosts. A canid may either consume an infected tadpole, or the tadpole may be consumed by a reptile, which serves as a paratenic host. In this case, the life cycle is completed when a canid consumes the reptile.

Next, one of two things happens. Occasionally, the definitive host consumes an infected tadpole. If so, mesocercariae are liberated during digestion and subsequently penetrate the intestinal epithelium, migrating to the lungs. After more than a month, a larval trematode, now called a **diplostomulum metacercaria**, moves up the trachea. After reaching the throat, it is swallowed, which returns it to the intestine. Back in the intestine, following sexual maturation in several weeks, the life cycle is completed.

However, canids or other appropriate definitive hosts eat amphibians only rarely. *A. americana* more commonly relies on an alternative Plan B to complete its life cycle. If a snake or lizard consumes an infected tadpole or frog, the reptile will serve as a paratenic host, harboring the still infective trematode mesocercaria, until the reptile is finally consumed by a mammal. As an added twist to this complex life cycle, once they reach their mammalian host, larval diplostomulum metacercariae can move from an infected female mammal to her nursing young through breast milk. Transmission from mother to offspring, either in milk or to a developing fetus is termed **vertical transmission**, to be discussed below. And if all this weren't complicated enough, *A. americana* may sometimes cause some particularly odd pathology in an infected paratenic host; the tail of an infected snake can actually fall off.

Note that although similar in many regards, life cycle variation is common among different digenetic trematode species; there is no typical single pattern of larval development. **Figure 3.9** summarizes some of the diversity on a common basic theme that is observed in this subclass of the phylum **Platyhelminthes**.

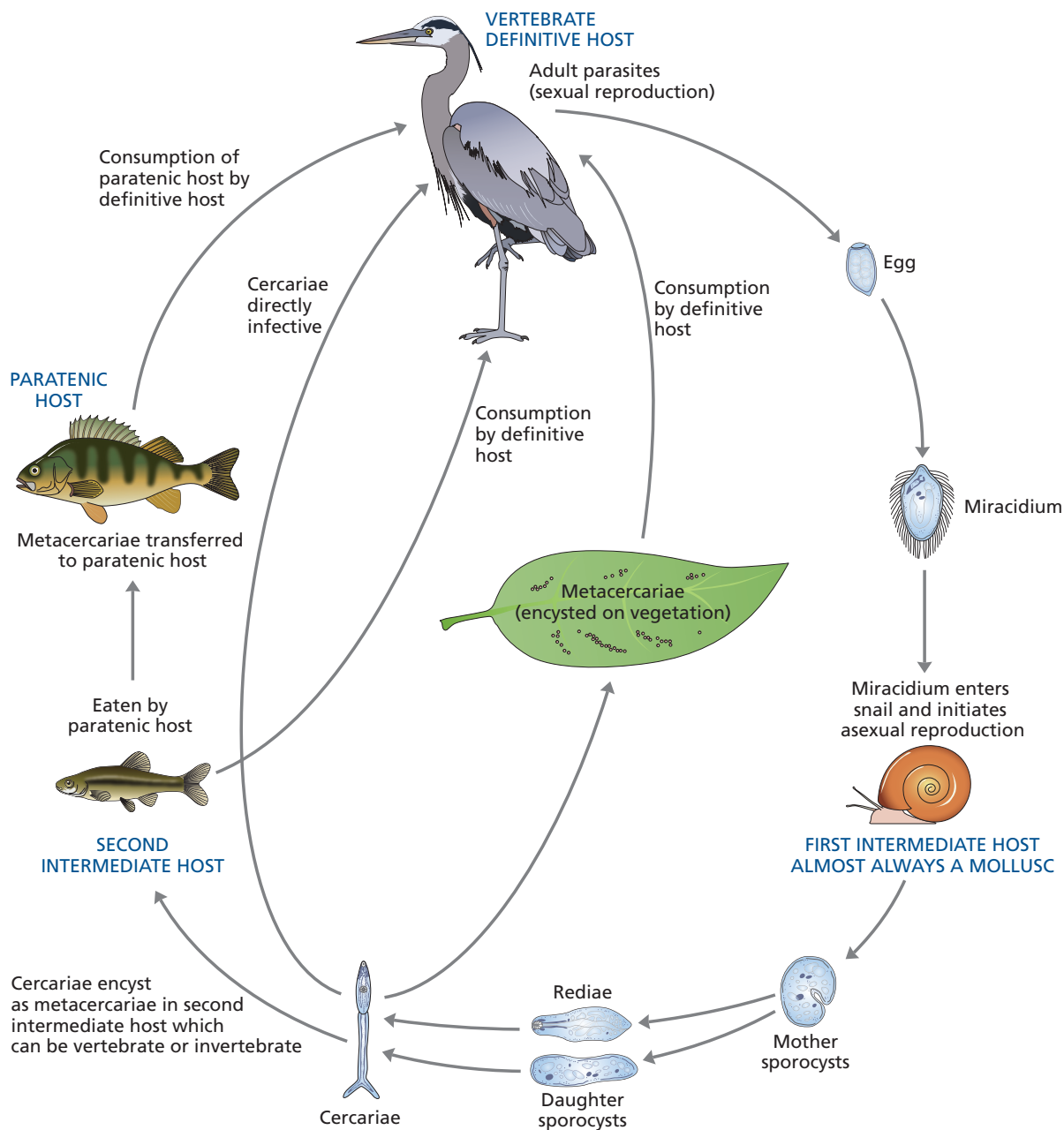


Figure 3.9 Diversity of life cycles in the digenetic trematodes. Digenetic trematodes show remarkable diversity in their life cycles. No single species takes all the paths shown here. Note that initially mother sporocysts produce either daughter sporocysts or rediae, but never both. If a paratenic host is used in the life cycle, little or no additional development occurs within this host. Not all possible life cycle variations are shown here. For instance, in the first intermediate host, there may be multiple generations of daughter sporocysts or rediae.

3.3 THE PARASITE'S TO DO LIST

Each stage of a parasite's life cycle comes with hurdles that the parasite must negotiate if it is to achieve its overriding objective of reproduction. To be successful a parasite must:

- Achieve transmission
- Enter its host
- Migrate to an appropriate target tissue or site within the host

- Maintain its position within the host
- Find a mate
- Successfully reproduce in and release progeny from the host
- Undergo essential developmental changes within the host
- Cope with ambient physiological conditions within the host
- Evade destruction by the host immune system, at least until reproduction is complete

As you might expect, different parasites use diverse strategies to overcome these various obstacles and not all requirements are identical for all parasites. Ectoparasites, for example, by definition do not enter their host. For some hermaphroditic parasites or for those lacking sexual reproduction, there is no absolute requirement to find a mate. We will now examine individually each life cycle component and its associated problems for the parasite. Immune evasion will be addressed in Chapter 4.

Effective transmission is essential for all parasites

Transmission, the passage of a parasite to a host, is the most fundamental imperative for any parasite. All other life cycle concerns are moot if the parasite cannot reach a required host in the first place.

Almost all organisms, both free living and parasitic, must disperse from their site of origin to reduce crowding, intraspecific competition and overuse of resources. However, for parasites mere dispersal is not enough; unless parasites achieve effective transmission, subsequent development and, ultimately reproduction, do not occur. Parasites employ a wide variety of strategies to achieve transmission.

The manner in which a parasite moves from host to host is defined as its **mode of transmission**. We next review the most common modes of transmission utilized by eukaryotic parasites. Other modes of transmission, including airborne, while common for bacteria and viruses, as well as some fungi, are rarely used by protozoa or helminths and are not discussed here. Keep in mind that many parasites utilize different modes in different stages of their life cycles.

Fecal–Oral Transmission

Most intestinal protozoa and helminths rely on **fecal–oral** transmission to complete at least part of their life cycle. Such transmission suggests that propagules, the stage responsible for achieving transmission, are released in the feces. They may then contaminate food or water, which may be consumed by the subsequent host. Often the propagule is a relatively metabolically inactive egg or cyst, which can withstand long periods of time in the external environment. Its consumption by the next host is largely fortuitous, and the parasite expends little if any energy as it simply waits to be eaten.

The flagellated protozoan *Giardia lamblia* provides a typical example (Figure 3.10). Like many intestinal protozoa, they live in their host's small intestine as an actively feeding and reproducing stage called the **trophozoite**. Reproduction by binary fission results in new generations of trophozoites. However, under specific physiological conditions within the host the trophozoite may transform into a nonreplicating and dormant **cyst**. The precise factors that initiate cyst formation are not completely understood. *In vitro* cyst formation can be induced by mimicking the normal decrease in lipid concentration and the increase in pH that occur along the gastrointestinal tract. It has also been shown in animal models that cyst formation

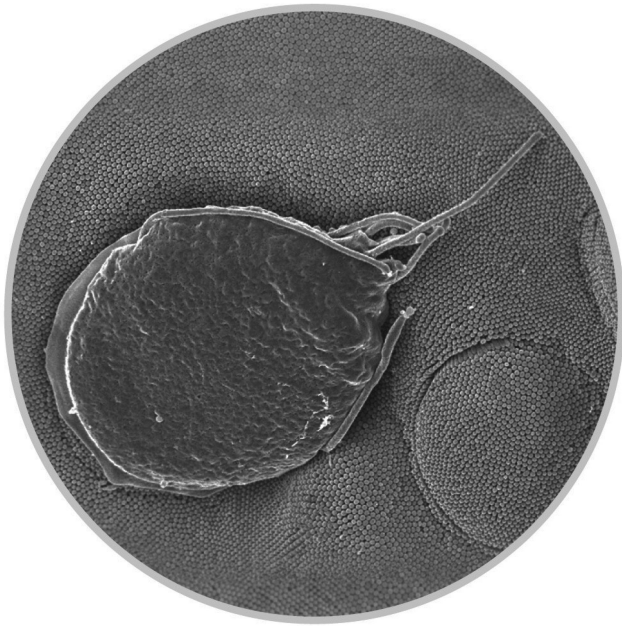


Figure 3.10 *Giardia lamblia*. In this electron micrograph of the trophozoite stage, both the ventral adhesive disk and flagella are clearly visible. Note the circular mark on the intestinal lining left by the adhesive disk. (Courtesy of CDC, Stan Erlandsen.)

commences when the trophozoites themselves achieve a certain threshold density in the intestine. This suggests that at least to some degree, *G. lamblia* relies on **quorum sensing** to initiate encystment. Quorum sensing is a mechanism of regulating gene expression that depends on the density of cells in a given area. The cells release signaling molecules and when the concentration of these molecules reaches a certain threshold level, genes dependent on this signaling are expressed. First discovered in bacteria, quorum sensing has now been demonstrated as a means of communication between eukaryotes as well.

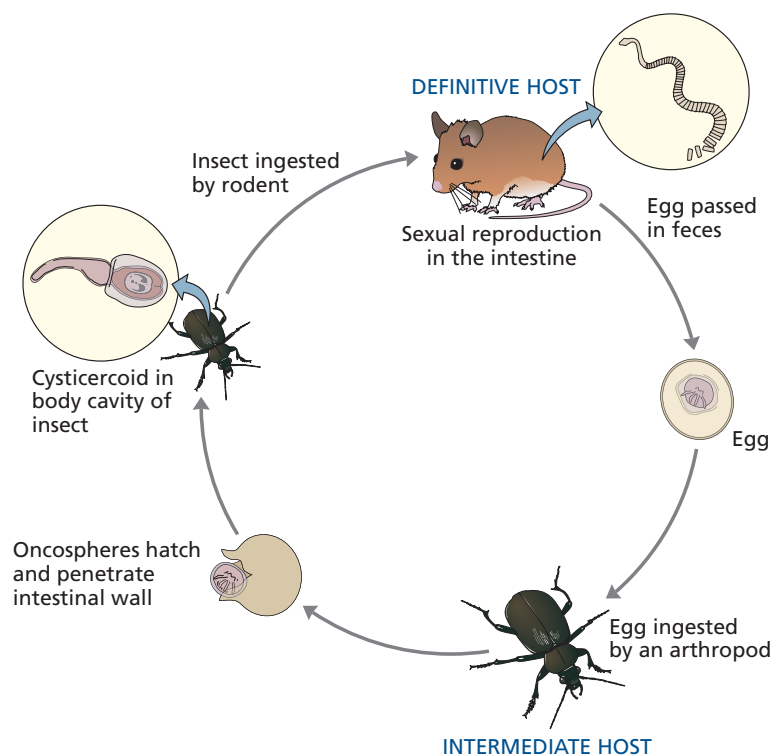
During cyst formation, the flagella and adhesive disk of the trophozoite are lost and various cyst wall proteins are excreted via exocytosis. The cyst wall, which also incorporates secreted carbohydrate polymers, forms as the proteins crosslink. Once passed with the feces, cysts can persist for at least a few months under appropriate conditions of humidity and temperature until a suitable host inadvertently consumes them.

Some parasites relying on fecal–oral transmission have evolved adaptations in addition to resistant propagules that to some degree stack the deck in their favor by enhancing the likelihood of transmission. Cestodes, for instance, have indirect life cycles, and for many tapeworms, the adult host passes eggs in its feces that are subsequently consumed by the intermediate host. In some tapeworms using small aquatic crustaceans called ostracods as hosts, the tapeworm eggs appear similar to the aquatic algae on which the ostracod feeds, tricking the intermediate host into consuming them. Other tapeworms use copepods as intermediate hosts, and their eggs either sink or float, depending on whether or not the specific copepod in question is benthic or pelagic.

Trophic Transmission

The fecal–oral transmission described above for tapeworms is only half of the story. Once the intermediate host is infected, transmission back to the definitive host is achieved when the definitive host consumes the intermediate host. That is, the parasite takes advantage of the established predator–prey relationship that exists between the definitive and intermediate hosts.

Figure 3.11 *Hymenolepis diminuta*, the rat tapeworm. The life cycle of this cestode relies on both fecal–oral transmission (for transmission from the definitive to the intermediate host) and trophic transmission (for transmission from the intermediate host back to the definitive host). Humans occasionally are infected when they inadvertently consume an infected insect that is serving as an intermediate host. (Courtesy of CDC.)



Such transmission, fecal–oral alternating with trophic, is typical of cestodes as well as many other parasites with indirect life cycles. *Hymenolepis diminuta*, the rat tapeworm, provides a representative example (Figure 3.11). Various insects that serve as intermediate hosts may consume eggs passed in a rat's feces. The eggs hatch in the insect's digestive system, releasing a larval stage known as an **oncosphere**. The oncosphere penetrates the insect's gut wall and enters the body cavity, where it develops into a **cysticercoid**. Transmission back to the definitive host occurs when a rat consumes the infected insect. Human infections are uncommon, but they can occasionally occur if a human inadvertently consumes an infected insect. This is most likely when a flour beetle (genus *Tribolium*) is acting as an intermediate host as these beetles readily colonize products such as flour or cereals.

A reliance on trophic transmission suggests that the parasite is relatively passive and that it does little or nothing other than wait to reach its next host. A fascinating aspect of transmission biology, however, is the way in which at least some trophically transmitted parasites actually manipulate the behavior of their hosts in a manner that increases the likelihood that a specific host will be consumed by the next host in the life cycle. To cite just one example, the acanthocephalan *Pomphorhynchus laevis* utilizes the aquatic amphipod *Gammarus pulex* as its intermediate host. Transmission to the definitive host, a freshwater fish such as a perch, is achieved when the fish eats the amphipod. Uninfected amphipods are generally photophobic, seeking out darker areas among rocks and vegetation, where there are hidden and less likely to be eaten. Amphipods infected with the acanthocephalan, however, actually seek out brightly lit open water, where they are *more* likely to be preyed upon. It is believed that the parasite, in some as-yet unclear way, alters the behavior of its intermediate host to facilitate its transmission to the definitive host. Other examples of such host manipulation will be more fully investigated in Chapters 5 and 6.

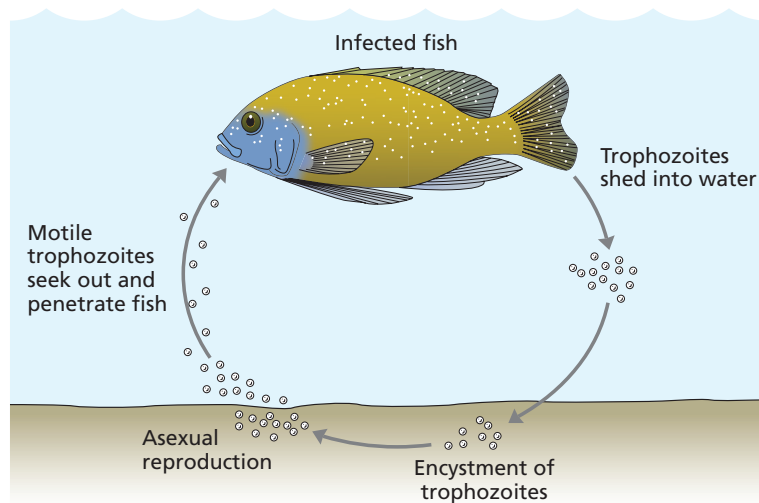


Figure 3.12 The life cycle of *Ichthyophthirius multifiliis*. Trophozoites released into the water form cysts in the bottom sediments, where they undergo extensive asexual reproduction. Free-swimming tomites released back into the water seek out a fish. Upon contact, they penetrate the epithelium and transform into trophozoites. The resulting inflammation and tissue damage form the characteristic white pustule, and the parasite feeds on nutrients released by dying host cells. The pustule eventually ruptures, releasing motile trophozoites back into the water, whereupon they return to the substrate.

Direct Penetration

Some parasites take a more proactive approach to their transmission; they actively seek out their host and once they find it, they bore their way in. An example familiar to many tropical fish hobbyists is *Ichthyophthirius multifiliis*, a parasitic ciliate that causes the condition known as ich or whitespot (Figure 3.12). The condition is easily recognized when small white nodules, looking like grains of salt, appear on the fish's body, fins, or gills.

The life cycle of *I. multifiliis* is unusual for a parasitic protozoan; reproduction does not occur in or on the host but via asexual binary fission in cystlike structures found on the bottom of a lake, pond or aquarium (see Figure 3.12). Between 50 and a few thousand new trophozoites are produced in each cyst and are subsequently released into the surrounding water. These free-swimming trophozoites are also called **tomites**. Once a tomite contacts a fish, it penetrates the epithelium. The resulting inflammation and tissue necrosis result in the typical white pustule. This parasitic stage lasts only a few days, during which the parasite feeds on nutrients released by dying epithelial cells. When the pustules finally rupture, motile trophozoites are again released back into the aquatic environment, returning to the substrate where they once again encyst.

Unlike those parasites relying on fecal–oral transmission, during which resistant cysts or eggs may simply tough it out until they are consumed, for parasites relying on direct penetration, the clock is ticking. Survival of motile and metabolically active penetration stages in the external environment is usually measured in hours. A typical miracidium, for example, as previously seen in the *A. americana* life cycle has only a few hours in which to locate and penetrate an appropriate snail before it succumbs to the rigors of the external environment, is eaten or depletes its energy reserves (see Figure 3.8). It is therefore not surprising that digenetic trematodes and other parasites with metabolically active external stages have evolved strategies to increase their likelihood of finding an appropriate host rapidly. Consider, for instance, the example with which we opened this chapter: the circadian rhythm that allows different species of schistosome cercariae to emerge when they are most likely to encounter the next host in their life cycles.

Cercariae often have other tricks up their sleeves. For example, they may position themselves in that part of the water column where they are most likely to encounter a host. To illustrate, consider the digenetic trematode *Cardiocephalus longicollis*. Like most such parasites, it uses a molluscan first

intermediate host. The cercariae that emerge from the marine, benthic-dwelling mollusc must find an appropriate second intermediate host—a fish such as a bream or porgie. Consequently, the cercariae swim from the bottom to an intermediate depth, where the suitable fish are most likely to be found. Once there, they allow themselves to fall back through the water column, repeating this vertical migration until the cercariae either contact a fish or die. Not surprisingly, the cercariae of other species utilizing surface-dwelling fish rise to the surface and remain there.

Vector Transmission

As discussed in Chapter 1 and the historical overview, many parasites rely on vectors to achieve transmission. Parasites typically move between vector and host as the vector takes a blood meal. Most vectors are arthropods and the roster of parasites (eukaryotic, prokaryotic or viral) that rely on arthropod vector transmission is long. The *Anopheles* mosquitoes that transmit *Plasmodium* may be the best known example. Mosquitoes also serve as vectors for the transmission of those nematodes in the genera *Wuchereria* and *Brugia* that cause filariasis. Other well-known examples include tsetse flies (genus *Glossina*), which transmit sleeping sickness (caused by kinetoplast protozoa in the *Trypanosoma brucei* species complex), cone-nosed or kissing bugs, which transmit Chagas disease (caused by *Trypanosoma cruzi*), and sand flies, which transmit leishmaniasis. Other parasites such as apicomplexans in the genus *Babesia* rely on non-insect arthropods, specifically ticks in the genera *Ixodes* or *Rhipicephalus*. As we already noted in Chapter 1 (see **Box 1.1**) the importance of ticks as vectors is increasing due to climate change, habitat alteration and other human-induced changes in the environment. Relapsing fever, caused by the spirochete bacterium *Borrelia miyamotoi*, is just one example of a newly emerging disease, resulting in part from the range expansion and increasing density of its ixodid tick vectors.

For information on tick-borne diseases, visit <https://www.cdc.gov/ticks/tickbornediseases/index.html>

Not all blood-feeding arthropods can act as vectors. An ingested parasite must not only be capable of survival in the vector's digestive tract, but it must also frequently be able to penetrate the vector's gut wall; reproduce, develop or both within the vector; migrate to the vector's salivary glands and achieve transmission when the vector takes its next blood meal. All of these tasks must be accomplished within the milieu of the vector's body and in the face of immune defense designed to impede parasite survival. It is therefore not surprising that various parasites often adapt to the selective pressures imposed by a particular vector, just as they adapt to their other specific hosts. The term **vector competence** describes the ability of a particular vector type to transmit a specific pathogen. Vector competence is assessed in the laboratory by allowing uninfected potential vectors to feed on animals infected with a particular pathogen. These putative agents of transmission are subsequently allowed to feed on uninfected animals. Should the second group of animals become infected, vector competence is demonstrated.

Competent vectors can conveniently be categorized into a few different types. **Mechanical vectors** are those in which the parasite undergoes neither replication nor development; it merely adheres to the vector's body or mouthparts and essentially hitches a ride without cost to the vector. *Trypanosoma evansi*, discussed in Chapter 1 provides an example.

A **biological vector** is one in which the parasite either develops or reproduces or both. A biological vector itself, unlike a mechanical vector, is infected with a specific parasite. *Wuchereria bancrofti* and other filarial worms, for instance, undergo essential larval molts within their mosquito vector but do not increase in number. This is an example of what is called **cyclodevelopmental transmission**. Arboviruses (a non-taxonomic

term describing any virus transmitted by arthropods), on the other hand, including dengue virus and yellow fever virus, multiply greatly within mosquitoes but undergo no specific developmental progression. This phenomenon is known as **propagative transmission**. Finally, parasites that pass through required development *and* multiplication within their vectors are exhibiting **cyclopropagative transmission**. Malaria parasites in the genus *Plasmodium*, utilizing mosquito biological vectors are an example.

Even a competent vector may be unimportant in disease transmission if it is, for instance, not abundant or if it rarely feeds on a particular host. These extrinsic factors determine what is called **vector capacity**. Some of the other characteristics that contribute to high vector capacity include:

- Regular feeding on the host in question
- Feeding for an extended period of time and taking a relatively large blood meal
- A life span long enough to allow the parasite time to reach its infective stage, to migrate to the salivary glands and to replicate sufficiently to allow the vector to infect the next host
- Abundance
- Good dispersal ability

Thus, competent vectors differ in terms of their vector capacity. A perfect vector would have all of these characteristics. Vectors with high capacity may serve as **primary vectors**—able to maintain a parasite transmission cycle in the absence of other vectors. Competent vectors with low capacity may act as **secondary vectors** because they may be able to transmit a parasite but are unable to maintain the transmission cycle in the absence of the primary vector.

Let's take a closer look at just one aspect of vector capacity. *Anopheles stephensi* is a competent mosquito vector for *Plasmodium falciparum*. Yet its capacity to vector the parasite is different at different temperatures (Figure 3.13). Based on temperature-dependent parameters such as mosquito survival and the developmental rate of *P. falciparum* in the mosquito, it has been predicted that the capacity of *A. stephensi* to vector the parasite should peak at about 26°C. We will revisit this topic in Chapter 6 with respect to modeling of parasite abundance and in Chapter 10, where we consider how climate change might alter vector capacity. Box 3.2 describes a different arthropod that is less able to transmit pathogens than one might guess.

Many vector-borne parasites also come equipped with various adaptations designed to increase the odds of transmission. For instance, adult *W. bancrofti* females release microfilariae into the lymphatic fluid of the human host. From there, they enter the circulatory system. In doing so, they demonstrate a circadian rhythm, not unlike the schistosomes with which we opened this chapter. At night, when mosquito vectors are most likely to feed, microfilariae migrate to peripheral blood vessels near the skin, well positioned to be ingested along with a blood meal. The cues the microfilariae use are probably either changes in body temperature or in blood gas composition that occur at night, when the human host is sleeping. Where filarial worms are transmitted by day-biting mosquitoes, this pattern is reversed. Such periodicity may also be seasonal rather than only daily. The causative agent of canine heartworm *Dirofilaria immitis* also releases microfilariae into the blood of an infected dog. These larval nematodes, infective to the mosquito vector, increasingly localize in surface blood vessels in the spring just as mosquito numbers begin to increase.

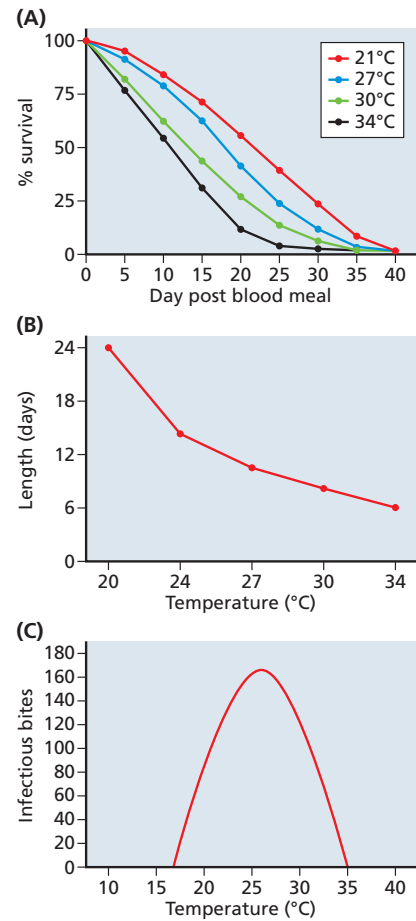


Figure 3.13 The effect of temperature on vector capacity. The ability of the Asian mosquito *Anopheles stephensi* to vector *Plasmodium falciparum* varies according to temperature. Mosquitoes must, for instance, live long enough to take more than one blood meal and for the parasite to develop into sporozoites, the infective stage for humans. (A) Mean survival of female mosquitoes following their first blood meal at 4 different temperatures. (B) The number of days required for *P. falciparum* to develop into infective sporozoites as a function of temperature. (C) The average number of infectious bites taken by a cohort of 100 infected female mosquitoes at different temperatures. Vector capacity is highest at approximately 26°C where one mosquito takes 160 infectious bites. At ambient temperatures below 16°C and above 35°C, parasite transmission cannot occur. (A, B & C modified from Shapiro LL, Whitehead SA & Thomas MB (2017) *PLoS Biol* 15:e2003489. doi:10.1371/journal.pbio.2003489.)

BOX 3.2**Return of the Bed Bugs**

Bed bugs are back. The most common of these small, blood-feeding insects (order Hemiptera) is *Cimex lectularius* (Figure 1). The name bed bug comes from their preferred habitat: dark, enclosed areas where people sleep. Mainly nocturnal, they emerge from cracks in the wall or mattresses to feed unnoticed on their human hosts.

When feeding, a bed bug penetrates the host's skin with two tubelike proboscises. The first proboscis injects saliva containing a mixture of anticoagulant and anesthetic. The other is used to obtain the blood meal, which requires approximately 5–10 minutes. After feeding, the insect returns to its hiding place. Humans may never see their tormenters, whose only calling card might be their small, granular, reddish-black feces that are released following engorgement. Severe blistering and itching may occur at the bite site, although in some individuals there are no visible symptoms (Figure 1).

Bed bugs have plagued humans for millennia. Even the ancient Egyptians had to put up with these unwelcome bedfellows 3,500 years ago. And recent evidence suggests they've been around a lot longer than that. Indeed, recent phylogenies place their origin at about 115 million years ago, long before the evolution of bats, which were previously thought to be the ancestral hosts. In other words, instead of first evolving on bats and later colonizing humans, the first bed bugs probably took blood from dinosaurs or ancient birds and mammals. Bats and humans are relatively recent additions to the bed bug menu.

In more developed countries, the insects were largely eradicated by the 1950s thanks mainly to the use of effective insecticides. Recently, however, bed bugs have been mounting a comeback. Severe infestations have been reported in many large North American cities (Figure 2). Across Europe as well, bed bugs have become an unpleasant part of life. Exactly why bed bugs are

resurging is unclear. Resistance to insecticides, increased travel and a simple neglect of bed bug control have all been suggested as potential factors.

Because they feed repeatedly, it has often been speculated that bed bugs should serve as effective biological vectors. Over the years, various researchers have proposed that bed bugs might transmit a variety of diseases, including filariasis and leishmaniasis. More recently the vector competence of bed bugs to transmit the human immunodeficiency virus (HIV) and the hepatitis B virus (HBV) has been evaluated. In no case has transmission of any infectious organism to humans been demonstrated. Regarding filariasis, for example, when bed bugs were allowed to feed on humans infected with *Wuchereria bancrofti*, although microfilariae were found in the insects, they uniformly died before developing into infective L3 larvae. HIV was detected in bed bugs up to eight days following their experimental engorgement with blood containing high concentrations of

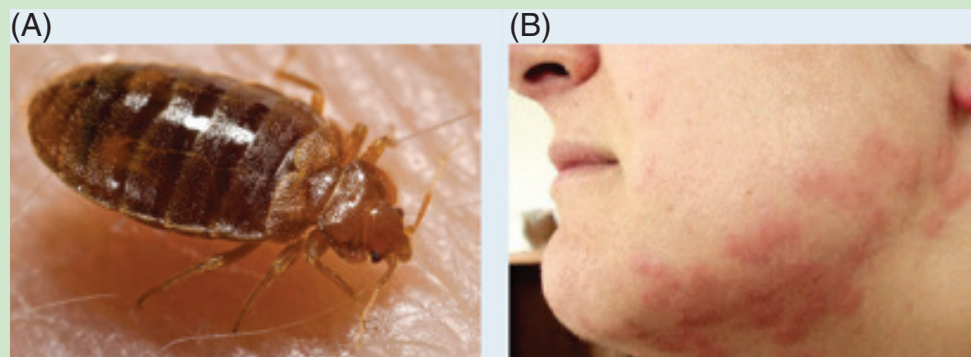


Figure 1 Bed bugs. (A) *Cimex lectularius*, the common bed bug. (B) Blistering on the face, caused by inflammation in response to bed bug feeding. Individuals vary considerably in the severity of these blisters. (A, Courtesy of CDC.)



Figure 2 Bed bugs in the Big Apple. New York City, as well as other cities across North America and Europe, have been plagued by an increase in bed bugs. (Courtesy of Rick Moser.)

virus. Yet the virus failed to replicate and no virus was isolated from insect saliva or feces. Bed bugs aren't entirely off the hook, however. Occasional transmission of hepatitis B virus between laboratory animals has been observed, but this transmission was judged to be inefficient and of limited or no importance in the natural transmission of this virus. Similarly, some reports have indicated that *Trypanosoma cruzi*, the causative agent of Chagas Disease, can be experimentally vectored between mice by *C. lectularius*.

In spite of recent high-profile court cases in which attorneys representing plaintiffs who were bitten by bed bugs in upscale hotels argued that their clients were at risk for various vector-borne diseases, there is little or no evidence indicating that they transmit disease to humans. Exactly why this is the case remains unclear. So remember; although nobody wishes to “let the bed bugs bite,” we can “sleep tight” in the knowledge that at least they're not infecting us with deadly parasites.

References

- Ibrahim O, Usama MS, & Tomecki KJ** (2017) Bed bugs: helping your patient through an infestation. *Cleveland Clinic J Med* **84**:207–211. doi:10.3949/ccjm.84a.15024.
- Roth S, Balvin O, Siva-Jothy MT et al.** (2019) Bedbugs evolved before their bat hosts and did not co-speciate with ancient humans. *Curr Biol* **29**:1847–1853. doi:10.1016/j.cub.2019.04.048.
- Salazar R, Castillo-Nevra, Tustin AW et al.** (2015) Bed bugs (*Cimex lectularius*) as vectors of *Trypanosoma cruzi*. *Am J Trop Med Hyg* **92**:331–335. doi:10.4269/ajtmh.14.0483.

Another example is provided by sand flies that when infected with *Leishmania* parasites feed more frequently. Likewise, trypanosome-infected tsetse flies seem to become more active and increase their feeding frequency, increasing the likelihood of transmission. Additionally, it may be no accident that vector-borne parasites such as trypanosomes are often among the most virulent. It has been suggested by some, for instance, that the extreme lethargy of humans infected with trypanosomes is actually a strategy on the part of the parasite; a comatose victim of sleeping sickness is in no condition to shoo away a tsetse fly in search of a meal. If this proposed scenario is in fact the case, enhanced virulence may be viewed as an adaptation to increase the likelihood of transmission. In Chapter 7 the relationship between virulence and transmission will be more fully explored.

Sexual Transmission

Eukaryotic parasites may also be transmitted via sexual contact. *Trypanosoma equiperdum*, the causative agent of dourine, a disease of equines, is an example. Another example is provided by *Tritrichomonas foetus*, a flagellated protozoan parasite that can cause serious disease in cattle. The parasite generally resides in the foreskin of bulls, in which symptoms are usually unapparent. When transferred to the female reproductive tract, however, it can cause uterine infection (pyometra), infertility and spontaneous abortion. In the past, this parasite imposed a large financial burden on the cattle industry. In recent years, the number of cases has declined as the use of artificial insemination has increased. Remarkably, however, it is now considered to be an emerging pathogen in cats, where it can cause chronic diarrhea. Unlike in cattle, it inhabits the intestinal tract and is excreted in the feces.

An additional example is *Pthirus pubis*. Commonly called crabs, these organisms are not crustaceans at all, but sucking lice—insects in the sub-order Anoplura. Crabs have prominent, grasping appendages reminiscent of crab claws, with which they hold tightly to their host's body hair (Figure 3.14). *P. pubis* only infects humans. The related *Pthirus gorillae* infects gorillas (see Figure 2.23). Although it can be found in armpits, eyebrows and other hairy body parts, it is most commonly found in the external genital region, where it feeds on the host's blood. The primary symptom is itching, which is sometimes severe. In some cases the skin takes on a characteristic bluish coloration. Transmission occurs through close contact between individuals, most commonly sexual intercourse. The lice adhere closely to their host and cannot survive for long away from the human body. Once the lice have mated, the female cements her eggs, called nits, to the host's pubic hair. Infection in children has a possible forensic

To learn more about bedbugs go to: www.cdc.gov/parasites/bedbugs/faqs.html



Figure 3.14 *Pthirus pubis*. A micrograph of a male pubic louse. Note the prominent appendages enabling the louse to hold tightly onto its host's pubic hair. They may also be found on coarse hair elsewhere on the body, such as eyelashes or armpits. *Pthirus pubis* is also known by a number of colorful names including crab louse (owing to its crablike appearance), cooties, and le papillon d'amour. Another human ectoparasitic louse, the head louse *Pediculus humanus capitis*, has a more elongated body. (Photo courtesy of CDC.)

angle; although certainly not conclusive, it at least raises the suspicion of sexual abuse.

Vertical Transmission

Vertical transmission refers to the transmission of parasites from mother to offspring, either across the placenta or through breast milk in the case of mammals or via infected gametes. *Toxocara canis*, for example, is a common nematode parasite of dogs. Puppies are frequently infected before birth as a consequence of transplacental transmission. Earlier in this chapter, we mentioned that *Alaria americana* can be transmitted through breast milk.

Babesia bigemina, the causative agent of redwater fever in cattle, relies on ticks in the genus *Rhipicephalus* as vectors. The parasite, however, can be passed from an infected female tick to her eggs, a type of vertical transmission known as **transovarian transmission**. Thus, newly hatched larval ticks are born already infected and can pass on the parasite to cattle when they take their first blood meal, making *Rhipicephalus* somewhat of a rarity among arthropod vectors. Typically, most blood-feeding arthropods must feed on at least two different hosts to serve as vectors—first to acquire an infection and then to pass the infection on to a new host. Ticks, depending on the species, use from one to three hosts and can consequently be categorized as one-host, two-host, or three-host ticks. One-host ticks are generally not involved in parasite transmission. But some *Rhipicephalus* spp. are one-host ticks, thereby breaking this rule: that is, the newly hatched and already infected larval ticks are able to transmit the parasite when they feed on their first (and only) host.

High reproductive rates are common in many parasite life cycles

A relatively high investment in reproduction is common among living things for which the likelihood that any single progeny will survive is low. Many plants and sessile aquatic animals, for instance, rely on **broadcast reproduction**. Propagules are released into the environment to be dispersed by wind or water currents. Most fail to survive. High fecundity allows these organisms to compensate for the high mortality incurred by their progeny.

Many parasites also produce large numbers of relatively small progeny to counterbalance the low probability of success of any single egg or larva. Parasites, like other organisms, must contend with various potentially lethal abiotic and biotic factors. Parasites, however, must cope with the additional problem of transmission. The high host specificity of many parasites further complicates the problem of finding a suitable host environment (see Chapter 6).

The egg-laying capacity of some helminths is truly astonishing. It is estimated that a mature female *Ascaris lumbricoides* can produce up to 200,000 eggs a day over the course of her 6–12 month life span. Even more impressive are some cestodes. The pork tapeworm, *Taenia solium*, can produce up to 300,000 eggs daily, whereas *T. saginata*, the beef tapeworm, produces as many as 700,000 eggs each day. Top honors, however, may go to *Tetragonoporus calyptocephalus*, a tapeworm of whales. These behemoths often achieve lengths of close to 40 meters. Each worm is composed of up to 45,000 segments, and we can only speculate about the vast number of eggs released each day. Cestodes have an added advantage over certain other dioecious helminths, including most nematodes and acanthocephalans, in which sexes are separate. Cestodes, on the other hand, are usually monoecious. Each segment, called a **proglottid**, is equipped with both testes and ovaries. In some species, such as *T. calyptocephalus*, there are even multiple sets per proglottid. Mating can occur between two proglottids in the same

individual or between two different tapeworms. Consequently for cestodes, the problem of finding a mate, a prerequisite for reproduction in dioecious organisms, is less of a requirement.

Most digenetic trematodes, which like cestodes are in the phylum *Platyhelminthes*, are also monoecious, although most are preferential out-crossers. Compared to cestodes, however, their egg production can appear somewhat paltry. *Schistosoma haematobium* adult females (schistosomes, unlike most trematodes, are dioecious), for example, can do no better than one or two hundred eggs daily. The other two principal schistosome species that infect humans, *S. mansoni* and *S. japonicum*, produce about 300 and between 1,500 and 3,500 eggs, respectively, each day. Certain other trematodes are more fecund. The liver fluke *Fasciola hepatica* is capable of producing over 20,000 eggs per day. Some of these trematodes live for many years, potentially resulting in a very high cumulative egg production. Yet the overall value of such a long lifespan is less clear than one might imagine. It has been known for some time that aging parasites produce fewer eggs as they become senescent. More recently it has been demonstrated that at least for *Heligmosomoides polygyrus*, an intestinal nematode of rodents, even the offspring of older parents had lower survival rates and reduced egg output, compared to worms with younger parents. Such a reduction in offspring quality may be characteristic of other parasites as well.

As previously mentioned, many trematodes undergo substantial asexual reproduction in their intermediate hosts. Some cestodes, most notably those in the genus *Echinococcus*, also reproduce asexually in the intermediate host, but this phenomenon is most pronounced in digenetic trematodes. Indeed, the name “digenean” refers to the two generations, sexual and asexual, that are characteristic of these trematodes.

In digenetic trematodes this characteristic asexual reproduction is known as **polyembryony**, the development of two or more embryos from a single fertilized egg. Following infection of the molluscan host by the miracidium, the offspring (cercariae) eventually derived from the miracidium are all clonal; except for mutations that may have occurred during cell division they are genetically identical.

Parasitic protozoa also are capable of impressive feats of reproduction. Asexual reproduction, usually via binary fission, is a common feature of the parasitic protozoan life cycle. *Entamoeba histolytica*, for instance, is the primary agent responsible for amebic dysentery in humans. This strictly human parasite has a direct life cycle. Transmission is via the fecal–oral route. The infective stage (the cyst) contains four trophozoites formed by two rounds of cell division. Following ingestion, the cyst passes through the stomach to the lower portion of the small intestine where excystation and trophozoite release occurs. These trophozoites migrate to the large intestine where they feed on cellular debris and bacteria and undergo multiple rounds of binary fission. Under certain conditions, many of which remain to be characterized, trophozoites encyst and are released with the feces.

Kinetoplastids are flagellated protozoa, which include among their ranks some of the most important human and animal parasites. Trypanosomes are responsible for both human and animal trypanosomiasis, whereas *Leishmania* spp. cause either cutaneous or visceral leishmaniasis. These parasites have indirect life cycles and use vector transmission. In all of them, there is extensive asexual reproduction via binary fission in both the vertebrate host and the arthropod vector.

The ability of kinetoplastids to undergo sexual, genetic recombination has been debated for decades; traditionally, these parasites were believed to reproduce primarily or exclusively asexually. Yet with the development

of modern techniques, numerous studies now indicate that most of these parasites also engage in genetic recombination. Evidence supporting sexual reproduction in trypanosomes was presented in [Figure 2.1](#). Similar evidence also exists for *Leishmania*. These parasites are transmitted by sand flies in the genera *Phlebotomus* (in Africa and Asia) and *Lutzomyia* (in the Americas). In the vector, replication of a flagellated stage known as a promastigote occurs in the sand fly gut. Promastigotes are ultimately transferred to the vertebrate host as the sand fly feeds. Within the vertebrate, promastigotes are ingested by phagocytic cells such as macrophages, where they develop into amastigotes. Amastigotes also undergo extensive binary fission within the phagocytic cell. Following their release from phagocytic cells, newly formed amastigotes, may be taken up by a different cell, resulting in a new round of replication. Ultimately a sand fly may ingest an infected phagocytic cell as it feeds, completing the cycle.

But in recent years, evidence has emerged indicating that *Leishmania* is also capable of genetic recombination and hence sexual reproduction. This evidence includes the direct observation of nuclear fusion between different *L. tropica* promastigotes in its sand fly vector. Similar observations have been made between different amastigotes in the mammalian host.

Do other protozoa, long believed to replicate only asexually, also at least occasionally undergo sexual reproduction? For some of these parasites, the jury is still out. To cite one such ambiguous example (see the Chapter 2 web callout on *Trypanosoma cruzi* for another), *Trichomonas vaginalis* is the sexually transmitted flagellate causing trichomoniasis in humans. Responsible for 250 million new cases of the disease annually, *T. vaginalis* is the most common sexually transmitted eukaryote in humans. Recent evidence, including surprisingly high within-population genetic variability, is suggestive of sexual reproduction. The observation that many genes associated with meiosis are found in the *T. vaginalis* genome further supports the case that this protozoan also at least occasionally reproduces sexually, or did so in its recent evolutionary past.

Apicomplexans utilize both sexual and asexual reproduction

In terms of their medical and veterinary importance, apicomplexans take a backseat to no one. We have already discussed a number of these intracellular parasites. *Plasmodium* spp. causing malaria, and *Toxoplasma gondii*, the causative agent of toxoplasmosis are two key examples. Others include *Cryptosporidium* spp., which may cause an acute form of diarrhea in both humans and animals, *Eimeria tenella*, an important cause of cecal coccidiosis in poultry, and *Babesia bigemina*, responsible for the serious cattle disease babesiosis, also known as Texas red-water fever. Life cycle details differ for each of these parasites (see the Rogues' gallery), but certain general features, including both sexual and asexual reproduction, are typical of all ([Figure 3.15](#)).

Both *T. gondii* and *Plasmodium*, for instance, undergo sexual reproduction in their definitive hosts (cats and other felines in the case of *T. gondii*, the mosquito vector in the case of *Plasmodium*). There is also a stage of asexual reproduction (**sporogony**) in each of these hosts. Reproduction in the intermediate hosts (birds or small mammals for *T. gondii*, a vertebrate for *Plasmodium*) is exclusively asexual and is known as **merogony**. Unlike binary fission, merogony is a type of multiple fission in which the nucleus and other organelles of the parasite divide several times before cell division, resulting in the simultaneous production of many **merozoites**. Merozoites may invade new host cells and undergo additional rounds of merogony or they may give rise to male and female gametes in a process called **gametogony**.

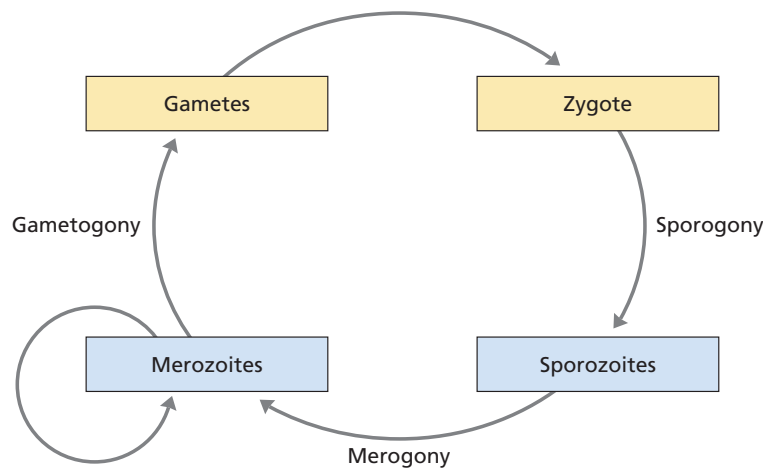


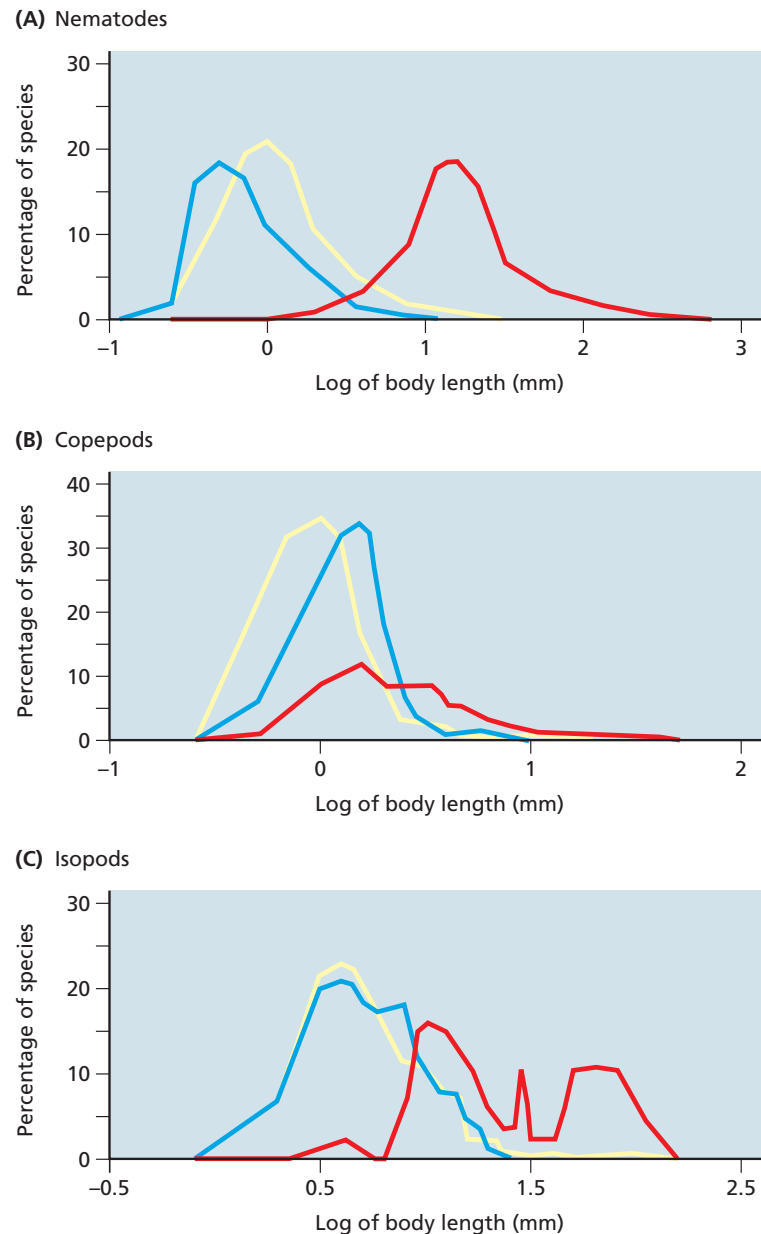
Figure 3.15 Generalized life cycle of apicomplexan parasites. Apicomplexans have a complex life cycle that alternates between sexual reproduction, during which gametes are formed, and asexual reproduction (sporogony and merogony). Merozoites and sporozoites are invasive forms resulting from merogony and sporogony, respectively. (From Wisner MF (2011) *Protozoa and Human Disease*, 1st ed. Garland Science, New York.)

Parasites may use strategies other than high fecundity to achieve transmission

A caveat must be added to our conclusions regarding the relationship between parasitism and high fecundity; things are not always as straightforward as one might assume. Like all living things, parasites must balance many competing metabolic demands, and the observed fecundity for any parasitic species represents a trade-off with other life history characteristics. For example, it has been found that parasitic copepods that use invertebrates as hosts produce far fewer eggs than those that parasitize fish. This difference is thought to reflect the relative ease of locating and attaching to a sedentary, benthic invertebrate as opposed to a highly mobile and active fish. Those relatively few eggs produced by species using invertebrates as hosts tend to be larger, reflecting the higher investment in each propagule. Numerous other factors might likewise act to influence a parasite's optimal fecundity. These factors include but are not limited to adult size, life span of both the parasite and the host and the host range of the parasite (Figure 3.16). In those species that engage in both asexual and sexual reproduction, there often seems to be a compromise of sorts regarding the amount of energy devoted to each of these two processes. Some cestodes in the genus *Taenia*, for example, reproduce asexually in the intermediate host, whereas others do not. In those that do, adult worms tend to be smaller. They also have shorter life spans relative to exclusively sexually reproducing species. Likewise, in mammalian schistosomes, some of the species with the highest rate of egg production in the definitive host produce the lowest number of cercariae per miracidium in the intermediate host.

The *Plasmodium* parasites causing malaria often show strikingly different patterns of investment in reproduction, depending on specific factors. Like other apicomplexans (see Figure 3.15 and the Rogues' gallery) cycles of merozoite formation are followed by the production of gametocytes. In the vertebrate host, asexual merogony occurs first in hepatocytes and later in red blood cells. At some point, merozoites that were produced asexually in red blood cells cease replicating and give rise to gametocytes (gametogony), the stage infective to vector mosquitoes. The optimal point of conversion from merogony to gametogony, where transmission to mosquitoes will reach a maximum, is variable according to ambient conditions experienced by the parasite. It has been predicted, for example, that if the host immune response levels off at some point, *Plasmodium* benefits from delaying gametogony and investing more heavily in merogony. Likewise, the presence of competing conspecific *Plasmodium* strains results in postponing gametogony. A steadily increasing and stronger immune response on the part of the

Figure 3.16 Frequency distribution of body sizes. Body-size distributions of free-living organisms are shown in yellow. Those parasitic on invertebrates are shown in blue, and those parasitizing vertebrates are shown in red. (A) Nematodes. (B) Copepods. (C) Isopods. Although there is much overlap, parasites of vertebrates tend to be the largest in all three taxa. Invertebrates, which are generally smaller than vertebrates, harbor somewhat smaller parasites. Free-living organisms are generally not larger than their parasitic relatives. (Adapted from Poulin R (1995) Evolution of parasite life history traits: myths and reality. *Parasitol Today* 11:342–345. With permission from Elsevier.)



host, on the other hand, favors an earlier switch to gametogony. Higher concentrations of blood-stage merozoites also result in an earlier onset of gametogony, as the parasite seeks the “sweet spot” between reproduction and transmission, where the likelihood of making it to the next host is greatest.

Many factors can complicate an understanding of parasite transmission

Obviously, transmission is a complex affair. Various factors, including those discussed in the previous section, influence the metabolic commitment that a specific parasite makes to reproduction and the likelihood of successful transmission to the next host. And that’s just the beginning. Many other variables further complicate transmission, making simple statements about its efficiency problematic.

Co-infection with other parasites is one such variable. Unlike the situation described above, where hosts are infected with multiple *Plasmodium* strains of the same species, mice co-infected with *Plasmodium* and digenetic

trematodes have more gametocytes in their blood compared to mice infected only with *Plasmodium*. Mosquitoes that feed on such mice ingest more gametocytes. These mosquitoes consequently produce more sporozoites that are subsequently passed on to the rodent host in greater numbers. Other parasites that use more than one mode of transmission might vary their transmission strategy based on specific environmental variables. The host's gender, for instance, influences transmission of the gregarine *Ascogregarina culicus*, which infects *Aedes aegypti* mosquitoes. The infective stage of the parasite, the oocyst, infects mosquito larvae. If a specific larva is female, the oocysts are retained during pupation, giving rise to an infected adult female. Oocysts are released either as the female oviposits in an aquatic habitat or when the infected mosquito dies at a breeding site. Male mosquitoes, of course, do not oviposit, and they are far less likely to visit and die at aquatic breeding sites. Infected male larvae generally die when they pupate, releasing oocysts as a consequence. Not only does this example indicate that the parasite detects the sex of its host and alters its transmission accordingly but that the parasite's virulence differs in hosts of different sexes as well. There is a long list of other factors that can influence the likelihood of successful transmission once infectious propagules have been released from a host. These include abiotic parameters such as environmental temperature, pH and salinity, as well as biotic factors such as the likelihood of hyperparasitism, of predation on parasite eggs or larvae, or of encountering and entering an inappropriate host. Some parasites can even skip life cycle stages when conditions dictate. When its definitive host is rare, the trematode *Coitocaeum parvum* dispenses with it entirely and can produce viable eggs in its second intermediate host, an aquatic crustacean instead. Miracidia released from these eggs infect the first intermediate host, a freshwater gastropod. This phenomenon of precocious maturation is called **progenesis** (see also **Figure 10.5**). Should the density of definitive hosts, freshwater fish in the genus *Gobiomorphus*, increase sufficiently, the trematode reverts to its normal three-host life cycle. Ecological and evolutionary aspects of transmission are considered in Chapters 6 and 7, respectively.

Mathematical models provide a useful tool to predict transmission rates

Because transmission can be so complex, the use of mathematical models in parasite transmission studies has great appeal. Models provide a tool with which to explain complex biological phenomena in a simplified manner. They can also reveal the critical factors that are required to fully understand transmission dynamics. The simplicity of models compared to the almost overwhelming complexity of genuine biological processes is both a strength and a weakness. It is a tall order indeed to reduce a biological phenomenon to a set of mathematical equations. Yet if the various assumptions inherent in any model are reasonable, mathematical models can have great predictive value and as such can provide valuable insight into the nature of parasite transmission.

For example, climatic conditions, specifically rainfall and temperature, affect both the survival and development of *Anopheles* mosquitoes and the *Plasmodium* species that they transmit. A model incorporating rainfall and temperature patterns in sub-Saharan Africa can predict how these climatic parameters affect the ability of mosquito vectors to transmit *P. falciparum*. This has allowed researchers to predict the intensity of malaria transmission in various geographic locations (**Figure 3.17**). Specifically, the impact of rainfall and temperature on R_0 , or the **basic reproductive rate** of infected mosquitoes, was considered. The R_0 value corresponds to the

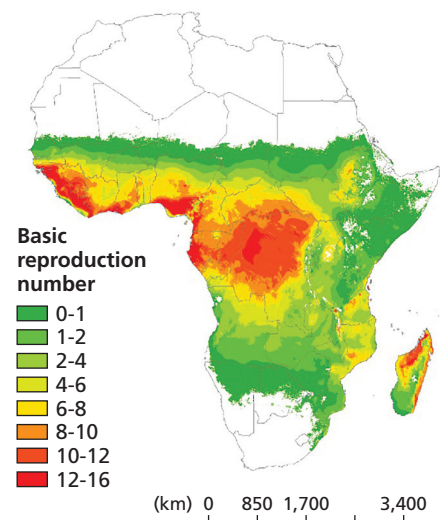


Figure 3.17 Malaria transmission based on climatic conditions. The map indicates the basic reproductive rate (R_0) for *Plasmodium falciparum* transmission, based on a model that incorporates rainfall, and temperature. Climatic conditions that promote the survival, development, and reproduction of both the parasite and the mosquito vector result in a higher R_0 . (Adapted from Ngwarakana-Gwasira ET, Bhunu CP, Masocha M et al. (2016) *Malar Res Treat* 2016:Article ID 7104291.)

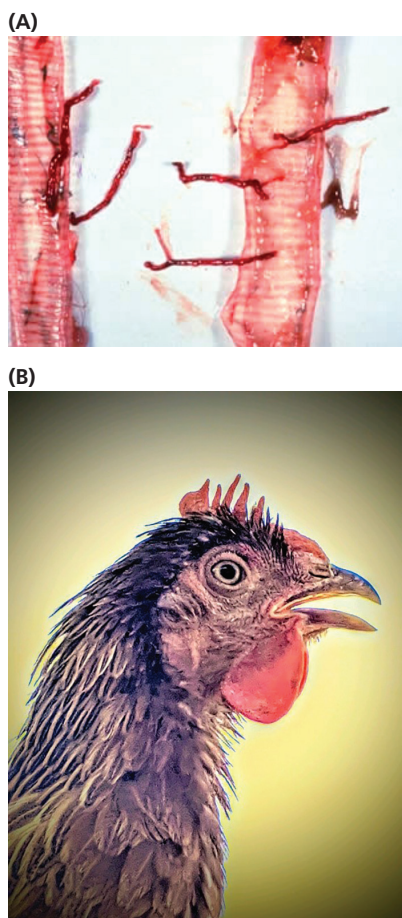


Figure 3.18 *Syngamus trachea*, the gapeworm. (A) *S. trachea* adults in the trachea of a chicken. (B) A chicken infected with gapeworm, struggling to breathe. Gapeworm in domestic birds can be prevented by keeping them from feeding on newly plowed fields where earthworms (the paratenic host) are easily available. Infected birds can successfully be treated with various anthelmintics, including ivermectin. (A Image from <https://www.merckvetmanual.com/poultry/helminthiasis/helminthiasis-in-poultry> reproduced by permission of Dr. Jean Sander. B Courtesy of Leslie Kranz.)

mean number of new cases that a single infected individual will cause in a population with no immunity to the disease in question. In this study, R_0 represented the average number of human *P. falciparum* infections caused by a single infected mosquito. When R_0 is less than one, transmission of any disease should eventually cease, provided infection rates remain constant. As R_0 rises above one, the disease will be able to spread in a population, and the larger the R_0 value becomes, the more intense the transmission. The researchers found that the ability of *Anopheles* mosquitoes to transmit malaria (that is, their vector capacity) could be predicted based on rainfall and temperature. Vector capacity was then used to develop a model that could predict R_0 , and consequently the likelihood of malaria transmission in any geographic location. It was suggested that such a model could be used to determine how transmission of malaria and other diseases might change in response to global climatic change. We will return to the topic of climate change in Chapters 8 and 10.

Mathematical models have repeatedly demonstrated their value in predicting the effectiveness of various intervention strategies to disrupt transmission. *Schistosoma japonicum*, for instance, is unusual in that, unlike other important human schistosomes, this species uses a wide variety of mammals as definitive hosts. By assessing the number of eggs released in the feces of various mammalian hosts, including humans, researchers developed a transmission model that assessed the importance of different mammalian hosts in overall *S. japonicum* transmission in the Philippines. The model predicted that larger animals like water buffaloes and dogs played a relatively slight role in eventual transmission to humans. Smaller mammals, including rats, were of much greater importance. This finding suggests that control efforts aimed at reducing infection in water buffaloes or dogs will have little impact on overall infection rates in humans. Rat control, however, could pay large dividends in reducing human disease prevalence and intensity. We will revisit the topic of modeling parasite transmission in Chapter 6.

Many parasites must migrate to specific sites or tissues within the host

Reaching and entering an appropriate host is just the beginning. Once they have gained entry, many parasites make elaborate and often bewildering migrations within the host in an effort to arrive at a particular location. Such **site specificity** is a common feature in many parasitic infections. *Leishmania* spp. are found only in macrophages within their vertebrate host, whereas *Plasmodium* is restricted to either hepatocytes or erythrocytes in mammals, depending on the developmental stage. Poultry raised in backyards is susceptible to blackhead, a serious disease caused by the flagellate protozoan *Histomonas meleagridis*. These parasites show a particular tropism for the cecum. They reach their favored location by infecting the eggs of a different parasite, *Heterakis gallinarum*, a nematode that also inhabits the cecum of ground-feeding poultry, like chickens and turkeys. As a parasite of a parasite, *H. meleagridis* is an example of a **hyperparasite**.

The protozoan trophozoites are extremely fragile and only remain viable for a few hours if released into the environment. After infecting the eggs of *H. gallinarum*, however, they are passed with the feces into the soil, where they can remain infective for up to two years. Birds become infected as they inadvertently ingest the nematode eggs while feeding. Once the nematode eggs hatch, the *H. gallinarum* larvae release *H. meleagridis* in the bird's cecum. Alternatively, the eggs may be consumed by an earthworm, which serves as a paratenic host that might eventually be consumed by a bird.

A related nematode of poultry, *Syngamus trachea*, infects not the cecum but the trachea. Here they cause respiratory symptoms resulting in gasping and coughing, giving rise to the common name for the parasite: the gape-worm (Figure 3.18). As an infected bird struggles to breathe, it may cough up eggs, which are subsequently swallowed. Larval worms molt twice inside the egg as they transit through the digestive system. Eggs are ultimately passed with the feces. As with *H. gallinarum*, eggs can either be ingested directly by a bird or they may reach a bird indirectly, after first being consumed by an earthworm. Once the bird consumes the eggs, they hatch in the gut, whereupon the infective larvae penetrate the gut wall. The larvae are then carried by the blood to the lungs, where they penetrate through the capillaries into the lung spaces. A final climb up the bronchi finally brings them to the trachea, where they remain as adults.

These few examples raise several questions. First and perhaps easiest to answer is, why are many parasites so site specific? From the parasite's perspective, any host offers hundreds or even thousands of habitats that may be colonized. Each of these potential habitats differs in pH, nutrient availability, oxygen concentration and other factors. All parasites must cope with the host immune response, and this response varies in different body regions. Considering all the variables that confront any parasite in the host environment, site specificity becomes easily understandable in terms of adaptation to a site-specific range of conditions. It is an unusual parasite indeed that can successfully survive and reproduce anywhere within the host environment. Complicating such adaptation to specific conditions are the other parasites with which any given species must contend. Although it has proven difficult to demonstrate that competition within a host takes place (to be discussed in Chapter 6), it seems likely that at least in heavy infections, interspecific competition further enforces site selectivity, resulting in parasite habitat specialists able to out-compete other species in their preferred location.

This is not to say that all parasites are restricted to highly specific niches within the host. The tissue cyst stages of tapeworms in the genera *Taenia* and *Echinococcus*, for instance, may be found in various tissues or organs. Yet such cysts tend to be in a highly predictable, relatively short list of potential sites within the intermediate host.

A second question is, how do parasites manage to find their preferred location? The ability to home in on such a site may seem less problematic for ectoparasites, many of which take site specificity to something of an extreme. Certain lice on birds, for example, only attach to feathers with barbs of a specific diameter (Figure 3.19). The monogenean trematodes that parasitize fish in some cases limit themselves to a particular gill filament. In such cases, specificity is often a consequence of morphological adaptations for attachment, which permit strong adherence only at particular sites.

Internal parasites have a more complex task. Navigation within an environment that is dark, churning with digested food or flowing blood, and full of molecules like antibodies designed to impede progress is far from trivial. The host environment, however, although complicated, is predictable. Because of anatomic similarity among members of the same host species and because of homeostatic mechanisms that maintain relatively constant chemical conditions in specific regions of the body, parasites are presented with a predictable set of navigational cues. Over evolutionary time, parasites have evolved to recognize such cues and alter their migration within the host accordingly.

It has been shown that those parasites undergoing complex migrations within their hosts actually key in on a very small number of the environmental stimuli to which they are exposed. That is, such parasites live in a relatively limited sensory world and respond to only a few of the environmental

For those wishing to know more about parasites of poultry, you may wish to visit: https://ohio4h.org/sites/ohio4h/files/imce/animal_science/Poultry/External%20Parasites%20of%20Poultry%20-%20eXtension.pdf and <https://midwestpoultry.com/wp-content/uploads/2019/03/Dr.-Fred-Dustan-Clark-MPF-2018.pdf>



Figure 3.19 Site specificity in feather lice. *Columbicola columbae* are lice specializing on rock pigeons. Like other feather lice, they are usually closely adapted to their avian host, with appendages that match the diameter of the individual feather barbs. By specifically attaching to barbs of a particular diameter, the lice become much less vulnerable to being removed by the bird as it preens its feathers. (Courtesy of Jack Scott, University of Alberta.)

signals that they encounter. Furthermore, unlike many animals that navigate by following a concentration gradient of airborne or waterborne chemicals, parasites are far less likely to rely on chemical gradients. In a tightly constrained environment, such as an intestine or blood vessel, such gradients cannot be established because fluid within the space is continually bounced back and forth as it encounters anatomical barriers. Consequently, animal parasites exhibit genetically programmed stereotypic behaviors when they encounter a particular threshold level of a given stimulus. Such behaviors help ensure that they respond in an appropriate manner.

As an example, consider the liver fluke *Fasciola hepatica*, and its movement from the intestine to the liver. When a mammal consumes the infective metacercaria, contact with low stomach pH initiates excystment. Once the partially excysted larva reaches the duodenum, it responds to the presence of bile with violent spasms that release it from the cyst. Only bile causes this response. These same spasms in tandem with secreted lytic enzymes allow the fluke to penetrate the intestinal lining into the peritoneal cavity. Within the cavity, the nonfeeding larva adheres via its anterior and posterior suckers to the endothelium lining the cavity. It subsequently begins to move along the peritoneal endothelium until it eventually reaches the liver. Interestingly, it does not necessarily navigate directly toward the liver and no sophisticated orientation is required. Because the peritoneal cavity is like the inside of a sphere, movement in any direction will eventually bring the fluke into contact with the liver, located in the anterior portion of the cavity, directly beneath the diaphragm. Once it finds the liver parenchyma, it begins to feed upon liver tissue.

Recent advances in genomics and transcriptomics are helping to further clarify the manner in which parasites recognize certain host physiological cues and utilize them in their migration. Phylum-wide genomic analysis in nematodes, for instance, confirms the fact that parasites have a greatly reduced complement of genes encoding chemoreceptors compared to free living species, although most of the downstream molecules involved with signaling pathways are retained. Furthermore, at least in certain filarial worms, including *Brugia malayi* (see the Rogues' Gallery for a description of its life cycle and migratory route through its hosts), a species causing human filariasis, different chemoreceptors are expressed at different points in the parasite's migration. These chemoreceptors belong to the G-protein linked receptor family and are found on specialized cilia in **amphids**, which are anterior innervated, invaginations of the cuticle serving as chemosensory organs in nematodes. Although many questions remain, these results suggest that different chemoreceptors recognize different host ligand molecules at key points in parasite migration, helping to guide the parasite through both its mosquito intermediate and human definitive hosts.

Less motile protozoan parasites often rely on an affinity between certain molecules on their surfaces and other molecules found on target tissue membranes to locate their preferred site within the host. *Toxoplasma gondii*, for instance, initially binds to the intestinal epithelium in the intermediate host, utilizing an adhesion protein called mic2, which binds the host protein ICAM-1. This interaction allows the parasite to cross the epithelia by squeezing between epithelial cells in a process called **diapedesis**. Other proteins in the mic family may also be involved as are other host molecules, most of which remain poorly described. Having traversed the intestinal epithelium, the parasite infects macrophages and dendritic cells found in the underlying lamina propria. *T. gondii* merozoites penetrate these phagocytic cells using **gliding motility**, a mode of locomotion unique to the apicomplexans. This type of movement occurs only on substrates

"OpenWorm," is an international open science project with the goal of developing a virtual simulation of the nematode *Caenorhabditis elegans*, in which all 302 neurons and 95 muscle cells are modeled. You can visit their website at: openworm.org. There you will find a model of the neural "connectome" (a comprehensive map of neural connections in the brain that may be thought of as its "wiring diagram") and a 3D model of the worm's anatomy.

and in the absence of locomotory organelles and involves the interaction of the parasite surface proteins with actinomyosin found beneath the parasite cell membrane in the cytoskeleton. In effect, the myosin motor proteins moving along actin filaments pull the apicomplexan along the substrate. In this case, such movement propels the parasite into the host cell. Infected phagocytic cells then disseminate the parasite throughout the host as they migrate. Interestingly, *T. gondii* has been found to release specific proteins within infected phagocytes that subsequently decrease the adherence of these infected host cells and enhance their motility. In effect, the parasite has reprogrammed the cell to further its own spread through the host.

Although *T. gondii* is an extreme generalist in its intermediate host, able to infect both birds and mammals, sexual reproduction only takes place in felines. A recent fascinating study reveals the mechanism accounting for this host specificity in the definitive host. Cats are unusual in that they lack an enzyme called Δ -6-desaturase in their intestine. In other mammals, this enzyme metabolizes linoleic acid. In felids, the absence of the enzyme results in an accumulation of this common, polyunsaturated fatty acid. And it is this buildup of linoleic acid, which occurs only in cats, that signals *T. gondii* to initiate its program of sexual development (Figure 3.20). This represents the first defined mechanism for species specificity in a eukaryotic parasite sexual cycle.

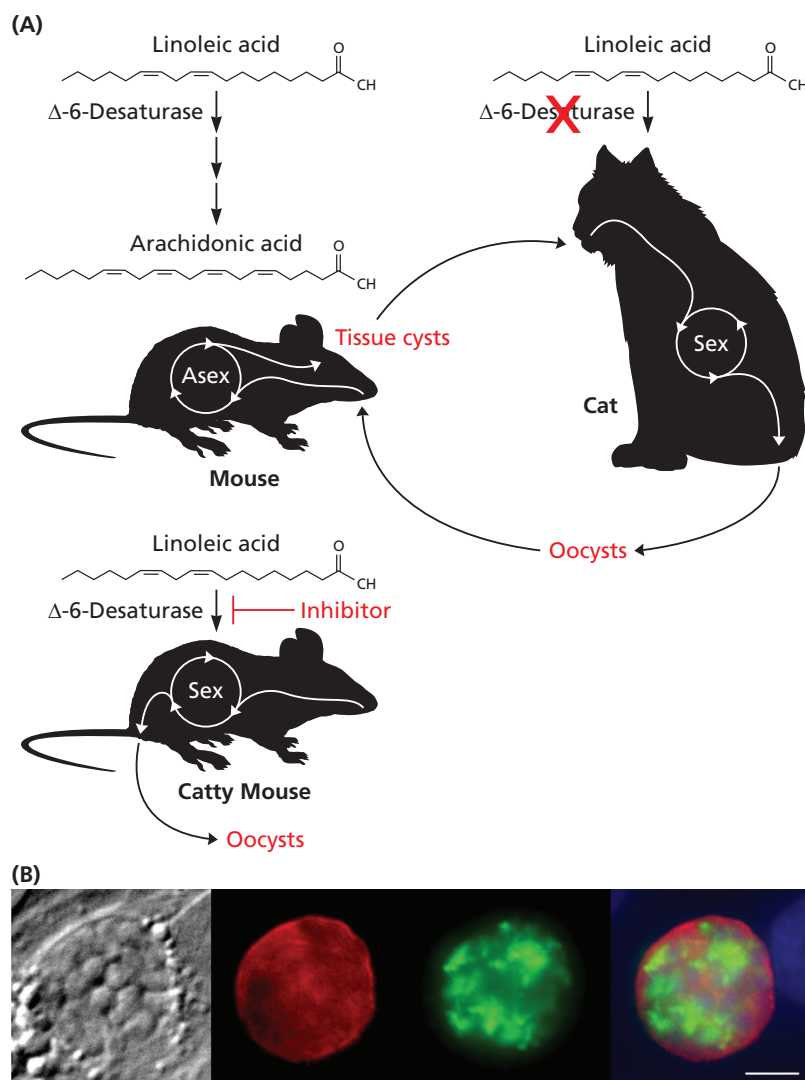


Figure 3.20 Explanation of mechanism controlling host specificity of *Toxoplasma gondii* in cats. (A). Normally *T. gondii* develops sexually only in cats, producing oocysts passed in the feces. Note the lack of the Δ -6-desaturase enzyme in the intestine of cats that allows linoleic acid to accumulate, unlike in the intestine of other mammals. In the “catty mouse,” artificially increasing linoleic acid levels permitted sexual development and oocyst production to occur. (B). Shown is a series of images of mouse intestinal cells infected with *T. gondii* in the presence of increased levels of linoleic acid. The use of red and green fluorescent stains for the parasite show clear evidence of developing parasite cells in the integrated image on the right. Scale bar 5 μ m. (A, Adapted from English ED & Striepen B (2019) *PLoS Biol* 17:e3000446. doi:10.1371/journal.pbio.3000446; B, from Martorelli DB, Wilson SK, Dubey JP & Knoll LJ (2019) *PLoS Biol* 17:e3000364. doi:10.1371/journal.pbio.3000364.)

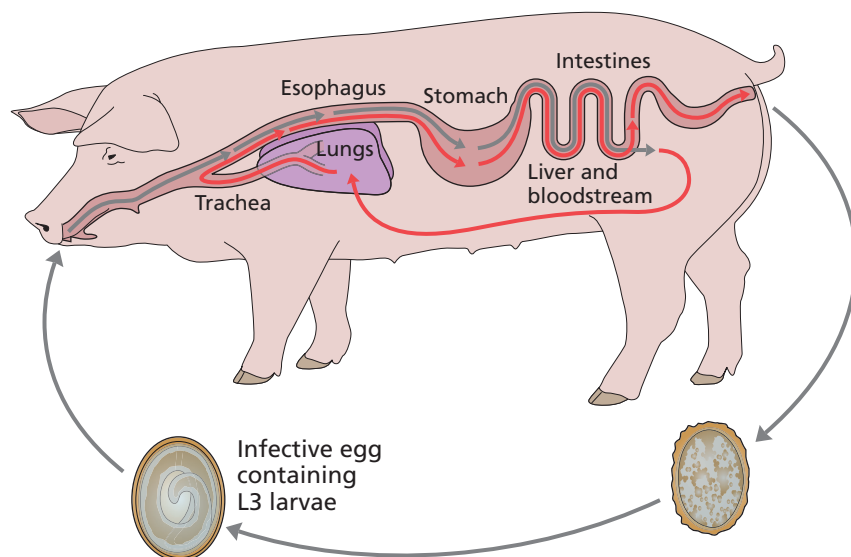
The evolution of complex migration within a host is not always clear

Another question relates to the evolution of the complex migration observed in some parasites. The tissue migration of many intestinal nematodes with direct life cycles, for example, appears to defy logic. *Ascaris suum* is illustrative (Figure 3.21). This species is a common intestinal roundworm of swine and is similar to (and arguably even the same species as) the human parasite *A. lumbricoides*. As in all nematodes, there are characteristic life cycle stages. Fertile eggs are produced by the adult female. A first stage larva, called an L1 larva, develops within the egg. The larva undergoes four molts that are followed by periods of growth giving rise to L2, L3 and L4 larvae and ultimately to an adult. The larval stage that emerges from the egg may be the L1, L2 or L3 larva, depending on the species. In the genus *Ascaris*, following ingestion by the host, an L3 larva, still covered by the L2 cuticle, emerges from the egg in the small intestine. Note that some researchers prefer to call immature nematodes juveniles instead of larvae and thus refer to the L1 to L4 stages as J1 to J4.

Following the ingestion of infective eggs, the L3 larvae emerge in the intestine and embark on a seemingly byzantine trek that starts when they penetrate the intestinal epithelium. They then enter the circulatory system, where they pass through the liver and are subsequently transported to the lungs. They next break into the alveoli, migrate up the trachea and return to the intestine via the esophagus, when they are swallowed. Why do the larvae leave the intestine, if their only goal is to return there? Why do emerging larvae not simply remain in the intestine, maturing to adulthood, without migrating through the tissues?

One possibility is that *A. suum* evolved from a species that had an indirect life cycle and tissue migration in the ancestral intermediate host was required for proper larval development. According to this hypothesis, the developmental requirement for tissue migration was retained as *A. suum* evolved a direct life cycle. Alternatively, it has been observed that nematodes such as *A. suum* that undergo extensive tissue migration grow faster and are larger than nonmigrating nematodes. Because larger nematodes are more fecund, the maintenance of tissue migration over evolutionary time may be a selectively advantageous life history strategy. Immune evasion has also been suggested as the selective pressure behind migration. Specifically,

Figure 3.21 Internal migration of *Ascaris suum* within the pig. *Ascaris suum* undergoes a complex migration within its host. Infection occurs when the pig consumes infective eggs in which the larvae have developed into infective third stage (L3) larvae, still covered with the L2 cuticle. The eggs hatch in the intestine, whereupon the larvae penetrate the intestinal epithelium. After entering the circulatory system, they pass through the liver where the L2 cuticle is lost, eventually reaching the lungs. After penetrating through the capillaries into the alveoli, larvae molt into L4 larvae. These L4 larvae migrate up the trachea. They then return to the intestine via the esophagus. Having returned to the intestine, L4 larvae undergo a final molt into the adult form. Adults remain in the intestine, where reproduction occurs.



migration within a host may have evolved as a way to avoid immune defenses on mucosal surfaces.

Finally, before we leave the topic of parasite migration, it is worth noting that such migration is often unsuccessful; migrating stages frequently become lost and many are killed as a consequence of host immune responses. Aberrant migration is particularly common when infective stages enter an inappropriate host, in which the anatomy, the necessary navigational cues or both are sufficiently different from those found in the normal host. A well-known example involves the infection of humans with hookworms that normally infect dogs or other animals. When encountering their normal host, hookworm larvae penetrate through the skin and subsequently migrate through the circulatory system and lungs, eventually reaching the intestine, where they mature into adults. Consider, however, *Ancylostoma braziliense*, which typically infects dogs. When infective *A. braziliense* larvae contact human skin, they may still penetrate the outermost epithelial layer. They cannot, however, move through the deeper basal layer. At this point they may simply wander aimlessly through the skin, leaving a trail of inflammation in their wake. The inflamed, itchy skin condition is called **cutaneous larval migrans** (Figure 3.22).

Parasites are adapted to maintain their position on or within the host

The host environment is not particularly conducive to a stationary existence. Parasites inhabiting the digestive tract must contend with powerful peristalsis. Those found in the circulatory system must cope with the surge of rushing blood. Ectoparasites are confronted with environmental factors such as wind or rain or host behaviors such as grooming that make remaining in place more problematic.

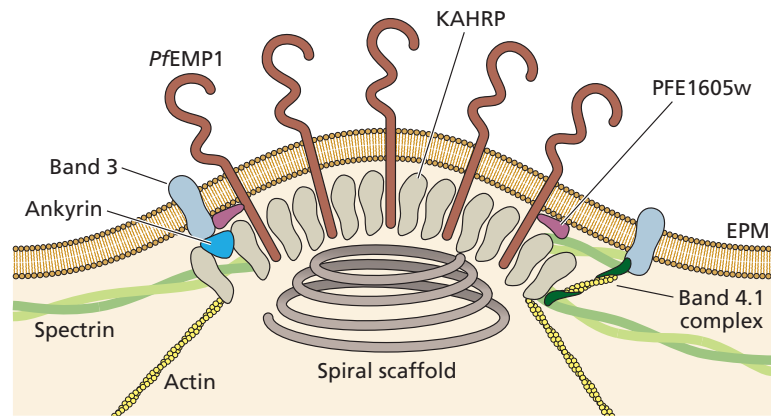
We have already mentioned some of the strategies used by various parasites to overcome this obstacle. Gut parasites such as *Toxoplasma gondii* may invade the intestinal epithelium, or like *Giardia lamblia*, they may adhere with adhesive disks (see Figure 3.9). Acanthocephalans and tapeworms rely on their proboscis or scolex, respectively, whereas many intestinal nematodes utilize powerful longitudinal muscles to maintain their location via vigorous sinusoidal motion. Trematodes generally come equipped with powerful ventral suckers or **acetabula** that assist in adherence. Male and female schistosomes form pairs with the female lying within the gynephoral canal of the male. A male with its more powerful musculature and with the aid of its oral sucker and acetabulum moves the pair upstream into smaller venules where the pair becomes lodged. Some *Plasmodium* species, alternatively, having adhered to and entered erythrocytes, enjoy a moveable feast of sorts, circulating through the host in the safety of their cellular shuttle. *P. falciparum*, on the other hand, the most pathogenic of the human malaria parasites, takes a different tack. Once inside erythrocytes, this species encodes a protein called *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) that protrudes from the surface of infected cells (Figure 3.23). This protein is involved in the formation of knobs, which bind the infected cells to capillary walls and consequently interferes with blood flow. Such interference, to be discussed more fully in Chapter 5, figures prominently in the severe pathology caused by *P. falciparum*.

Ectoparasites also employ various adaptations to maintain their position in a specific site. Of particular interest are the parasitic copepods, some of which appear to have stretched morphological plasticity to the limit in terms of their specific adaptations for adherence. For instance in



Figure 3.22 Cutaneous larval migrans. When infective L3 larvae of canine or feline hookworms such as *Ancylostoma braziliense* penetrate human skin, they are unable to reach the circulatory system. As the larvae migrate aimlessly, they provoke a telltale trail of inflammation. Older homes often have crawl spaces underneath them, where pets may defecate. Because plumbers often had to enter crawl spaces to work on piping, they were frequently at elevated risk for this condition, which in consequence is sometimes referred to as plumber's itch. An alternative common name is creeping eruption.

Figure 3.23 *Plasmodium falciparum* adherence. Once it has invaded red blood cells, the parasite produces the protein pfEMP 1, which, in association with other host and parasite proteins, protrudes from the host cell membrane where it binds to capillary epithelial cells. By binding to capillaries in this way, *P. falciparum* avoids transport to the spleen, where parasite-infected cells might be detected and destroyed. Parasite-infected erythrocytes may accumulate in the capillaries of the central nervous or renal system, resulting in much of the severe pathology associated with *P. falciparum* infection. (Adapted from Gesa Helms, Anil Kumar Dasanna, Ulrich S. Schwarz, Michael Lanzer, published under CC BY-SA 4.0.)



Chapter 1 we discussed *Ommatokoita elongata*, the females of which imbed themselves permanently in the cornea of Greenland and Pacific sleeper sharks (see **Figure 1.16**). Another noteworthy parasitic copepod is *Pennella balaenopterae*, the colossus of the copepod world. Over 30 cm in length, these copepods along with other members of the family Penellidae have the distinction of being the only copepods to parasitize whales (**Figure 3.24**). Like other members of its family, *P. balaenopterae* has lost all traces of external segmentation, and its locomotory appendages are largely vestigial in adults. Females of this species, however, more than compensate for these anatomical losses with the development of two prominent anterior anchoring appendages. Each of the two processes expands and bifurcates, allowing the female to anchor herself into the host's skin.

Finding a mate is a requirement for many sexually reproducing parasites

The site specificity we have been discussing, as well as the adaptations that permit it, serve another purpose; for sexually reproducing parasites it facilitates finding a mate. This, of course, is not a problem for asexual or nonreproducing life cycle stages. Monoecious species are often preferential outcrossers, but for those that readily self-fertilize, finding a mate is of reduced importance. However, for dioecious sexually reproducing parasites, finding a member of the opposite sex is less problematic when both sexes



Figure 3.24 *Pennella balaenopterae*. Female copepods embedded in the blubber of a striped dolphin (*Stenella coeruleoalba*) via their modified anterior appendages. (From Fraija-Fernandez N, Hernandez-Hortelano A, Ahuir-Baraja AE et al. (2018) *Dis Aquatic Orgs* 128:249–258. doi:10.3354/dao03226. With permission.)

are homing in on the same target tissue. In this sense, parasites conform to the **Allee effect**. This population biology concept notes that in small populations, individual fitness will increase as population density increases. It has even been proposed that for dioecious parasites with indirect life cycles, trophic transmission evolved as an efficient way for parasites to meet a sexual partner, as it serves to concentrate parasites at a single location.

Once they have arrived at their site of choice, evidence suggests that many parasites rely on chemoattractants to bring them into physical contact. In echinostomes, for example, lipophilic chemoattractant factors have been characterized. Schistosomes, in particular, have attracted considerable attention in this regard. As we will see in Chapter 5, egg production by female schistosomes is central to the pathology caused by these digenetic trematodes. Consequently, if the signals responsible for mate pairing could be elucidated and somehow disrupted, female egg production would be negatively impacted. This would in turn lessen both pathology and transmission. Although the identification of the factors that attract the sexes to one another and that the male utilizes to induce egg production in the female remains elusive, considerable progress has been made in identifying a role for organic bases called biogenic amines, potentially including serotonin, dopamine or histamine. G protein-coupled receptors, the largest group of transmembrane receptors in animals, have also been implicated in male–female pairing.

Propagules are released through a portal of exit

Following parasite reproduction within a host, eggs or larvae must often be released to the external environment or must pass directly to the next host in the life cycle. The anatomical structure through which propagules move is called the **portal of exit**. For parasites of the gastrointestinal tract, the usual portal of exit is the anus. Vector-borne parasites rely on the feeding behavior of an arthropod both for host ingress and egress. Genital contact serves this same dual function for sexually transmitted parasites such as *Trichomonas vaginalis*. It is not always true, however, that the portal of exit is part of the same anatomical structure or even the same organ system as the one through which the parasite enters a host. Hookworms, for instance, infect their definitive host when larvae burrow through the skin. Following an extensive migration, they ultimately arrive in the intestine where they mature sexually. Fertile eggs exit the definitive host with the feces. In the schistosomes, cercariae enter the definitive host by direct penetration of the skin. Eggs, however, are released through the feces or the urine, depending on species (Figure 3.25).

Parasites undergo complex developmental changes in response to environmental cues

The developmental changes that occur in all living things during their lives are especially noticeable in those organisms that use very different habitats at different points in their life cycle. Striking changes, for instance, in both body plan and physiology are typical of insects with aquatic larvae and terrestrial adults. Many parasites also undergo dramatic developmental changes as they move from one habitat or host to the next. We know a great deal about precisely *what* developmental changes transpire during a parasite's life cycle. Although we know considerably less about *how* these changes occur, in recent years substantial progress has been made regarding the mechanisms underlying developmental change. Advances in fields such as transcriptomics have started to allow the identification of the



Figure 3.25 Portal of exit for *Schistosoma haematobium*. Although the infective stage, the cercaria, enters a human by direct penetration of the skin, the portal of exit for *S. haematobium* is the urine. Bloody urine is a common sign of *S. haematobium* infection, caused by damage to the circulatory epithelium during the transit of eggs into the bladder. Children in Niger holding urine samples indicative of heavy infections. (Courtesy of Juerg Utzinger, University of Basel.)

To learn more about schistosomiasis and how the Carter Center is involved in its control, visit: <https://www.cartercenter.org/health/schistosomiasis/index.html>

environmental signals used by parasites to initiate developmental change, as well as how these signals are detected, transduced, and how they impact gene expression at various life cycle stages.

Not surprisingly, most developmental changes occur either in anticipation of transmission or upon arrival in the body of a new host. These, of course, correspond to those times in the life cycle of any parasite when the environment is either about to change or has suddenly changed most radically. And it is evident that parasites actively monitor their environment and, in doing so, regulate their developmental changes to best ensure either successful transmission or survival in the new host.

Parasite development does not occur on a strict timetable; parasites do not simply charge ahead and undergo developmental changes at a defined time, no matter the circumstances. On the contrary, parasites interact with a complex array of signals of both host and parasite origin. These signals indicate when the time is right to proceed to the next developmental stage. As previously discussed, for instance, *Plasmodium* spp. switch from merogony to gametogony in response to host immune response, as a consequence of competition with other blood-borne parasites, or other factors.

The mammalian bloodstream form of *Trypanosoma brucei* undergoes a change from long and slender to a shorter form known as “stumpy” in preparation for transmission back to the tsetse fly vector. Development into this transmissible form is controlled by the parasites themselves, which monitor their density in the blood. In a process very similar to the quorum sensing observed in many bacteria, the slender forms release a signal called stumpy induction factor (SIF). This soluble protein has only recently been characterized. When slender forms are exposed to enough SIF, indicating that the population of parasites in the blood is sufficiently high, the signal to develop into the stumpy form passes a threshold and development proceeds (Figure 3.26).

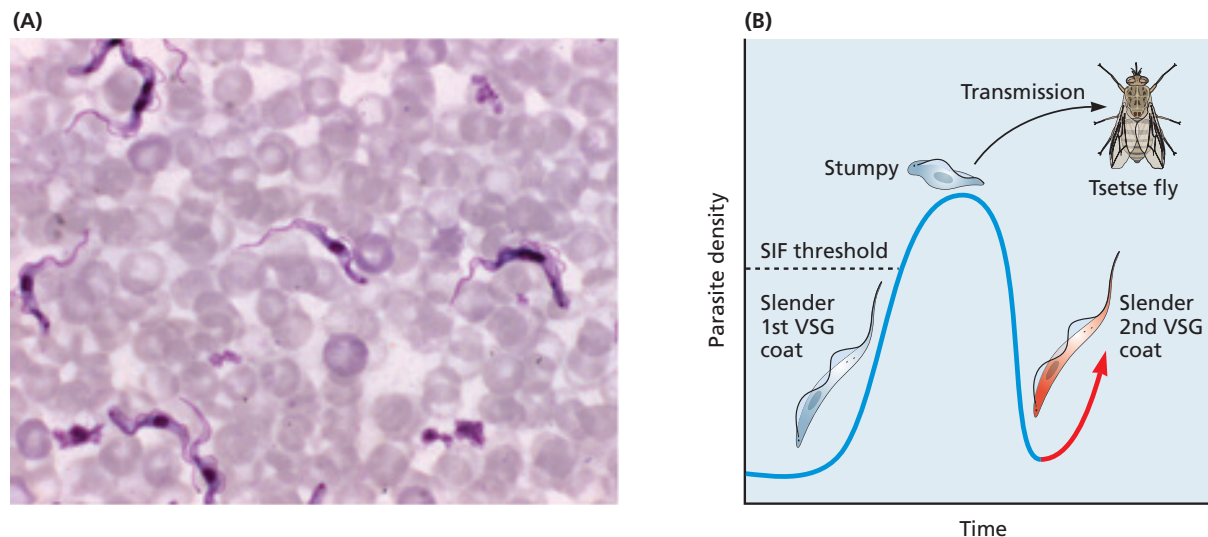


Figure 3.26 Trypanosome developmental stages. (A) Blood smear from an animal infected with *Trypanosoma brucei*. Slender forms of the parasite are visible. The stumpy form, as the name suggests, has a somewhat shorter, thicker morphology. (B) The progression from slender to stumpy form is in response to stumpy induction factor SIF, released by slender forms. As slender forms replicate in the blood, parasitemia rises, as does the concentration of SIF, inducing some parasites to differentiate into nonreplicating stumpy forms that are transmissible to the tsetse fly vector, once a threshold in the blood concentration of SIF is reached. As slender forms differentiate into stumpy forms, most of them are cleared by the host immune response, which recognizes a surface antigen on the parasite called VSG. This leads to a crash in parasitemia. However, some slender forms are able to switch the form of VSG that they display on their surface, and these parasites are not recognized immunologically. This allows a second wave of parasitemia, as parasite numbers once again increase. (A, Courtesy of CDC, Mae Melvin; B, Adapted from Pollitt LC, MacGregor P, Matthews K & Reece SE (2011) *Trends Parasitol* 27:197–202. With permission from Elsevier.)

Metazoans too seem to adjust their life cycle in response to such environmental cues. Filarial worms, for instance, increase their production of microfilariae in response to more aggressive host responses.

Parasitic nematodes with free-living larval stages often arrest the development of those stages when external environmental conditions are not compatible with larval survival. Such developmental arrest, called **hypobiosis**, makes sense from an adaptive perspective, as development in parasites is usually irreversible; once a parasite undergoes a developmental change, there is no going back. Consequently, the parasite must regulate development carefully to prevent premature development that may not allow survival or transmission. Hypobiosis may either be induced or ended as specific environmental cues are detected.

Both hookworms and strongyloids are well-known examples. The stimuli that induce such arrest vary according to species, but they are generally assumed to include factors such as temperature, soil moisture, changing photoperiod, or some combination of these and other abiotic factors. Many nematodes are likewise able to arrest their development inside the host when conditions warrant. The microfilariae of filarial worms, for instance, may circulate in the blood for months without further development until their arthropod vector consumes them. It has more recently become clear that developmental arrest may occur in protozoa as well. In *T. brucei*, for example, progression to the transmissible stumpy form described above is repressed by a tyrosine phosphatase, which maintains the parasites poised for developmental change once the appropriate signal is received.

Developmental change is associated with changes in gene expression

The dramatic changes occurring in parasites as they transition through life cycle stages are accompanied by substantial alteration of gene expression and protein synthesis. *Giardia lamblia* provides a protozoan example. As previously discussed, the *G. lamblia* life cycle consists of two stages: the actively feeding and reproducing trophozoite and the transmission stage cyst. The encystation process consists of an early and a late phase. During the early phase, the adhesive disc and the cytoskeleton disassemble and the cell starts to round up. During the late phase the nuclei divide, DNA is replicated and the cyst wall is completed. As illustrated in **Figure 3.27**, these structural changes are accompanied by extensive changes in gene expression.

G. lamblia is by no means unique in this regard. Stage-specific gene expression has been demonstrated for a wide range of protozoa. The apicomplexan *Cryptosporidium parvum*, for instance, is an epicellular parasite of the intestinal epithelium. Its life cycle consists of both an extracellular,

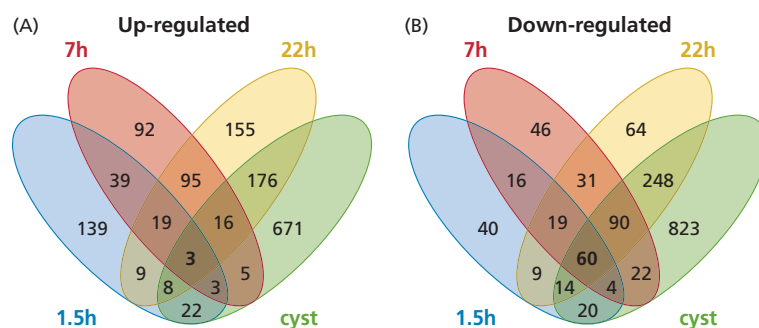


Figure 3.27 Differential gene expression during encystation in *Giardia lamblia*. Venn diagrams showing the number of genes that are up-regulated (A) or down-regulated (B) compared to non-encysting trophozoites at different time points following the induction of encystation. Proteomic and transcriptomic data were obtained at 3-time points following the induction of encystation (1.5, 7 and 22 hours) and from mature cysts. For example, compared to non-encysting trophozoites, 139 genes were uniquely upregulated at 1.5 hours post-induction, while 39 genes were expressed at both 1.5 and 7 hours post-induction. Other values similarly show the number of genes up- or down-regulated in one or more times following induction compared to trophozoites. (Adapted from Einarsson E, Troell K, Hoepfner MP et al. (2016) *PLoS Negl Trop Dis* 10:e0004571. doi:10.1371/journal.pntd.0004571.)

invasive stage (the sporozoite) and intracellular stages (the merozoite or the gamont-see the Rogues' gallery for details of the life cycle). This later stage is often referred to as **epicellular** rather than intracellular, as the parasite remains surrounded by host membrane material, which forms the parasitophorous vacuole. There are 3,774 known protein-encoding genes in its genome. Transcriptomic analysis has shown that 173 of these genes are upregulated during the sporozoite stage, with 1,259 upregulated during intracellular stages. In *Leishmania major* approximately 9% of whole-genome expression is altered as the parasite moves from sand fly vector to mammalian host.

Differential gene expression in different life cycle stages is also common among helminth parasites. Schistosomes, for example, undergo dramatic changes in morphology and cellular composition in the course of their life cycle. These changes are accompanied by extensive alterations in gene expression patterns (see Chapter 2, **Figure 2.35**). Genes that are up- or down-regulated in a stage-specific manner often have functions related to nutrient acquisition, calcium signaling, lipid metabolism and subversion of the host immune response. In *Clonorchis sinensis*, metacercariae express high levels of genes coding for cytoskeletal proteins, relative to adult worms. In both the hookworm *Ancylostoma caninum* and the strongyloid nematode *Strongyloides ratti*, transition from the free-living to the parasitic state is accompanied by significant transcriptome change. In *A. caninum* specifically, infective L3 larvae were found to up-regulate over 600 genes. These genes include many related to pathogenesis and proteases, which are thought to be involved in both morphological restructuring and host penetration.

Once a parasite successfully reaches a new host, rapid development is often imperative. Conditions within the newly acquired host may differ radically from those in the previous environment, necessitating speedy change. One common strategy seems to be to hold pre-made mRNA molecules ready for translation as soon as needed. In *Plasmodium*, for instance, mRNA is held quiescent in gametocytes inside cytoplasmic granules called P granules. These mRNA transcripts will be translated following ingestion of gametocytes by the mosquito vector. The signals that activate translation may include the sudden drop of temperature within the mosquito vector, the sudden rise in pH within the gut, and the presence of xanthurenic acid, a component in the mosquito pigment pathway. This strategy allows the parasite to remain poised for action as soon as transmission to a new host is achieved. A comprehensive analysis of protein kinases in the rodent parasite *Plasmodium berghei* identified 66 such enzymes as components of signal transduction cascades regulating development. Twenty-three of these kinases had redundant functions related to asexual development within erythrocytes. Others were required for gametogenesis prior to transmission from rodent to mosquito host and formation of sporozoites, the infective stage for the mammalian host. Because kinases are largely conserved among *Plasmodium* species, it is likely that these identified enzymes are involved in the regulation of development and stage-specific gene expression in other malaria parasites as well.

Mechanisms responsible for differential gene expression during development are being revealed

How exactly is gene activity regulated during a parasite's transition between life cycle stages? With the advent of biotechnologies such as the use of RNAi to silence specific gene expression, answers to this complex question are

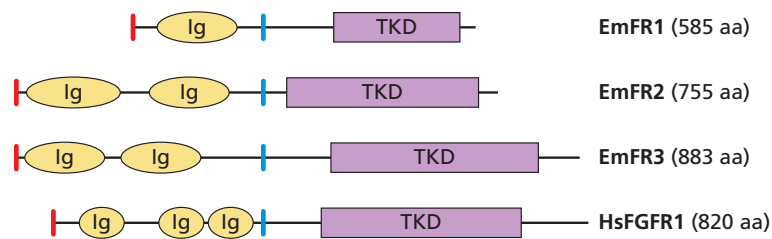


Figure 3.28 Structure of *Echinococcus multilocularis* fibroblast growth factor receptors. A schematic representation of three *E. multilocularis* receptors for mammalian Fibroblast growth factor (FGF) receptors (EmFR1, EmFR2 & EmFR3). The analogous human FGF receptor (HsFGFR1) is shown for comparison. The enzymatically active, intracellular Tyrosine Kinase Domains (TKD) are in purple. The extracellular Ig domains are shown in yellow. Ig domains have a structure similar to those found in antibodies (immunoglobulins). The number of amino acids in the receptors is shown at the far right. (Adapted from Forster S, Koziol U, Schafer T et al. (2019) *PLoS Neg Trop Diseases* 13:e0006959. doi:10.1371/journal.pntd.0006959. With permission.)

beginning to be revealed. One increasingly evident trend is that many parasites co-opt host hormones or immunomodulatory signals to induce signal transduction cascades. *Echinococcus multilocularis*, for instance, encodes at least three receptors that are activated by mammalian fibroblast growth factor (FGF). These tyrosine kinase receptors are structurally analogous to those found in the mammalian host (Figure 3.28). Their activation by host FGF stimulates the development of protoscoleces within hydatid cysts. In *Schistosoma mansoni*, free-swimming, infective cercariae transform rapidly within human hosts into schistosomulae, which migrate within the skin, ultimately reaching the circulatory system. The survival and development of these schistosomulae rely upon certain kinases, the activity of which is initiated by host molecules including epidermal growth factor and insulin. Box 3.3 highlights an example from the plant kingdom of how parasites can co-opt host signaling molecules.

BOX 3.3 Witchweed

Evil spirit. Vampire. Witch. All of these are direct translations from the Latin “*Striga*.” And *Striga* more than lives up to its name, or as it’s commonly called “witchweed.” This genus of parasitic plants (*Striga asiatica*, *S. gesnerioides* and *S. hermonthica* are the three worst offenders) is native to Africa, Asia and Australia where it wreaks havoc on crops including corn, sorghum, rice and sugarcane. It causes problems elsewhere as well, including tropical and subtropical regions in the Americas. Entire crops are often lost due to this agricultural pest.

The plant looks innocent enough (Figure 1). With its bright green stems and leaves along with small, brightly colored and attractive flowers, witchweed looks like something anyone might want in their flower garden. But don’t let its good looks fool you. Once it locates its victim, witchweed rapidly springs into action. The *Striga* root first releases an enzyme that digests the host root surface. The parasite then starts to develop its haustorium (see Chapter 2, Figure 2.14), which uses

mechanical force and chemical digestion to penetrate the host root. In less than 72 hours the haustorium reaches the host plant’s vascular system when finger-like projections on the haustorium called oscula (Latin for “little mouth”) penetrate the host xylem, allowing the *Striga* seedling to rob its hosts of fluid and nutrients. The now parasitized and probably doomed host suffers from stunting, wilting and loss of chlorophyll as also seen during severe drought, or nutrient deficiency.

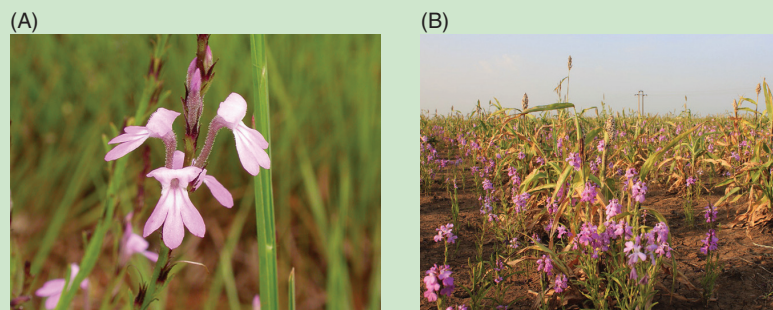


Figure 1 Beauty and the Beast. (A) Flowering *Striga hermonthica*, commonly known as purple witchweed. This obligate parasite is a major problem for subsistence farmers in sub-Saharan Africa. (B) The same species, infecting a sorghum field in South Sudan. (B, With permission from Professor Yoshida Satoko.)

How does *Striga* zero in on its host? Many plants release a hormone through their roots called strigolactone. This hormone attracts symbiotic fungi, which establish a mutualistic association, providing phosphate and other nutrients to the plant in exchange for sugars. It also acts as a signal spurring the growth of the plant itself.

But in an excellent botanical example of how parasites can utilize host molecules for their own purposes, it has been found that *Striga* seeds have approximately a dozen different strigolactone receptors of their own. Some of these receptors are sensitive to

extremely low picomolar concentrations of the hormone, making them in some cases, more sensitive to strigolactone than the host plant is to its own hormone. When the parasite's receptors bind the host hormone, signal transduction that results in seed germination is initiated. In effect, the parasite has hijacked a host signaling system to promote the progression of its own life cycle.

Yet this nefarious strategy on the part of the parasite may turn out to be its Achilles Heel. Because of the economic impact caused by *Striga*, considerable research continues to probe the parasite's life cycle for weak links. For

instance, it has been proposed that a practice called "suicide germination" could be utilized, in which fields without host crops are sprayed with a strigolactone-like molecule, tricking *Striga* into germinating when there are no hosts around to parasitize.

References

Khosla A & Nelson DC (2016) Strigolactones, super hormones in the fight against *Striga*. *Curr Opin Plant Bio* **33**:57–63. doi:10.1016/j.pbi.2016.06.001.

Samejima H, Babiker AG, Takikawa H et al. (2016). Practicality of the suicidal germination approach for controlling *Striga hermonthica*. *Pest Manag Sci* **72**:2035–2042. doi:10.1002/ps.4215.

Once signaling pathways have been initiated, they often result in epigenetic changes that up or down-regulate specific genes. **Epigenetics** refers to heritable changes in phenotype or gene expression caused by mechanisms other than an alteration in the DNA sequence (see **Box 2.4**). One epigenetic phenomenon that appears to play a key role in parasite developmental change is the modification of chromatin structure. Chromatin refers to the complex of DNA and protein found in the cells of eukaryotes. By wrapping around these proteins, DNA is packaged into a more compact form, which prevents the DNA strands from becoming tangled. The characteristic shapes of chromosomes visible during mitosis are the result of DNA being coiled into highly condensed networks of chromatin.

For example, earlier in this chapter we discussed the transition of *Plasmodium* blood-stage merozoites into gametocytes, which are infective to blood-feeding mosquitoes. The conversion of merozoites to gametocytes requires the activity of a transcription factor called AP2-G. However, during repeated rounds of merozoite replication, the gene encoding this requisite transcription factor is associated with heterochromatin and is consequently not expressed. Maintenance of heterochromatin and continued silencing of the gene for AP2-G requires the association of a different protein called Heterochromatin Protein 1 (HP1), which forms part of the DNA-protein chromatin complex. As long as HP1 associates with chromatin, AP2-G cannot be produced, meaning that gametogenesis cannot take place.

However, certain ambient environmental conditions result in the transcription of yet another protein, *Plasmodium falciparum* gametocyte development 1 protein (GDV1) (**Figure 3.29**). GDV1 interacts with HP1, removing it from chromatin. The chromatin containing the gene for the transcription factor AP2-G now assumes a looser configuration, permitting transcription of the gene and subsequent gametogenesis to proceed. The precise environmental cues that result in GDV1 production are not yet fully elucidated, but they may include markers of host immune response or an overall high density of merozoites.

As discussed in Chapter 2 (see **Figure 2.34**) **histones** are an especially important type of DNA-associated protein found in chromatin. The addition of methyl groups (**methylation**) to histone proteins is well-known as a mechanism that regulates whether associated DNA is in the heterochromatin or euchromatin form. Methyl groups are added to specific amino acid

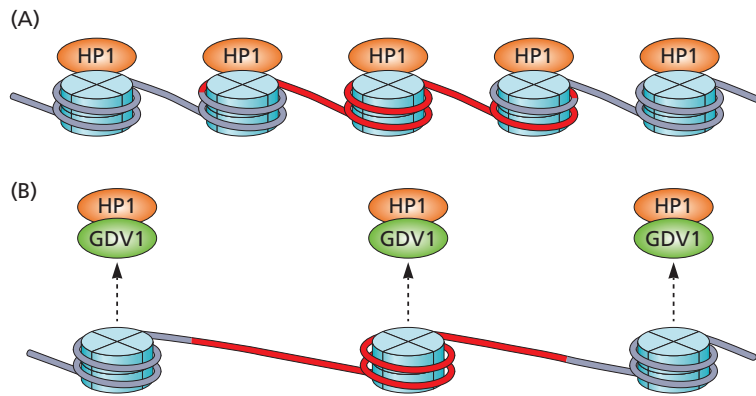


Figure 3.29 Activation of a *Plasmodium* gene required for gametogony. To convert from merogony to gametogony, *P. falciparum* requires the production of a protein, for which a specific transcription factor AP2-G is required. (A) The gene encoding AP2-G (in red) is tightly wrapped around histone proteins (in blue), which form dense heterochromatin. As heterochromatin, the gene for AP2-G remains silenced. The dense, heterochromatin form is maintained by a different protein, HP1 (in orange), that associates with the histones. (B) In response to a certain environmental cue, a protein called GDV-1 (in green) is produced. GDV-1 binds to HP1 and removes it from the heterochromatin complex. This allows the DNA, including the associated gene for AP2-G to associate more loosely with histone proteins, forming euchromatin, which can be expressed. As the gene for AP2-G is expressed, levels of the necessary transcription factor go up, and development transitions from the merozoite to the gametocyte stage. (Adapted from Rea E, Le Roch, K & Tewari R (2018) *Trends Parasit* 34:450-452. doi:10.1016/j.p.t.2018.04.006. With permission from Elsevier.)

residues by a group of enzymes called methyltransferases. Histone methylation can either increase or decrease transcription of genes, depending on which amino acids in the histones are methylated and how many methyl groups are added. Methylation events that weaken chemical attractions between histones and DNA increase transcription because they enable the DNA to uncoil, allowing transcription factors and RNA polymerase to access the DNA.

Histone methylation has been shown to play a role in the development of diverse parasites. In *Schistosoma mansoni*, for instance, different patterns of histone methylation have been observed in each of 5 important life cycle stages (Figure 3.30, and see also the Rogues' Gallery for a detailed description of the schistosome life cycle). Several of these methylation events have been shown to be crucial for life cycle progression. For example, the transition of a cercaria to a schistosomulum requires the addition of 3 methyl groups to lysine #4 on Histone #3 (H3K4me3). If the methyltransferase responsible for this trimethylation is inhibited, transcription of the genes associated with histone #4 remains suppressed, and continued development is blocked. Similarly, the trimethylation of Lysine #27 on Histone #3 is required for the transition from the miracidium stage to a sporocyst in the gastropod intermediate host.

Many questions remain regarding the various mechanisms that underlie parasite development. As we continue to tease apart the details of such mechanisms, we can expect a more coherent picture of parasite development to emerge. Not only will such a picture enhance our knowledge of development in general, but it opens the door to new and innovative strategies to control parasitic diseases by interfering with specific required events that occur in the course of a parasite's life cycle.

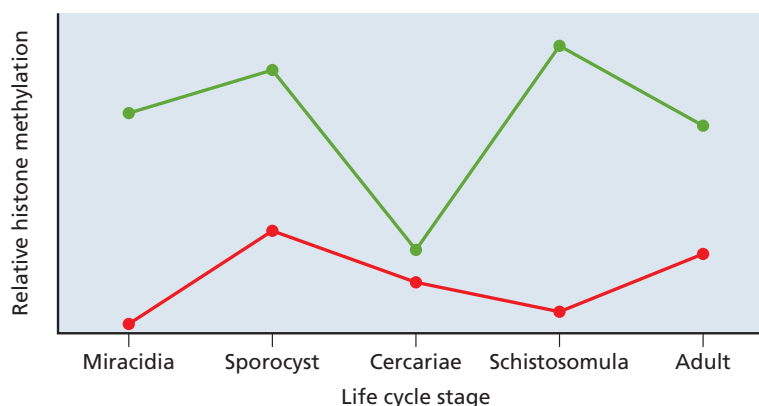


Figure 3.30 Histone methylation during 5 *Schistosoma mansoni* life cycle stages. Histone methylation frequency for histone protein H3 is shown for miracidia, sporocysts, cercariae, schistosomula and adult life cycle stages. The red line indicates the frequency of trimethylation of H3 at the lysine in position number 27 (H3K27me3). The green line shows the frequency of H3 trimethylation at Lysine number 4 (H3K4me3). (Adapted from Roquis D, Tautz A, Geyer KK et al. (2018). Histone methylation changes are required for life cycle progression in the human parasite *Schistosoma mansoni*. *PLoS Pathog* 14:e1007066. doi:10.1371/journal.ppat.1007066. With permission.)

SUMMARY

In general, the problems confronting parasites are similar to those of all living things, but the parasitic lifestyle requires adaptation to the unique host environment, which differs considerably from more familiar and conventional habitats. The manner in which parasites have adapted to the host environment and evolved in response to the overriding need to be transmitted between hosts has resulted in various life cycle patterns. Understanding these life cycles provides insight into numerous other aspects of the parasitic mode of existence.

As the problems that any parasite must overcome are essentially the same, there are basic patterns to parasite life cycles, but there is seemingly no end to the number of ways different parasite species modify these patterns to suit their specific needs. Parasites transmitted via the fecal-oral route need eggs or larvae that are capable of temporary survival in the external environment, their infective stages must be able to cope with the low pH encountered in the stomach, and the life cycle stages inhabiting the intestine have to withstand powerful digestive enzymes and peristalsis. Other parasites, using other modes of transmission, encounter somewhat different environmental conditions that necessitate different solutions.

Many of the generalities historically accepted about parasites are not necessarily true and sometimes the adaptations observed in a particular parasite seem at first glance to be counterintuitive. For instance, contrary to the commonly accepted belief, parasites are not always more fecund relative to their free-living relatives. Such examples remind us that simple statements about what constitutes a parasite rarely stand up to close inspection.

Like all living things, parasites must juggle a large number of competing selection pressures and the result is frequently a compromise between these competing demands. The variety of selection pressures acting on parasites ensures that they evolve along a wide variety of trajectories, just as free-living organisms do.

REVIEW QUESTIONS

1. *Gnathostoma procyonis* is a dioecious, sexually reproducing nematode that parasitizes the stomach of raccoons. Raccoons, which serve as definitive hosts, become infected when they consume either fish serving as second intermediate hosts or paratenic hosts (other vertebrates that consumed the infected fish). Imagine a raccoon that is lightly infected with this parasite early in the spring. As spring progresses, the intensity of infection rises, as the raccoon eats additional infected fish. How do you predict that this increasing parasite intensity will impact the reproduction rate of *G. procyonis*?
2. You are studying a previously undescribed apicomplexan parasite, about which almost nothing is currently known. In the course of your investigations, you observe that the life cycle of this parasite includes a metabolically inactive “cyst-like” stage. Which mode or modes of transmission can you likely rule out, based on this observation?
3. Parasites relying on vector-borne transmission are often among the most pathogenic. Can you think of a mode of transmission, for which selection might work to lessen pathogenicity?

4. The R-value, related to the basic reproduction number (R_0) but taking into account the immune status of the population, describes the average number of new cases in susceptible individuals derived from one infected individual. If members of the potential host population are immune to the infectious agent in question, they cannot become infected, and this causes R to change as the proportion of individuals that are immune changes. In the following cases would R be expected to rise or fall:
 - a. If a sudden influx of new potential host individuals who have not previously been exposed to the parasite in question entered the population?
 - b. If a number of hosts became infected with the parasite, and subsequently became immune to later infections with the same parasite?
 - c. If a number of potential hosts were vaccinated against this particular parasite?
 - d. If a number of new babies are born?

5. *Onchocerca lupi* is an emerging parasite of veterinary importance. Like the related *O. volvulus*, it can result in vision loss for the definitive host. Whereas *O. volvulus* is a human parasite, *O. lupi* appears to affect mainly dogs and other canines. The vectors of this parasite have been identified as various species of black fly (genus *Simulium*) but the exact species in most areas have yet to be determined. Suppose that you wished to identify potential vectors in a particular geographic area and found that a species of black fly was at least occasionally infected with larval stages of *O. lupi*. What would be your next step, if you wished to further investigate the role of this fly as a potential *O. lupi* vector?

6. In schistosomes, it has been shown that cytokines (chemical messengers involved in immune responses) released by macrophages are recognized by the parasite and stimulate egg production in female worms. With an understanding that egg production correlates with pathology in human schistosomiasis, can you think of the way a drug might interfere with the co-option of a host signal molecule by a parasite to reduce pathology during a schistosome infection?

REFERENCES

Rijo-Ferreria F, Takahashi JS & Figueiredo LM (2017). Circadian rhythms in parasites. *PLoS Pathogens* **13**:e1006590. doi:10.1371/journal.ppat.1006590.

A HISTORICAL PERSPECTIVE OF THE PARASITE LIFE CYCLE

Early medical and natural history studies gave rise to an understanding of parasite life cycles

Cox FEG (2002) A history of human parasitology. *Clin Microbiol Rev* **15**:595–612.

Roncagli AR (2001) The history of Italian parasitology. *Veterinary Parasit* **98**:1–3. doi:10.1016/S0304-4017(01)00420-4.

Arthropod transmission for filarial worms suggested that other diseases may be similarly transmitted

Cox FEG (2010) The history of the discovery of the malaria parasites and their vectors. *Parasit Vectors* **3**:5. doi:10.1186/1756-3305-3-5.

An Overview of Parasite Life Cycles

Blasco-Costa I & Poulin R (2017). Parasite life cycle studies: a plea to resurrect an old parasitological tradition. *J Helminthology* **91**:647–656. doi:10.1017/S0022149X16000924.

Parasites with direct life cycles use only a single host

Page W, Judd JA & Bradbury RS (2018) The unique life cycle of *Strongyloides stercoralis* and implications for public health action. *Trop Med Infect Dis* **3**:doi:10.3390/tropicalmed3020053.

Two or more hosts are necessary for those parasites with indirect life cycles

Benesh D (2016) Autonomy and integration in complex parasite life cycles. *Parasitology* **143**:1824–1846. doi:10.1017/S0031182016001311.

Shoop WL & Corkum KC (1984) Transmammary infection of newborns by larval trematodes. *Science* **223**:1082–1083.

Zelmer E, Wetzel J & Esch GW (1999) The role of habitat in structuring *Haliplus occidialis* metapopulations in the green frog. *J Parasitol* **85**:19–24.

THE PARASITE'S TO DO LIST

Effective transmission is essential for all parasites

Antonovics J, Wilson AJ, Forbes MR et al. (2017) The evolution of transmission mode. *Phil Trans R Soc* **372**:doi:10.1098/rstb.2016.0083.

Bakker TC, Frommen JG & Thunken T (2017). Adaptive parasitic manipulation as exemplified by acanthocephalans. *Ethology* **123**:779–784. doi:10.1111/eth.12660.

Barash NR, Nosala C, Pham JK et al. (2017) *Giardia* colonizes and encysts in high-density foci in the murine small intestine. *Mosphere* **2**:e00343–16.

Boulanger N, Boyer P, Talagrand-Reboul E et al. (2019). Ticks and tick-borne diseases. *Medicine et Maladies Infectieuses* **49**:87–97. doi:10.1016/j.medmal.2019.01.007.

Cernikova L, Faso C, & Hehl AB (2018). Five facts about *Giardia lamblia*. *PLoS Pathog* **14**:e1007250. doi:10.1371/journal.ppat.1007250.

Goddard J (2018) Dynamics of arthropod-borne diseases. In: *Infectious Diseases and Arthropods*, pp. 27–36. Infectious Disease. Humana Press, Cham.

Henni L, Molyneux DH, Livesey L & Galun R (1980) Feeding behavior of tsetse flies infected with salivarian trypanosomes. *Nature* **283**:383–385.

Jarecka L (1961) Morphological adaptations of tapeworm eggs and their importance in life cycles. *Acta Parasitol* **9**:409–426.

Lauwaet T, Davids BJ, Reiner DS & Gillin FD (2007) Encystation of *Giardia lamblia*: a model for other parasites. *Curr Opin Microbiol* **10**:554–559.

Little TJ, Shuker DM, Colegrave N et al. (2010) The coevolution of virulence: tolerance in perspective. *PLoS Pathog* **6**(9):e1001006. doi:10.1371/journal.ppat.1001006.

Shapiro LL, Whitehead SA & Thomas MB (2017). Quantifying the effects of temperature on mosquito and parasite traits that determine the transmission potential of human malaria. *PLoS Biol* **15**:e2003489. doi:10.1371/journal.pbio.2003489.

High reproductive rates are common in many parasite life cycles

Bradic M & Carlton JM (2018). Does the common sexually transmitted parasite *Trichomonas vaginalis* have sex? *PLoS Pathog*. **14**:e1006831. doi:10.1371/journal.ppath.1006831.

Haag ES & Lo TW (2018). How to make a billion parasites. *Dev Cell* **45**:147–148. doi:10.1016/j.devcel.2018.04.006.

Koprivnikar J & Randhawa HS (2013). Benefits of fidelity: does host specialization impact nematode parasite life history and fecundity? *Parasitology* **140**:587–597. doi:10.1017/S0031182012002132.

Lippens C, Faivre B, Lechenault C et al. (2017). Aging parasites produce offspring with poor fitness prospects. *Biol Lett* doi:10.1098/rsbl.2016.0888.

Loker ES (1983) A comparative study of the life-histories of some mammalian schistosomes. *Parasitology* **87**:343–369.

Moore J (1981) Asexual reproduction and environmental predictability in cestodes (Cyclophyllidae: Taeniidae). *Evolution (N Y)* **35**:723–741.

Poulin R (1995) Clutch size and egg size in free-living and parasitic copepods: a comparative analysis. *Evolution (N Y)* **49**:325–336.

Poulin R (1995) Evolution of parasite life history traits: myth and reality. *Parasitol Today* **11**:342–345.

Rougeron V, De Meeus T & Banuls AL (2017). Reproduction in *Leishmania*: a focus on genetic exchange. *Infect Genet Evol* **50**:128–132. doi:10.1016/j.meegid.2016.10.013.

Both sexual and asexual reproduction are used by apicomplexans such as *Toxoplasma gondii*

Szabo EK & Finney CM (2017). *Toxoplasma gondii*: one organism, multiple models. *Trends Parasit* **33**:113–127. doi:10.1016/j.pt.2016.11.007.

Parasites may use strategies other than high fecundity to achieve transmission

Greischar MA, Mideo N, Read AF et al. (2016). Predicting optimal transmission investment in malaria. *Evolution* **70**:1542–1558. doi:10.1111/evo.12969.

Reece, SE, Ramiro RS & Nussey DH (2009) Plastic parasites: sophisticated strategies for survival and reproduction? *Evol. Appl.* **2**:11–23.

Many factors can complicate an understanding of parasite transmission

Fellous S & Koella JC (2009) Different transmission strategies of a parasite in male and female hosts. *J Evol Biol* **22**:582–588.

Laguerre C, Rinnevalli R & Poulin R (2017) Smelling the future; subtle life-history adjustments in response to environmental conditions and perceived transmission opportunities in a trematode. *Parasit* **144**:464–474. doi:10.1017/S003118201600192X.

Noland GS, Graczyk TK, Fried B & Nirbhay K (2007) Enhanced malaria parasite transmission from helminth co-infected mice. *Am J Trop Med Hyg* **76**:1052–1056.

Poulin R & Lefebvre F (2006) Alternative life-history and transmission strategies in a parasite: first come, first served? *Parasitology* **132**:135–141.

Mathematical models provide a useful tool to predict transmission rates

Craig MH, Snow RW & Le Sueur D (1999) A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitol Today* **15**:105–111.

Ngwarakana-Gwasira ET, Bhunu CP, Masocha M et al. (2016) Assessing the role of climate change in malaria transmission in Africa. *Malar Res Treat* **2016**:1–8. Article ID 7104291.

Riley S, Carabin H, Bélisle P et al. (2008) Multihost transmission dynamics of *Schistosoma japonicum* in Samar province, the Philippines. *PLoS Med* **5**(1):e18. doi:10.1371/journal.pmed.0050018.

Many parasites must migrate to specific sites or tissues within the host

Drewry LL & Sibley LD (2017) *Toxoplasma gondii* infection reprograms monocyte adherence and motility. *FASEB J* **31**(Suppl.1):776–779.

Dubey JP & Knoll LJ (2019) Intestinal delta-6-desaturase activity determines host range for *Toxoplasma* sexual reproduction. *PLoS Biol* **17**:e3000364. doi:10.1371/journal.pbio.3000364.

Moreira CK, Templeton TJ, Lavazec C et al. (2008) The *Plasmodium* TRAP/MIC2 family member, TRAP-like protein (TLP), is involved

- in tissue traversal by sporozoites. *Cell Microbiol* **10**:1505–1516. doi:10.1111/j.1462-5822.2008.01143.x.
- Sukhdeo MVH & Sukhdeo SC** (2002) Fixed behaviors and migration in parasitic flatworms. *Int J Parasitol* **32**:329–342.
- Wheeler NJ, Heimark ZW, Airs PM et al.** (2019) Genetic and functional diversification of chemosensory pathway receptors in filarial nematode parasites. *bioRxiv*:doi:10.1101/683060.
- Wong W, Huang R, Menant S et al.** (2019) Structure of *Plasmodium falciparum* Rh5-CyRPA-Ripr invasion complex. *Nature* **565**:118–121. doi:10.1038/s41586-018-0779-6.
- The manner in which complex migration within the host evolved is not always clear**
- Mulcahy G, O'Neil S, Fanning J et al.** (2005) Tissue migration by parasitic helminths – an immunoevasive strategy? *Trends Parasit* **21**:273–277. doi:10.1016/j.pt.2005.04.003.
- Read AF & Sharping A** (1995) The evolution of tissue migration by parasitic nematode larvae. *Parasitology* **111**:359–371. doi:10.1017/S0031182000081919.
- Finding a mate is a requirement for many sexually reproducing parasites**
- Brown SP, Renaud F, Gueagan JF et al.** (2001) Evolution of trophic transmission in parasites: the need to reach a mating place? *J Evol Biol* **14**:815–820.
- Hahnel S, Wheeler N, Lu Z et al.** (2018) Tissue-specific transcriptome analyses provide new insights into GPCR signaling in adult *Schistosoma mansoni*. *PLoS Pathog* **14**:e1006718. doi:10.1371/journal.ppat.1006718.
- Kanev I, Sterner M, Radeu V et al.** (2000) An overview of the biology of echinostomes. In: *Echinostomes as Experimental Models for Biological Research*, pp. 1–29. Edited by Fried B & Graczyk TK. Kluwer.
- Wang J, Yu Y, Shen H et al.** (2017) Dynamic transcriptomes identify biogenic amines and insect-like hormonal regulation for mediating reproduction in *Schistosoma japonicum*. *Nat Com* **8**:14693. doi:10.1038/ncomms14693. PMID: 28287085.
- Parasites undergo complex developmental changes in response to environmental cues**
- Billker O, Lindo V, Panico M et al.** (1998) Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. *Nature* **392**:289–292. doi:10.1038/32667.
- Radke JR, Behnke MS, Mackey AJ et al.** (2005) The transcriptome of *Toxoplasma gondii*. *BMC Biol* **3**:26. doi:10.1186/1741-7007-3-26.
- Sollelis L & Matthias M** (2019). A major step towards defining the elusive stumpy inducing factor in *Trypanosoma brucei*. *Trends Parasit* **35**:6–8. doi:10.1016/j.pt.2018.11.009.
- Developmental change is associated with changes in gene expression**
- Cho PY, Kim TI, Whang SM & Hong SJ** (2009) Gene expression profile of *Clonorchis sinensis* metacercariae. *Parasitol Res* **102**:277–282.
- Clayton CE** (2016) Gene expression in Kinetoplastids. *Curr Opin Micro* **32**:46–51. doi:10.1016/j.mib.2016.04.018.
- Datu BJD, Gasser RB, Nagaraj SH et al.** (2008) Transcriptional changes in the hookworm *Ancylostoma caninum* during the transition from a free-living to a parasitic larva. *PLoS Negl Trop Dis* **2**(1):e130. doi:10.1371/journal.pntd.0000130.
- Einarsson E, Troell K, Hoepfner MP et al.** (2016) Coordinated changes in gene expression throughout encystation of *Giardia intestinalis*. *PLoS Negl Trop Dis* **10**:e0004571. doi:10.1371/journal.pntd.0004571.
- Gobert GN, Moertel L, Brindley PJ & McManus DP** (2009) Developmental gene expression profiles of the human pathogen *Schistosoma japonicum*. *BMC Genomics* **10**:128. doi:10.1186/1471-2164-10-128.
- Lippuner C, Rannakrishan C, Basso WU et al.** (2018) RNA-seq analysis during the life cycle of *Cryptosporidium parvum* reveals significant difference in gene expression between proliferating stages in the intestine and infectious sporozoites. *Int J Parasit* **48**:413–422. doi:10.1016/j.i.jpara.2017.10.007.
- Rochette A, Raymond F, Ubeda JM et al.** (2008) Genome-wide gene expression profiling analysis of *Leishmania major* and *L. infantum* developmental stages reveals substantial differences between the two species. *BMC Genomics* **9**:255. doi:10.1186/1471-2164-9-255.
- Tewari R, Straschil U, Bateman A et al.** (2010) The systematic functional analysis of *Plasmodium* protein kinases identifies essential regulators of mosquito transmission. *Cell Host Microbe* **8**:377. doi:10.1016/j.chom.2010.09.006.
- Mechanisms responsible for differential gene expression during development are being revealed**
- Cheeseman K & Weitzman JB** (2015) Host-parasite interactions: an intimate epigenetic relationship. *Cellular Microbiol* **17**:1121–1132. doi:10.1111/cmi.12471.
- Filarsky M, Fraschka SA, Niederwieser I et al.** (2018) GDV1 induces sexual commitment of malaria parasites by antagonizing HP1-dependent gene silencing. *Science* **359**:1259–1263. doi:10.1126/science.aan6042.
- Forster S, Koziol U & Schafer T** (2019) The role of fibroblast growth factor signaling in *Echinococcus multilocularis* development and host-parasite interaction. *PLoS Negl Trop Dis* **13**:e0006959. doi:10.1371/journal.pntd.0006959.
- Mair GR, Lasonder E, Garver LS et al.** (2010) Universal features of post-transcriptional gene regulation are critical for *Plasmodium* zygote development. *PLoS Pathog* **6**:e1000767. doi:10.1371/journal.ppat.1000767.
- Reece SE, Drew DR & Gardner A** (2009) Sex ratio adjustment and kin discrimination in malaria parasites. *Nature* **453**:609–614. doi:10.1038/nature06954.
- Ressurreicao M, Elbeyioglu F, Kirk RS et al.** (2016). Molecular characterization of host-parasite cell signaling in *Schistosoma mansoni* during early development. *Scientific Reports* **6**:35614. doi:10.1038/srep35614.
- Roquis D, Taudt A, Geyer KK et al.** (2018). Histone methylation changes are required for life cycle progression in the human parasite *Schistosoma mansoni*. *PLoS Pathog* **14**:e1007066. doi:10.1371/journal.ppat.1007066.

