Part I

INTRODUCTORY CONCEPTS

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Introduction to Animal Parasitology

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Chapter 1

Introduction to Animal Parasitology

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Introduction

One of the most fascinating things that a person can experience in the complex realm of biology is the discovery of an animal living inside another animal. If this discovery takes place at an early enough stage in the development of a young person's view of the world, that is, before the rules and regulations of what of society thinks, and before what is good and what is bad are perfused into a learner's mind, the first discovery of living-motile trematode worms living inside the lungs of a frog or of tapeworms inhabiting the gut of a rodent can be exhilarating and a positively unforgettable experience. The questions that arise when these kinds of animals are encountered for the first time are innumerable and, if answered carefully and perhaps fully, may lead to more and more questions, and hopefully, more and more answers.

Many students of biology first begin to investigate parasites and parasitism via the initial study of the ecology, behavior, or systematics of a species of a free-living organism. That is, the free-living animal (pick your favorite species) is being studied for any of a myriad of reasons and during the investigations, those doing the work discover that there may be several species of parasites occurring in or on (or, more likely, both) their study animals. This discovery can occur for other reasons not related to **parasitology** at first, but then leads to investigation of parasitism.

True **parasitologists**—those who are intrigued with the intimate associations of parasites and are interested in the biology of the parasite itself—may become intensely focused on a single group, like tapeworms of rodents or gregarines of beetles or damselflies, for instance. Other students of parasitology may focus on the complete endoparasite fauna of a group of insects, fish, mammals, birds, amphibians, or reptiles. It is not unusual for a parasitologist to spend their whole career studying a single group of parasites pretty much to the exclusion of other parasites, as did Odile Bain, who worked on filarioid nematodes (phylum Nemata: superfamily Filarioidea) and Marie Claude Durette-Desset who works on trichostrongyloids (phylum Nemata: superfamily Trichostrongyloidea), both in the Laboratoire des vers, French National Museum of Natural History. Another example of a working parasitologist is Donald W. Duszynski from the University of New Mexico, who followed the path initially laid out by his mentor, William C. Marquardt at Colorado State University. Duszynski chose to focus the bulk of his entire career on protozoan parasites called the coccidia.

Humans—Including Scientists—Beginning to Notice Parasites

Even though the recognition of parasites and of parasitism had a recorded beginning in ancient Greece and China (Hoeppli, 1959), there is no doubt that parasites were known as part of the natural fauna by the earliest of peoples. For example, in the early 1950s, the nomadic Nunamiut Eskimo hunters in the Brooks Range of Alaska knew of and routinely recognized the strobilar (adult) stages of cestodes in the intestines of carnivores and other mammals and they recognized the larval stages of the cestodes in the viscera of the caribou that they prepared and used for food and shelter (Robert L. Rausch, personal communication; Rausch, 1993).

The first studies of parasites of animals and resulting scientific publications started during the late 1700s and early 1800s with formal publications by Johann Gottfried Bremser, Carl Asmund Rudolphi, Karl Moriz Diesing, Raphaele Molin, A. F. Schneider, R. von Drasche, Peter Simon Pallas (shown in Figure 1), Karl Theodor Ernst von Siebold, Johann August Ephraim Goeze, Karl Georg Friedrich Leuckart, Constantine Janicki, Otto von Linstow, and others. Much of the work that was originally published by Molin and Rudolphi originated from the collections made by Johann Natterer (see Guerrero, 2021) and Hermann von Ihering (see Klassen, 1992; Brooks and McLennan, 2002) during collecting expeditions into the

Figure 1. Portrait of Peter Simon Pallas. Source: Artist, Ambroise Tardieu; reproduced by Raikov, 1952; digitized by Kouprianov, 2006. Public domain.

Amazon region of Brazil. In the late 1800s, Leuckart trained many **helminthologists** in his parasitology laboratory in Leipzig, Germany including Henry Baldwin Ward and others.

As scientific knowledge of the natural world increased during the early 1800s, studies of the natural history of parasites produced increasing numbers of publications. Scientists wanted to know what these animals were and how they got where they were being discovered. It was soon revealed that some parasites had very complex **life cycles** and that parasites were extremely common in nature. Through time, as students of parasite diversity studied **transmission patterns**, **life histories**, and **pathologies**, and then much later, researchers put these together in **phylogenies**, more knowledge was generated that enabled new testable ideas to develop in ecology and evolution (Brooks and McLennan, 2002). The development of the ecological and evolutionary ideas that used parasites as indicators of both biogeographical and ecological relationships was aptly named **parascript** by Harold Winfred Manter (1966). Manter's research program in parasite **systematics** was foundational in the field of parasitology for the subsequent development of parasite phylogenetics and ecology which was ultimately articulated as a research program called **Historical Ecology** that was first outlined in a talk by Daniel R. Brooks (1985) at the Systematics Symposium of the Missouri Botanical Garden organized by Peter Raven. Brooks realized that Manter's insight was derived from his deep knowledge and understanding of the **biological diversity** of trematodes that occurred in marine fishes on both sides of the isthmus of Panama, even though at the time, there was not a firmly established method (in the English-speaking world) of consistent analysis of phylogeny (Manter, 1966). Subsequent groundbreaking work in the area of parasite phylogenetics and biodiversity was done in parasite systematics with the publication of the book Parascript: Parasites and the Language of Evolution by Brooks and McLennan (1993). For more information on the history of animal parasitology, see Janovy's chapter (Chapter 68) in this volume, as well as Sattmann (2002; in German) and Hoeppli (1959). As mentioned above, the parasitic way of life is one of the most common—if not the most common—way of protozoan and animal life that exists. It is likely that more than half of all species of organisms are parasites, and many are of very great economic and medical importance. Some of the most devastating diseases of humans, such as malaria, trypanosomiasis, and filariasis, are caused by parasites, and the economic loss caused by parasites of plants and animals worldwide reaches the equivalent of billions of United States dollars every year.

Definition of a Parasite

The concept of a **parasite** and its **host** essentially refers to the biological tension between 2 organisms that live physically adjacent to one another. With the classical definition of a **parasite** as **an organism living on or in another organism (the host) and usually causing some harm to the host**, the parasite sounds like it is merely a bad thing with respect to the host; and this definition works for the most part since most parasites probably *do* harm the host. In some species, the harm can be minuscule and undetectable, without causing discomfort to the host, or the damage can be significant, actually killing the host. For example, pinworm nematodes probably don't do very much to decrease the ability of their hosts to go about their daily lives or produce a normal number of offspring and live to old age. On the other hand, species of the phylum Acanthocephala, known as the thorny-headed worms, can cause a great deal of harm to their **definitive** or **final hosts** by penetrating the mucosal layer of the small intestine with their proboscis and sometimes the proboscis may penetrate the muscularis mucosa through the serosa into the peritoneal cavity, causing peritonitis, and when this occurs, the host usually dies.

Parasitism, beyond the classical definition provided above, can be defined in a very wide sense, that is, as **a close association between 2 organisms, in which a parasite depends on a host that provides some benefit to it** (usually nutrition or food, depending on the group of parasites), and the parasite does not always damage the host (as noted above, pinworms of rodents are good examples of this). A parasite can be very small relative to the size of the host—and most parasites *are* much smaller than the host; however, some parasites can reach huge sizes, and those that become numerous or are very large can even drain their host's blood of essential nutrients.

Parasitology is usually restricted to single celled eukaryotic, or protozoan (also called protistan) and multicellular or metazoan parasites, whereas many groups of organisms that lead a parasitic way of life, such as some fungi and bacteria, are usually instead included in the domain of microbiology, while viruses are studied in virology. However, it really depends on convention. In France, for example, fungi are often studied by parasitologists in addition to the helminths and protozoans or protistans.

Different authors use different definitions for parasitism, depending on their perspective or research interests. Thus, a medical parasitologist will stress that a parasite causes certain diseases and will exclude certain species from the definition which have no apparent ill effect on the host. A zoologist might be more interested in the physiological and morphological adaptations of a parasite to its host or of the host to its parasite. An ecologist may be more interested in the interactions of the parasite on its host and the animal populations with which parasites live, while an evolutionary biologist may be interested in the evolutionary interactions among parasites and their hosts without too much regard for the individual species of animals that are being studied. The definitions presented here are from the general perspective of a parasite **systematist**, one who is primarily concerned with the understanding of parasitism from the aspect of parasite biodiversity, how they evolved and are evolving, and any and all relationships among them (and their hosts).

Associations Related to Parasitism

Some types of ecological associations resemble parasitism in various aspects and cannot always be unambiguously distinguished from a parasitic relationship, either because little is known about a particular species or because intermediate forms exist. Such ecological associations include: Predation, commensalism, phoresis, mutualism, and symbiosis sensu stricto (meaning, in the strict sense). In the case of **predation**, the predator usually kills and eats another animal, the prey. In the case of **commensalism**, an organism associated with a host uses food found in the internal or external environment of the host and there may be no close phylogenetically determined relationship with the host or host group. For example, many species of barnacles and isopods can take up residence on the external surfaces of whales. These can then be termed **ectocommensals** (**ecto** = outside of the host). In **phoresis**, one organism uses another only for transport and/or protection. Barnacles can again serve as an example: Some species live attached to the skin of whales, by which they are carried around finding new sources of pelagic food (plankton). A **mutualistic** association is one in which both host organism and the associated species benefit. The Australian mistletoe bird *Dicaeum hirundinaceum* feeds on the seeds of mistletoes which are plants that derive most of their sustenance from their host plants, and the mistletoe depends on the bird for dispersal of its seeds through space. **Symbiosis** (sensu stricto) is an extreme form of mutualism, in which the association is compulsory, that is, both partners (symbionts) benefit and cannot live without each other. Very ancient examples of symbiosis are organelles (specialized cell components) of all protozoan (unicellular) and metazoan (multicellular) animals and plants, which are thought to have arisen by the joining of originally free-living organisms. However, the term symbiosis is also occasionally used in a wider sense that can include the phenomena of parasitism, commensalism, phoresis, and mutualism.

That a distinction between the various kinds of associations is sometimes difficult to make is shown by the observation that the same organism may sometimes be a parasite, commensal, mutualist, or predator, depending on the circumstances. Thus, oftentimes, the amoeba *Entamoeba histolytica* may feed on bacteria in the intestine of humans without causing any damage, or it may live as an often-fatal pathogenic parasite ingesting red blood cells and sometimes penetrating through the gut wall into the abdominal cavity, with fatal consequences. Some parasites may even improve the well-being of their hosts when infection intensities are low, but this is an understudied area.

Kinds of Parasitism

Lice, ticks, fleas, some monogeneans, and many crustaceans such as isopods and barnacles, as alluded to above, are **ectoparasites** that live on the surface of animals. Nematodes (such as species of Oxyurida or Oxyuroidea), tapeworms (such as fish, beef, and pork tapeworms), flukes (also known as trematodes, such as liver flukes, eye flukes, and blood flukes), and coccidian parasites (such as *Plasmodium*, which causes the disease malaria in humans) are examples of **endoparasites** found in the tissues or within the organs of their hosts. Cestodes and trematodes are **obligate**

parasites which cannot survive without a host at least for part of their life cycle, whereas some maggots (larvae of flies that usually feed on decaying organic matter) may be **facultative parasites**, which infect living animals only occasionally (note that there are plenty of species of flies in which their larval stages are parasitic in vertebrates and cannot live anywhere else). **Permanent parasites**, such as most parasitic helminths, including trematodes, cestodes, and nematodes, are organisms that are parasitic on or in a host over long time spans, whereas **temporary parasites,** such as most leeches, are parasitic only intermittently.

An example of a **sexually dimorphic** parasite is the chigoe flea *Tunga penetrans* Linnaeus in which only the female is a permanent parasite—usually on the toes of some hapless human or some other mammal—and the male may move around from toe to toe and from host to host. Some species of parasites are selective in their parasitic existence such as species of the phylum Arthropoda that range in diversity from marine gnathiid isopods (phylum Arthropoda: subphylum Crustacea: class Isopoda) to terrestrial chigger mites (class Acari: family Trombiculidae). Some species in these 2 groups are parasites only as larvae, thus they are referred to as **larval parasites**. In this example, the isopod larvae live on marine fish and suck their blood, yet when they molt to the adult stage they live the rest of their lives eating detritus in the benthic zone of the sea floor. The trombiculid mites (family Trombiculidae) exist as adults that eat detritus in the soil and they lay eggs there that hatch into larvae called chiggers that are the torment of humans and other mammals worldwide. Other larval parasites include the cysticercoids of hymenolepidid tapeworms (phylum Platyhelminthes: class Cestoda: family Hymenolepididae) that live in mites or beetles as larvae and mature to adults in their rodent final or definitive hosts. However, many organisms are **parasitic only as adults** and they are associated with a host for all, or at least part, of their sexually reproductive phase.

Female mosquitoes and some fly larvae like the Congo floor maggot (*Auchmeromyia luteola*; see Zumpt, 1965) are **periodic parasites** which visit a host periodically. In this example, the *A. luteola* maggot comes out of its daytime hiding place in the evening and fills up on the blood of a sleeping human, and then goes back into the floor to wait until the next feeding session. When individuals of the same species parasitize other individuals of the same species, they are referred to as **intraspecific parasites**. This type of parasitism is not very common but does occur. An example is that of males of some deep sea fish that live permanently attached to females of the same species, absorbing food and deriving physical protection from the female. **Hyperparasites** (of the primary, secondary, tertiary, etc. degrees) are parasites of other parasites. For example, some protozoans infect helminths (worms) in the intestine or tissues of fishes, and this also occurs in nematodes that have flagellated protozoa (*Histomonas meleagridis*) in the uterus of females that are actually transmitted to the next galliform bird host such as chickens and turkeys (class Aves: order Galliformes) and are protected in the eggs of the nematode. **Kleptoparasites** are animals which force others to regurgitate or drop their food and then steal and eat their prize, and this is an example of behavorial parasitism. Frigate birds and some hawks chase other birds in flight. Cowbirds and about 50 species of cuckoos are **brood parasites**, that is, they lay their eggs in the nests of other birds where they are incubated by and cared for by the parental birds of the nest they have invaded. **Microparasites** include viruses, bacteria, protozoans, and some small worms (helminths), which reproduce in or on the host, sometimes inducing immune responses in vertebrate hosts. **Macroparasites**, that is, large-bodied parasites, include most helminths and arthropods; most do not multiply within the host.

There are many species of hymenopterans (phylum Arthropoda: class Insecta: order Hymenoptera) that are considered **parasitoids.** These are animals that lay their eggs in insect or other arthropod hosts and the egg hatches and begins to feed on the host tissues. Here, the host may survive for some time before it is eventually killed by the feeding and growing larval parasitoid. In some cases, several levels of **hyperparasitism** have been identified in which parasitoids are parasitized, such as by a wasp.

Mechanisms of Infection

Specific **mechanisms of infection** are truly numerous and are well-studied in many species of parasites (Table 1). Some species of parasites possess conspicuous morphological adaptations that increase the probability that the life cycle will be completed. For example, eggs of some blood flukes of humans (namely, schistosomes causing schistosomiasis also known as bilharzia or bilharziasis) have spines which contribute, together with enzymes produced by the larva within the egg, to eroding the walls of blood vessels where the adults live, thus facilitating escape of eggs produced by the female directly into the bloodstream. The eggs then travel from the bloodstream through the walls of the blood vessels into the feces or urine, depending on the species of *Schistosoma* (adults of *S. haematobium* live in blood vessels around the urinary bladder while adults of *S. mansoni* live in the blood vessels of the intestines).

Adaptations to Parasitism

Each parasite species has adaptations that increase the probability of the parasite to infect, or make it to, a new host

Mechanism	Example organism(s)
Autoinfection (for example, eggs hatching and the larvae maturing in the host's intestine)	*Taenia solium* or *Strongyloides stercoralis*
Contact transfer	Mange mites of various species
Fecal contamination of wounds, mucosa, or lacrimal surfaces	*Trypanosoma cruzi* transmitted by reduviid bugs/kissing bugs
Ingestion of infected intermediate hosts	Trematodes and cestodes of various species
Ingestion of parasite eggs	*Trichuris*, *Ascaris*, *Taenia*, *Echinococcus*
Ingestion of parasite cysts from undercooked muscle of vertebrates	*Toxoplasma*, *Taenia*, *Echinococcus*
Ingestion of spores and trypomastigotes	Protozoans of various species
Ingestion of transport hosts, such the muscle of uncooked, never-frozen fish	Anasakine nematodes
Inhalation and swallowing of eggs	Phylum Nemata: Species *Enterobius vermicularis*
Inoculation	*Plasmodium* spp. transmitted by mosquitoes
Kissing	Flagellated protozoan *Trichomonas tenax*
Penetration into the nasal passage	Protozoan *Naegleria fowleri*
Penetration through skin	Phylum Nemata: Family Ancylostimatidae
Sexual intercourse	Flagellated protozoan *Trichomonas vaginalis*
Transmammary transmission via milk	Nematodes of various species
Transplacental transmission	*Plasmodium*, *Toxoplasma*, *Ancylostoma*, *Toxocara*

and increases the chance of survival in it. For example, *Plasmodium* species in birds cannot normally survive in primates, and the species of human pinworm (oxyurid nematode) *Enterobius vermicularis* is known only from humans, although other species of *Enterobius* occur in primates with 1 species being reported from rodents (Brooks and McLennan, 2002). In other words, each of these species possesses characteristics enabling it to complete its life cycle using these hosts. Such characteristics (in the very few cases analyzed in some detail) determine not only the species of host(s) used, but also the degree of **host range**, that is, how many host species a parasite can utilize (Brooks et al., 2022).

Like all animal species, parasites must be able to disperse, as populations with a small numerical density and limited geographic distribution may be at risk of extinction when environmental conditions become unfavorable or they may succumb to inbreeding depression via loss of genetic heterozygosity, and (perhaps) run the risk of overinfecting a local and restricted animal-host population. In parasites, dispersal may be mostly, or even entirely, passive; that is, the parasite is spread to new geographic areas and new hosts via the geographic dispersal of the host. Many parasites have elaborate dispersal mechanisms, such as flotation organs of larval flukes (cercariae), polar filaments on the eggs of some cestodes that live in water birds, and some parasites can even modify the behavior of their host to increase the probability that the parasite will make it to the next host.

Aggregation, Hermaphroditism, Parthenogenesis, and Asexual Reproduction

Surveys of the distribution of parasites in animal populations always find that not all potential host individuals are infected to the same degree. Most parasites are usually concentrated in a few individuals of the host population. This is what is meant by distributions being **aggregated** or **overdispersed**. There has been some debate about whether aggregation has a biological function, such as facilitating the finding of mates, or limiting the damage done overall to the host population. Statistically speaking, in the negative binomial distribution, the variance is greater than the mean, so the variance divided by the mean is greater than 1. Since these are counts of numbers of parasites in hosts that were examined, the fact that few hosts have many parasites shows an overdispersed or an aggregation distribution of the parasites in or on a few hosts. The parasites are not dispersed evenly throughout the host population. Whenever the variance/mean is greater than 1, it is said that the distribution is overdispersed or aggregated.

Overdispersion characterizes a phenomenon of aggregation of a majority of parasites in a minority of the host individuals in a certain population. Thus, the majority of hosts have no or few parasites. A very small number of hosts, however, carry a great number of parasites. Crofton (1971) first showed that overdispersion was present for parasite populations. Since then, overdispersion has been defined as axiomatic among parasites of a variety of vertebrate and invertebrate hosts (Knight et al., 1977; Anderson and May, 1985; Crompton et al., 1984). Patterns of overdispersion have also been discovered in populations of managed species of wildlife (Shaw et al., 1998; Wilson et al., 2002).

Additional research shows that the same general pattern occurs across several other species of animals. For example, cestodes of the species *Triaenophorus nodulosus* (class Cestoda: family Bothriocephalidea) in perch fish (*Perca fluviatilis*) show less aggregated distributions with only 54% of these worms occurring in 18.5% of hosts with 81.5% of fish

remaining uninfected or lightly infected. Data accumulated relative to infections by the nematode *Porrocaecum ensicaudatum* (phylum Nemata: superfamily Ascaridoidea) in populations of the European starling (*Sturnus vulgaris*) from 1 study, 89% of the hosts were uninfected or lightly infected, and 69% of the parasites were recorded in just a few (11%) of the hosts. In pond frogs *Rana nigromaculata* harboring nematodes of the species *Spiroxys japonica* (phylum Nemata: class Spirurata: family Gnathostomatidae), it was found that 70% of the parasites were recorded in just 4% of the frogs examined while 88% of the frogs were found to be uninfected and 8% had light infections (Shaw et al., 1998).

Overdispersion was also recorded for 4 species of the most common human-infecting **geohelminths** (Croll and Ghadirian, 1981) and a search of the literature shows that almost invariably, parasites are distributed through animal populations in a non-random way, but what determines this is still poorly understood. For summaries of this topic in helminth parasites, see Churcher et al. (2005) and Lester (2012).

General Reproductive Biology of Parasites

Common among parasites are the various methods of reproducing that are found in the Kingdom Animalia, including: **Hermaphroditism** (1 individual has fully functioning male and female organs), **parthenogenesis** (females are able to produce offspring without mating), and **asexual reproduction** (an individual reproduces by budding or spores in which there is no recombination of genes on the chromosomes). Thus, in asexual modes of reproduction, the resulting new individuals are clones of the original organism. Among most species of parasites, only a single individual or very few individuals will reach and successfully infect or colonize a new host. In this case, populations of parasites may establish and then increase in numerical density from just a few founder individuals, or even from a single founder individual, that makes it to a new animal that it can then utilize as a host. It is a paradigm of evolutionary theory that sexual reproduction creates new combinations of genes that provide the raw material for evolution via natural selection (Williams, 1966; Williams, 1992). However, in reproduction that requires no mating and thus no sexual recombination of genes via the mixing of chromosomes, the advantage of rapid population growth from a single propagule in a new environment may in the short term outweigh the advantages of sex (Ghiselin, 1969; Williams, 1992; Kearney, 2022).

An example of **asexual reproduction** in a parasite occurs in species of *Plasmodium* (the causative agent of malaria in people). This example illustrates the stage that occurs in the vertebrate intermediate host, in the red blood cells after the infective stages first multiply in liver hepatocytes and are released into the bloodstream. In the bloodstream, these parasites develop in the red blood cells (RBCs) and multiply by mitotic division of the nucleus and other cell organelles but not the cytoplasm. These then escape the RBCs into the bloodstream to invade more RBCs and undergo more cycles of development and multiplication (depending on the species).

Parasitic platyhelminths, including trematodes, cestodes, and monogeneans, with a few exceptions, are hermaphroditic and individuals can, if necessary (such as when there are no mates nearby), fertilize their own eggs, although they usually cross-fertilize due to several morphological and developmental stages that decrease probability of self-fertilization in these groups. Some species of nematodes, including those in the genera *Steinernema* and *Heterorhabditis* (entomopathogenic nematodes, namely, those that infect insects as part of their life history) also have hermaphroditic stages (Cao et al., 2022). Many other species have been shown to exhibit various methods besides sexual reproduction and some of these are reviewed in Triantaphyllou and Hirschmann (1964) and Maggenti (1981).

Parthenogenesis is the growth and development of an animal from an ovum without fertilization and this occurs commonly in species of *Strongyloides* (phylum Nemata: family Strongylidae) which infect mammals (see Cable, 1971; also see the definition of parthenogenesis in Maggenti, 1981).

Host Range

Some parasites are known to occur in or on a few or, in some cases, only 1 species of free-living animal. Definitions are always problematic, and defining species of parasites with limited host range (formerly, or at times still, referred to as host specificity) depends on vast knowledge that can only be based on extensive collections of animals conducted over broad geographic spaces and includes complete data for the specimens of both parasites and their hosts (note that if an animal is not parasitized, it is not a host, but is only a potential host). In order for these data to be useful, the specimens that are collected and processed and their associated data must be deposited in museums that maintain both specimens and their data in perpetuity. The reason that the host and parasite are both stored in museums after collection is to enable tests of the hypotheses of host-range by actually looking at, and using data for, both the host and parasite. Many times, the host group is misidentified in the field and the species name can only be positively known by comparative methods using museum collections (Brooks et al., 2015; Galbreath et al., 2019).

Most species of parasites show some level of limited host range, although the extent of limits among species is

variable. For example, the large human nematode *Ascaris lumbricoides* (phylum Nemata: order Ascaridida) has a direct life cycle and occurs in both humans and pigs (Araújo et al., 2015). The apicomplexan protozoan *Toxoplasma gondii* (phylum Apicomplexa: family Sarcocystidae) has been shown to occur in a wide range of mammals and birds and shows broad infectivity on those groups of potential hosts (Dubey, 2008).

As a more detailed example of host range, the nematode *Ransomus rodentorum* (phylum Nemata: superfamily Strongyloidea) had been reported to occur only in the cecum of pocket gophers while a related species of *Ransomus* occurs in species of mole rats in China and perhaps Mongolia. The pocket gophers are rodents with a subterranean lifestyle restricted to North America, Central America, and extreme northern South America (Nearctic). Chinese mole rats are also subterranean rodents, but they have a distribution in the Palearctic and northern Ethiopian regions with no known history of either the Chinese mole rats occurring in the Nearctic nor of the pocket gophers occurring in the Palearctic (Thenius, 1972). Relative to *R. rodentorum* in pocket gophers, this strongyloid species has never been reported from other sympatric species of rodents within the geographic ranges of the nematode, and despite intense field collecting in several areas in North America, this species has never been shown to occur in rodents that are phylogenetically close to gophers. It is interesting that no instances of infection with these nematodes have been reported from rodents that share burrow systems with pocket gophers, even from those that are phylogenetically related, such as the kangaroo rats or pocket mice. These groups are related at a basal level, all with a common ancestor linking the heteromyids (such as kangaroo rats) with the geomyids (pocket gophers) in the superfamily Geomyoidea, one major shared derived trait (**synapomorphy**) being external fur-lined cheek pouches. This is a case where the other species of rodents are both **sympatric** (meaning, occurring in the same geographic space; Brooks and McLennan, 2002) and **syntopic** (meaning, occurring in the same ecological space; see the definition of syntopic in Rivas, 1964. See also an explanation synapomorphy in Chapter 2.).

Attempts to understand patterns of diversity of parasites that have both wide and narrow host ranges have been ongoing with concentrated work and summaries presented first by Baer and Mayr (1957). This work has been one of the foundations of systematic and ecological parasitology since the beginning of the scientific study of parasites (Guerrero, 2021; Hoeppli, 1959); however, the collections of individual parasites from vertebrates representing myriad species and their deposition into museums (as well as depositing individual host animals) has not kept pace with the same work on the vertebrates themselves (Galbreath et al., 2019). In a summary of mammal collections in museums in the United States (Dunnum et al., 2018), there were estimated to be about 5,275,000 individual cataloged mammal specimens distributed through 395 active mammal collections. However, there are only a handful of major collections of parasites of mammals in the United States and, of those, only 2 collections have significantly large reciprocal collections of both mammals and the parasites that were found during geographically focused surveys and inventories of the mammals themselves. Thus, without excellent reciprocal collections of parasites and their hosts with their data available in museums, it is difficult to say very much about host range. Until more data are collected, certain questions will remain unanswered.

Rausch (personal communication) considered that the concept of host specificity was imprecise at best because the noun *specificity* implies an unvarying quality, and he considered that the degree of specificity cannot be easily expressed or measured and any experimental test of the concept would be biased in so many different ways that the results of tests would be invalid, or at best equivocal. Phylogenetic specificity was a term that was used by Baer (1951) to refer to helminths and their hosts that were shown to have coevolved. Baer considered ecological specificity to occur when opportunistic infections were involved. This is what is now called **ecological fitting** sensu Janzen (1985).

Species Richness of Parasites and Distribution of Parasites

Arndt (1940) was the first ecologist who counted the number of parasites as a proportion of a total fauna. In Germany, he found 10,000 parasitic species out of a total of 40,000 species, but did not include insects parasitizing plants, as he classified them as herbivores. Price (1977) included such species but excluded temporary parasites (for example, mosquitoes and leeches) in his survey of the British fauna. Price estimated that more than half of all British species are parasitic.

Thirteen large taxa (phyla, subphyla, or classes) consist entirely of parasites, and many other groups include a high proportion of parasitic species. Even among the vertebrates several species are parasitic, such as the sea lamprey *Petromyzon marinus*.

Virulence of Parasites

Virulence of parasites can be defined as the degree of damage done by the parasite to the host. There are 2 opposing trends which determine the degree of virulence: 1) Usually it is not a selective advantage to severely damage or kill its host, because this would also affect the fitness of the parasite;

2) parasite transmission to another host may be facilitated by such damage: A weak host may be easier prey for a predatory final host than a strong one. Therefore, evolution will lead to an increase or a decrease in the virulent nature of various parasites, depending on the circumstances.

Life Cycles

Many parasites have a **direct life cycle** (lice, fleas, monogenean flukes, and many nematodes) and they use a single host which harbors larval/juvenile stages as well as adults. Other parasites have **complex life cycles** and use a final (= **definitive**) host which harbors the mature stage, as well as 1 or several **intermediate hosts** which harbor the larvae, that is, they have **indirect life cycles** (for example all digenean flukes, all of which are from class Trematoda). An example of a trematode with 2 intermediate hosts is the lancoleate trematode *Dicrocoelium dendriticum*. In certain parasite species, alternative life cycles are possible. For example, in the aspidogastrean fluke *Aspidogaster conchicola*, both a direct and an indirect life cycle are possible: Adult worms in the mollusc produce eggs which are inhaled by other molluscs, but fish can also become infected by eating infected molluscs. In other aspidogastreans, and in the amphilinid tapeworm *Austramphiina elongata*, among many others, the life cycle is always indirect, involving both an intermediate and final host. In the amphilinid tapeworm, turtles serve as final hosts, eggs escape from the host in an unknown way, larvae hatch in freshwater and penetrate into a crayfish intermediate host, which is then eaten by a turtle.

Many species of parasites possess varied and diverse **behavioral adaptations** that facilitate completion of their life cycle and entrance into the next host in the cycle. Adult *Dicrocoelium dendriticum* (phylum Platyhelminthes: class Trematoda: subclass Digenea) infect the liver mainly of sheep, but other ungulates are also parasitized by these trematodes. These trematodes produce eggs which pass out of the host with the feces and are eaten by land snails, in which various larval stages are produced. The last stage is the tailed larva, or cercaria, many of which cluster in slime balls which are left behind in the mucus trail of the snail as it speeds to its objective, whatever that may be. If the trematode larvae are lucky, these slime balls are then eaten by ants. If not eaten, they dry up and die. After being ingested, the cercariae move from the intestinal tract to various parts of the ant. The first cercariae getting into an ant penetrate into the ant's subesophageal ganglion, inducing the ant to climb up a grass stem and, when the temperature drops, the cercaria induces cramp-like behavior in the ant, which consequently clings to the grass stem with its mandibles. This behavior increases the likelihood of the infected ant being eaten by a passing sheep or another ungulate.

Host-Parasite Interactions, Example: Cleaning Symbiosis

A considerable range of behavioral patterns leading to (or thought to lead to) the removal of parasites has been observed among animals. They include **preening and bathing of birds** in dust and water, and **passive and active anting**, where ants are allowed to passively crawl over the body, or where ants are actively squeezed over the plumage. Also, dogs rubbing their skin against rough surfaces, jumping of fish and whales out of water, and so on, may have a cleaning function. Best known is **cleaning symbiosis**, in which one animal (the cleaner) cleans another (the host) from parasites and diseased (necrotic) tissues. For example, cleaning behavior has been observed in birds which remove ectoparasites from cattle, hippopotamuses, large marine fish floating on the ocean surface, several species of shrimp, and some freshwater fish. Hosts are freshwater and marine fishes, whales and dolphins, and invertebrates, among others. Many cleaner fish possess special morphological adaptations which enable them to pick parasites off of the host skin or even gills (the mouth is located terminally to facilitate picking up of parasites, the anterior teeth are fused to form cutting plates, and color patterns are conspicuous, useful in signaling to hosts: "I am a cleaner!"). The cleaner fish *Labroides dimidiatus* even performs a cleaning dance to attract host fish. Invitation postures of hosts signal, in turn, to the cleaner that they are ready to be cleaned.

Generalization of Parasitism: Stockholm Paradigm

Parasites can be specialists or generalists depending on how much of their fundamental **host fitness space** is occupied in a population of animals (Agosta et al., 2010; Brooks et al., 2019). The smaller the fitness space being occupied by a parasite, the more specialized the parasite appears. The following is a short summary of the general ideas of the **Stockholm Paradigm** that deals with host-range and parasite use of animal populations. A more detailed explanation can be found in the book of the same title by Brooks et al. (2019). See also Agosta (2022) and Brooks et al. (2022).

The concept of host range infections has undergone rapid change in the past few years with the ideas of Brooks et al. (2015; 2019) forging new ground towards the interpretation of parasite-host relationships. It now appears that most parasites retain genetically deep phylogenetic signals of host or habitat exploitation that enable the parasites to cross potential host-species boundaries when ecological opportunities arise. Mutations or genetic modifications a priori *are not needed* as the underlying **symplesiomorphic** (meaning, shared ancestral) traits enable cross-species transmission to new hosts when they are available (that is, **syntopic**). These

opportunities arise due to climate and geographic range-oscillations (the **oscillation hypothesis**; Brooks et al., 2019), **taxon pulses**, manifested by both multiplication and extinction of species (Erwin, 1985), and ecological fitting in sloppy fitness space (Janzen, 1980; Agosta, 2006; Agosta and Klemens, 2008; Agosta et al., 2010). Putting all of these together, Brooks and his colleagues (2019) have termed this the Stockholm Paradigm in honor of the researchers at the University of Stockholm in Sweden who first put these synthetic ideas into the literature stream.

Capacity

What is meant by capacity? As noted earlier, every species, including all parasites, have specific environmental resources they need in order to survive and reproduce. In the case of parasites, those resources are specific attributes of their hosts. For a given parasite species, if only 1 host species has the required resources, the parasite can survive only in association with that species, and its survival is tied to the survival of that species. But the vast majority of inherited attributes of all species are evolutionarily conservative, meaning they occur in more than 1 species of host.

All parasites live in association with a restricted number of hosts, and some not so restricted, as seen in *Toxoplasma*, and sometimes only 1 host species is infected. Sometimes parasites are restricted to a potential or actual species of host by limited **capacity** but mostly parasites are restricted by limited **opportunity**. And so, when the conditions change—say, as a result of climate change or intrusion of humans and their domestic animals into previously uncut forest—new opportunities are created and the parasites move into hosts they had the capacity to infect but never before had the opportunity to (this could be the result of trophic changes locally or of geographic dispersal into new areas).

Ecological Fitting

Ecological fitting (sensu Janzen, 1985) refers to cases when a parasite has the opportunity to encounter a new potential host that has the required resources for survival, the parasite will then be expected to add that species to its repertoire. This, by the way, eliminates the need for the right mutation to show up at the right time to allow or enable the parasite to jump into a new species of animal to make it a new host.

Fundamental Host Fitness Space

For any given parasite, the range of all hosts that have the required resources is called the **fundamental host fitness space** (in accordance with Hutchinson's notion of fundamental niche space; Hutchinson, 1959), which Agosta called fundamental fitness space in order to relate it directly to evolution (Agosta, 2006; Agosta et al., 2010). The actual hosts inhabited by the parasite at any given time represent the **realized host fitness space** (in accordance with Hutchinson's realized niche space and Agosta's use of the term *fitness* rather than *niche*). One of the keys to the evolutionary success of parasites is that the fewer species of animals used as actual hosts (that is, the smaller the realized fitness space), the more potential opportunities to inhabit new species of hosts exist. In other words, given the opportunity to come into contact with a suitable but previously unexposed (potential) host species, a parasite would add the new host to its host range and survive even if the original species of host went extinct. The fewer hosts actually used, the smaller the proportion of actual host fitness space compared to fundamental host fitness space and consequently the sloppier (meaning, more filled with potential opportunities) the host fitness space. At the same time, the more restricted the realized host fitness space, the more specialized the parasite is within that fitness space. Alternatively, the more species of potential hosts used, the larger the proportion of actual host fitness space compared to fundamental hosts space, the less sloppy the fitness space, the fewer new potential hosts, and the more generalized the parasite is in fitness space.

This insight, developed by Agosta (2006) and elaborated by Agosta et al. (2010) and Brooks and Agosta (2020), obviates the need to define or even discuss host specificity since it is basically impossible to look at host specificity in an evolutionary sense. Conversely, the idea of fitness space has a Darwinian evolutionary origin that can be tested in an evolutionary context.

Oscillation Hypothesis

Periods of climatic/environmental stability are usually associated with events of local geographic isolation, hence, specialization of parasites occurring in limited geographic areas and many potential hosts unexposed in other similar but separate geographic areas; periods of environmental perturbations are usually associated with increased or expanded species-level geographic distribution, hence, generalization may occur with fewer potential hosts. Parasites thus tend to oscillate between specializing and generalizing in host fitness space, depending on environmental conditions; this is called the **oscillation hypothesis** that was developed by Janz and Nylin (1998).

Taxon Pulse

All species of parasites and their actual and potential hosts alternate between geographic isolation (geographic contraction in space) and geographic expansion through space via dispersal. This is called the **taxon pulse** (Erwin, 1985).

Environmental perturbations drive taxon pulses, which drive host range oscillations, which drive parasite diversification by ecological fitting in sloppy fitness space, reinforced by natural selection (Agosta et al., 2010). Well worked-out examples that show these various parts of the Stockholm Paradigm include those presented in Brooks et al. (2006; 2015; 2019) and Malicka et al. (2015).

Ecological Fitting Example

Surveys and inventories are the primary ways that large scale and complete collections of parasites and their actual and potential hosts are accumulated over large geographic scales in short periods of time (Gardner, 1996; Gardner and Jiménez-Ruiz, 2009; Gardner et al., 2012; Galbreath et al., 2019). A final example of ecological fitting presented here stems from survey and inventory work on mammals and their parasites funded by the National Science Foundation (grant numbers BSR-9024816 and DEB-9496263), from a collection locality labeled 7 km S, 4 km E Cruce Ventilla in the Department of Oruro, Bolivia (read as "7 kilometers south and 4 kilometers east of Cruce Ventilla in the Department of Oruro, Bolivia"). The specific locality, referred to here as near Cruce Ventilla, was visited by a field team from the Museum of Southwestern Biology (Albuquerque, New Mexico, United States) and the American Museum of Natural History (New York, New York, United States), September 29–30, 1986 (Anderson, 1997).

Several species of mammals and their parasites were obtained at this locality. Of particular interest, 3 of the species of mammals were collected from the same burrow systems that had been constructed and were being actively used and maintained by subterranean rodents called highland tuco-tucos; species name *Ctenomys opimus* (Wagner). At this locality, several specimens of *C. opimus* were collected from the burrows, as well as several individuals of yellow-toothed cavy, species name *Galea musteloides* Meyen, and many individuals of 1 species of leaf eared mice, species name *Auliscomys boliviensis* (Waterhouse, 1846). Specimens of the mammals were collected sequentially or simultaneously, and all of the mammals were recorded as using the same burrow systems using the same entrances and exits. Great care was taken in performing the collections and necropsy on the specimens at this site because it appeared to be an opportune chance to identify any parasites that potentially could be shared among the 3 syntopic species of rodents that were occurring in the same micro-geographic space, using the same ecological space, and using the same resources (Rivas, 1964).

After collections were made using standard methods and necropsies performed (see Gardner and Jiménez-Ruiz, 2009; Galbreath et al., 2019), it was immediately evident that a single species of parasite was shared among 2 of the species of rodents but not all 3. The metacestodes were found only in *Ctenomys* and *Auliscomys*. This cestode was identified later as the larval form of *Taenia talicei* Dollfus, 1960, a polycephalic (meaning, having many scolexes) taeniid (order Cyclophyllidea: family Taeniidae: genus *Taenia*) identified by the morphology of the hooks and the multi-strobilate (many strobila associated with a single infection) nature of the larvae. Pinworms of the genus *Helminthoxys* were found in the cecum of the *Galea* but not in the cecum of individuals of *C. opimus*. However, many individuals of *C. opimus* were infected with a species of *Paraspidodera* that occurred in their cecae and large intestines. The individuals of *A. boliviensis* that were examined were shown to be infected with trichostrongylid nematodes (phylum Nemata: superfamily Trichostrongyloidea) in the small intestine and pinworms of the genus *Syphacia* (phylum Nemata: order Oxyurata) in the cecum. Current investigations are under way on both the endoparasites and the ectoparasites of this same host assemblage near Cruce Ventilla, Bolivia. This sharing of metacestodes among several species of rodents of widely divergent phylogenetic lineages illustrates the phenomenon of **ecological fitting** and the fact that metacestodes of *Taenia talicei* have broad host-range tolerances while the adults probably are more restricted (although no carnivores were collected and examined at or near this locality). It is generally observed that adult cestodes in the genus *Taenia* show host range use that is somewhat narrow, and this may partly be due to the effects of sympatric or syntopic species of intermediate hosts.

Economic and Hygienic Importance of Parasites

Some of the most important tropical diseases of humans are caused by parasites, such as schistosomiasis (bilharziasis) (caused by the blood fluke *Schistosoma*), filariasis (caused by several different species of filarioid nematodes), amebic dysentery (the protozoan *Entamoeba histolytica* is the causative agent of this one), and, in particular, malaria (at least 5 species of the protozoan *Plasmodium*). Annually, more than 247 million people are infected with various species of *Plasmodium*, the causative agent of malaria, and around 619,000 people die from it every year worldwide, particularly children in sub-Saharan Africa. The webpages of the World Health Organization (WHO), Division of Tropical Diseases and of the United States Centers for Disease Control and Prevention (CDC) contain information about the current status of the important parasitic diseases, which is continually updated. Information on prevalences of infection with various parasites and their geographical distribution are available at the CDC web site (https://cdc.gov) and at the WHO site (https://platform.

who.int/mortality/themes/theme-details/topics/topic-details/MDB/infectious-and-parasitic-diseases).

Global warming will lead to a spread of parasitic infections into some countries and increase prevalences of parasites in others that already have high parasite loads in their populations, especially in tropical and subtropical regions that will continue to warm over the next few hundred years (Brooks et al., 2019).

Parting Thought

The rest of this book provides an in-depth overview of many species of parasites, how they are related to one another, their adaptations, effects on hosts, and their importance as fellow inhabitants on Earth.

This introduction is fittingly ended with a quote from Harold W. Manter (Figure 2), one of the leaders in parasitology from the late 1920s through 1970 and the namesake of the Harold W. Manter Laboratory of Parasitology, one of the world's leading laboratories of systematic parasitology. Manter was an early proponent of the mutability of continents and plate tectonics and worked to provide evidence of continental movement with data from parasites and their hosts. From this work, he proposed the idea of **parascript** (Brooks and McLennan, 1993). Extracted from the book Host-Parasite Relationships (McCauley, 1966), Manter stated:

> Thus, parasites reflect both current environmental conditions and also the influences of ancient times—both ecology and phylogeny … Parasites of fishes, particularly such an abundant and diverse group as the Trematoda, furnish information about present-day habits and ecology of their individual hosts. These same parasites also hold promise of telling us something about host and geographical connections of long ago. They are simultaneously the product of an immediate environment and of a long ancestry reflecting associations of millions of years. The messages they carry are thus always bilingual and usually garbled. Today, we know only a few selected pieces of the code. As our knowledge grows, studies based on adequate collections, correctly classified and correlated with knowledge of the hosts and life cycles involved should lead to a deciphering of the messages now so obscure. Eventually there may be enough pieces to form a meaningful language which could be called ***PARASCRIPT: The language of parasites which tells of themselves and their hosts both of today and yesteryear***.

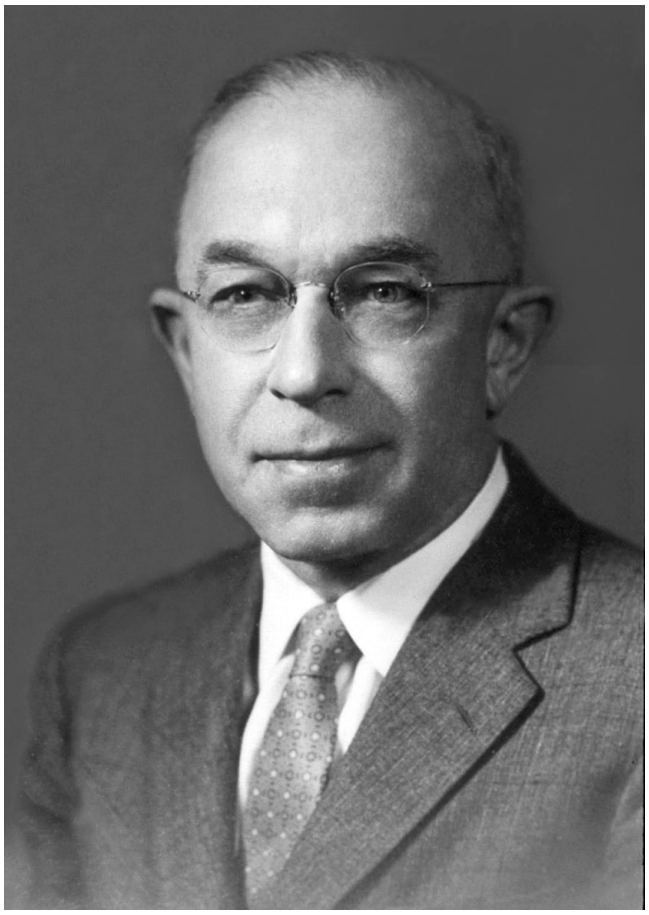

Figure 2. Harold Winfred Manter (1898–1971), circa 1960. Manter was a professor in the Department of Zoology, University of Nebraska (Lincoln campus; Lincoln, Nebraska, United States) from 1925 to 1971. He worked on systematics and biogeography of parasites of fishes, although during his tenure at Nebraska, he trained dozens of students in other areas of parasitology. The Harold W. Manter Laboratory of Parasitology (HWML) was named after him, having been established after his death in 1971 by Curator of the Parasitology Division of the University of Nebraska State Museum, Mary Lou Hanson Pritchard. Source: HWML. License: CC BY.

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2

Phylogenetic Systematics in Parasitology

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Chapter 2

Phylogenetic Systematics in Parasitology

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Connection Between Phylogenetic Systematics, Taxonomy, and Classification: A Review

Every species, whether it is the bacterium *Escherichia coli*, the malaria-causing *Plasmodium falciparum*, or the blue whale, *Balaenoptera musculus*, has a formal, given scientific name. Each name is in 2 parts, and in Latin, hence this formal name is also called the organism's Latin binomen (**bi** = 2-part, **nomen** = name). The first part of this 2-part name is the genus of the organism (for example, *Plasmodium*) and the second one, the specific or species name (for example, *falciparum*). However, it is conventional (and important) to use both parts of the scientific name (for example, *P. falciparum*), together when referring to the species. Because these names are in Latin, it is also conventional to *italicize* the scientific name in print or underline them when they are hand-written. The practice of giving each organism a formal name is **taxonomy** and relies on a set of methods.

Taxonomy goes hand in hand with, and is part of, a related scientific practice of placing organisms into formally named sets using a hierarchical system. This system of grouping, familiar to all of us since our biology classes in high school (Figure 1a), is **classification**. Early naturalists placed organisms that broadly shared common features first in larger groups and then ones that shared a smaller subset of features into progressively smaller groups, so there could be some order in describing and cataloging the vast diversity of life on Earth. A common formal classification scheme was devised (Figure 1a) and originally began with the category kingdom. It is a scheme that we follow to this day. Deciding what to name a species (taxonomy) when describing it for the first time or revising/changing the name depends on correctly classifying that organism. The ranks or categories of classification called the **genus** and **species** come at the very end of formal classification (Figure 1a). Here are examples of 2 species, the human broad tapeworm (*Dibothriocephalus latus*) (Figure 1b) and humans (*Homo sapiens*) (Figure 1c), formally classified.

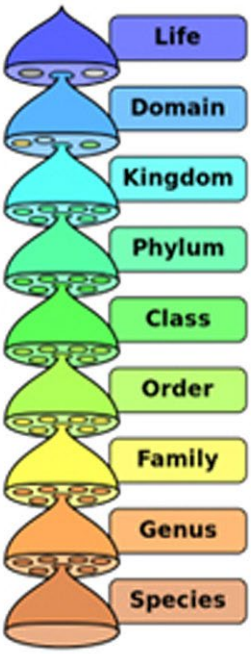

Figure 1a

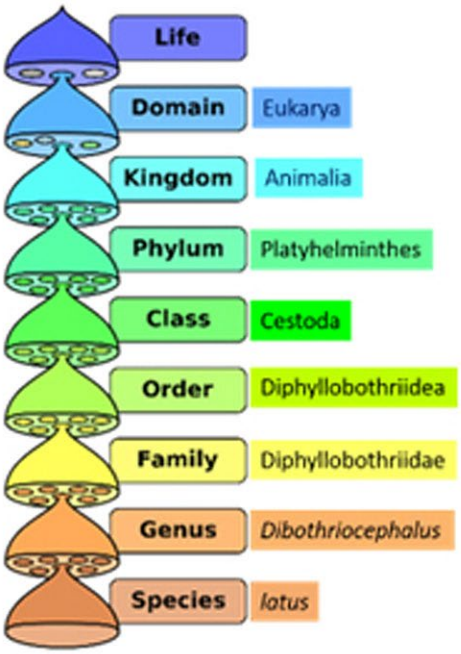

Figure 1b

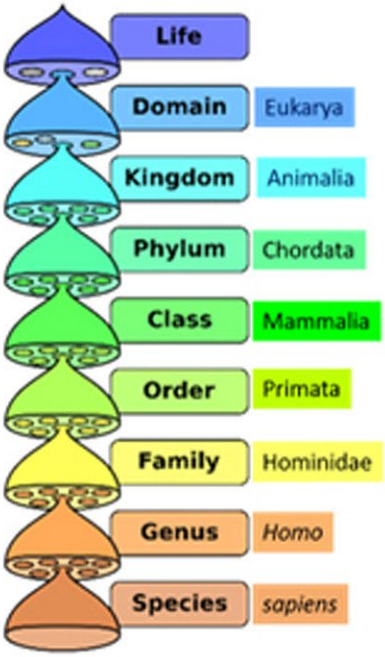

Figure 1c

Figure 1a, b, c. Names of taxonomic classification of organisms. Source: Adapted from Ideophagous, 2021. License: CC BY-SA 4.0 International.

Between these major categories or ranks (kingdom, phylum, class, order, family, genus, and species), taxonomists created subgroups to further fine-tune the classification. Examples of other categories are ranks such as subphylum, lower in rank than phylum but higher than class, or suborder, lower in rank than order but higher than family. In other words, a phylum could contain several subphyla, each with their own set of classes, and orders with their own set of suborders, etc. Note that this does not change the actual hierarchical nature of the classification but refines it. For example, the phylum Chordata is subdivided into 3 subphyla; 1 subphylum is the very familiar Vertebrata (vertebrates, a group to which we humans belong). The word vertebrate is used more often than the word chordate (for the phylum Chordata) because the other non-vertebrate chordates are rarely encountered in nature.

Taxonomy and classification fall under a broader branch of science called **systematics**, and scientists engaged in this research are called **systematists**. The following sections will include a brief review how systematics developed and flourished in the 20th century, and what impact it had on parasitology.

Cultivating a Deeper Understanding

What does **classification**—the formal grouping of organisms—imply and what methods are used to classify and place organisms in their correct groups and give them their appropriate scientific names?

Before scientists knew about evolution and genetics, organisms were classified based on their similarities. More common and general similarities were used for higher ranks or categories (for example, phylum) and similarities that were more limited to particular groups were used for lower ranks, such as class. For example, naturalists and anatomists noted that a large group of animals, including lampreys, jawed fishes, amphibians, lizards, snakes, turtles, mammals, birds, crocodilians, and even varieties of extinct fossil animals such as dinosaurs, pterosaurs, and others, possessed a stiff rod-like structure in their backs. Anatomists proposed that this structure, called the notochord or its modified version, a bony vertebral column, could be used as a unifying feature to group all organisms that possessed it, so they established the phylum Chordata (chordates). For chordates that possessed a bony spine, the vertebral column, they established the subphylum Vertebrata (vertebrates) to distinguish them at the time from chordates such as hagfish and lampreys that only had an unmodified notochord, which they considered primitive. The notion that some organisms and their features were ancient or primitive was well established because of the fossil record and the work of paleontologists. Naturalists also noted that only a subset of vertebrates possessed hair and mammary glands, so they grouped the ones that did into the next available lower taxonomic category, class, and named them Mammalia (mammals). Similarly, only a subset of vertebrates, birds, possess feathers, so for those vertebrates, naturalists established the formal class Aves. They also did this for amphibians (class Amphibia) and reptiles (class Reptilia). It is worth noting, albeit obviously, that naturalists were basing their classification on **comparative anatomy**.

Soon after formal classification was established in the 18th century, naturalists began thinking about the diversity of life on Earth as the product of **evolution**. Evolution proposed that all natural kinds of organisms–species–originated from previously existing natural kinds by modification, which led to the inevitable conclusion that all of life on Earth is related in the form of a giant family tree. As a result, taxonomists recognized that **similarity** among species was because of **evolutionary relatedness**. In other words, evolution provided, and for the first time, a unifying basis for understanding *why* species were more or less similar to one another.

Once evolutionary biology became widely accepted as the unifying theory in biology, taxonomists strove to produce **natural classifications**, that is, classifications that reflected the evolutionary, or genealogical, relationships of organisms. What this meant for the formal classification scheme (Figure 1a) was that when taxonomists examined the existing classification of species, or placed organisms they were discovering and describing in a particular class or family or genus, they needed to be reasonably confident that the placement reflected the evolutionary relationships of the species in question.

For several decades since the widespread acceptance of evolution in the early 1900s, taxonomists continued to use a combination of anatomical features, often newly discovered ones, to propose or revise the existing classifications of a wide range of organisms. Nevertheless, the practice suffered from the lack of a clear and objective methodology that could challenge or supplant the expert opinions and assertions made by leading taxonomists and systematists of the time. In other words, there was no consistent method of producing new classifications or testing existing ones. This problem was true for higher classifications, whether a species belonged to a particular order or family, as well as for lower-level classification, for example deciding whether a species belonged in one genus or another.

In 1963, Robert R. Sokal and Peter H. A. Sneath provided the first detailed objective method: **Numerical taxonomy**. In this once widely-used method, taxonomists tabulated data from as many morphological features of the species they were studying as they could and then analyzed those data using a particular mathematical algorithm (a set of computational

rules). This was akin to a **cluster analysis**, whereby species sharing the greatest number of characteristics would be grouped together. In other words, the method produced groupings based on overall similarity. The method in which groupings of species was based on such overall similarity came to be known as **phenetics**. The method had the advantage that both data and analyses were explicit, and hence, repeatable. Furthermore, the analyses could be improved by adding more data.

Phylogenetic Systematics

German entomologist Willi Hennig developed a fundamentally different method, called **phylogenetic systematics**, first published in German in 1950. Once it was translated into English in 1966 and became more widely accessible, it fundamentally transformed the practice of systematics, including how taxonomy and classification are practiced. Describing Hennig's approach, Brooks (1985), who first introduced phylogenetic systematics to parasitology, put it succinctly (emphases and word in brackets added):

> [Hennig] asserted that all species are composites of **ancestral** and **derived** traits; therefore, there are no such things as archetypes that, by definition, are all-primitive. This assertion led directly to Hennig's proposed methodology. If the traits exhibited by any species are a combination of primitive and derived features, then the traits shared by two or more species will be indicators of phylogenetic relationship. **Shared primitive traits** indicate general phylogenetic relationships while **shared derived traits** indicate more particular phylogenetic relationships. Two species that share a derived trait or traits that are unique to them are each other's closest relatives.

The idea in the last sentence from Brooks (1985) can also be applied to any taxon, whether it is a species or genus or any rank higher than that. For example, if 2 genera share a **derived** trait *unique* to them, the genera are each other's closest relatives.

In the technical language of phylogenetic systematics, relatively primitive or ancestral traits are called **plesiomorphies** (singular: plesiomorphy) or **plesiomorphic traits**, whereas relatively derived, that is, more recently evolved traits, are called **apomorphies** (singular: apomorphy) or **apomorphic traits**. Shared plesiomorphic traits are called **symplesiomorphies**, whereas shared derived traits are called **synapomorphies**. In phylogenetic systematics, synapomorphies are all important, and finding synapomorphies is a critical step in discovering true relationships among taxa.

The effect of phylogenetic systematics on classification was profound. Henceforth, valid natural groups could only be recognized or diagnosed by their synapomorphies, not by shared plesiomorphy. For example, if we want to examine the relationships among tetrapods, then the vertebral column is not a useful trait because all tetrapods have one, so it can't be used to distinguish some tetrapods from others. The vertebral column is a plesiomorphic trait for tetrapods. It is plesiomorphic because the common ancestor of tetrapods possessed this feature. Similarly, the presence of 4 limbs with digits is also not useful when trying to find out which tetrapods are related to which others either because the condition of having 4 limbs with digits is the ancestral tetrapod condition. On the other hand, an amniotic egg, found only in a subset of tetrapods, is a relatively more recently evolved type of egg compared to the ancestral egg of tetrapods that did not have an amnion surrounding the developing embryo. So, an amniotic egg can be used as a synapomorphy to relate mammals and sauropsids (birds, crocodilians, lizards, snakes, and turtles). Going a step further, within this amniote group, only a subset of amniotes have hair and mammary glands. Hair and mammary glands must then have evolved after the amniotic egg, and so can be used as synapomorphies for this group called mammals. Using phylogenetic systematics, the evolutionary relationships of the major groups of vertebrates can be depicted in the form of a branching diagram or **phylogenetic tree** and the synapomorphies placed on it (Figure 2).

In this phylogenetic tree (Figure 2), each group that is diagnosed by at least 1 synapomorphy is called a **monophyletic group**, often referred to as a **clade**. Thus, amniotes form a monophyletic group, comprising the common ancestor of all amniotes and all of the group's descendants. The clade amniotes is nested within a larger clade, the tetrapods, which in turn is nested within an even larger clade, the osteichthyans, and so on. Note the hierarchical nature of the relationship; there are groups within groups. This hierarchical relationship can be used to develop natural classifications, that is, classifications that reflect evolutionary relationships rather than arbitrary criteria or overall similarity.

What this foregoing example also illustrates is that every species, indeed every organism, is a mixture of very ancient anatomical (and biochemical) features, some that are not so ancient, and others that are quite recent. Humans, *Homo sapiens*, are able to produce collagen, a trait that is shared by every animal, including sponges. Collagen production actually defines what it means to be an animal; and, as such, it is one of humans' oldest traits. Humans' bony spine is ancient too, but not as ancient as our ability to produce collagen. Human jaws are also ancient, but not as ancient as the spine. Humans'

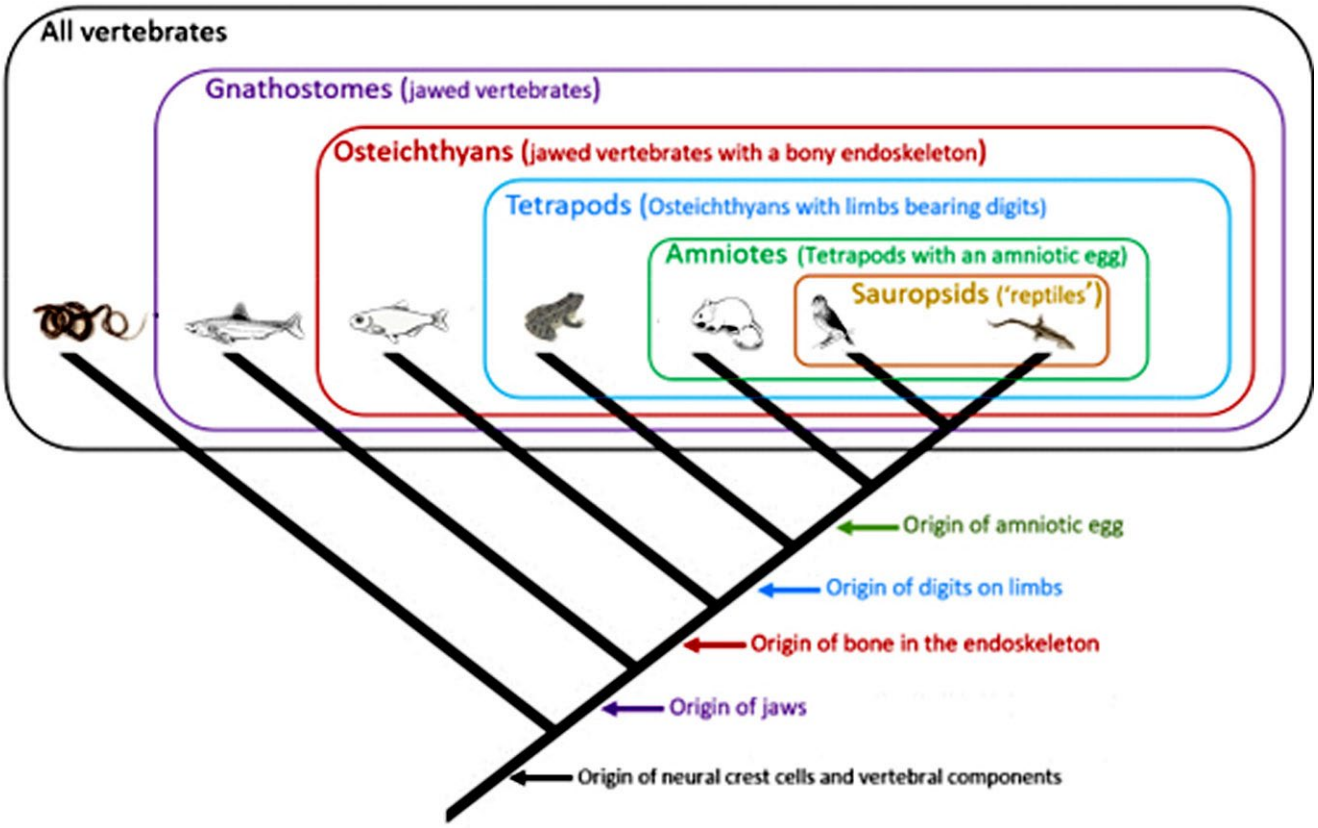

Figure 2. Basic phylogenetic tree of vertebrates. Snake image source: S. Stone, ca. 1789–1790, from the State Library of New South Wales, Australia. Public domain. Shark image source: P. S. Foresman, 2020. Public domain. Fish image source: Mrmw, 2021. Public domain. Frog image source: Z. Thompson, 1842. Public domain. Mouse image source: Gwilz, 2013. License: CC BY-SA 4.0. Bird image source: P. S. Foreman, 2020. Public domain. Lizard image source: J. de Graag, 1954. Public domain.

4 limbs with digits (fingers and toes) are also old, but not as old as the jaws that arose in distant ancestors some 450 million years ago. Human hair is a relative newcomer, only about 135 million years old. Humans' opposable thumb is much more recent, perhaps only about 2 million years old, and humans developed the ability for speech and, subsequently, language less than 200,000 years ago. These (and many other) traits can be **ordered**, ranging from the most ancient (earliest evolved; also called **plesiomorphic**) to the most recently evolved (**apomorphic**) as follows:

Collagen → vertebral column → jaws → limbs with digits → hair → opposable thumb → speech/language

Notice, too, that any feature/trait can be plesiomorphic or apomorphic relative to another feature/trait in this ordered series. For example, jaws are apomorphic relative to the vertebral column, but plesiomorphic relative to the tetrapod limb. Understanding the order of traits is an important part of phylogenetic thinking and practice. A word of caution here; sometimes the same traits/features may evolve independently in species that are distantly related by convergent evolution. These instances can be confusing; ornithischian dinosaurs have hip bones like those of birds hence the name Ornithischia (from the Greek **ornith** = of a bird). But birds share a greater number of synapomorphies with the theropod dinosaurs even though those dinosaurs have a hip that is unspecialized and is unlike that of birds and ornithischians. Therefore, the phylogeny of birds places them with theropod dinosaurs rather than with ornithischians.

There are online resources that provide useful overviews of phylogenetic systematics and related topics. The University of California, Berkeley hosts one such easily accessible and user-friendly resource, available at https://evolution.berkeley.edu/evolibrary/article/phylogenetics_01.

With advances in biotechnology and the ability to obtain DNA (deoxyribonucleic acid) and amino acid sequences, a new and rich source of data has become available. In molecular datasets, individual bases (nucleotides) or amino acids serve as characters and changes in these components (bases or amino acids) are conceptually treated in the same way as

changes in morphological characters. These data can thus be used for phylogenetic analyses. Molecular phylogenetics has now superseded morphology-based phylogenetic systematics in most areas, with the obvious exception of paleontology. Although both morphological and molecular data can be combined in an analysis, molecular data by their very nature (hundreds or thousands of bases or amino acids as characters) vastly outnumber morphological data.

Methods for Constructing Phylogenetic Trees

Several methods are currently used to analyze the relationships of taxa. These include Neighbor Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). Each method has its own set of assumptions. Neighbor Joining is considered a phenetic method by many because it uses a distance matrix of characters, and although computationally fast, is often inaccurate. It serves as an adequate first pass in an analysis and can be used in an exploratory manner, but has been supplanted by other, more powerful phylogenetic methods. Maximum Parsimony was originally developed for morphological data and is the oldest of the true phylogenetic methods. It is still preferred by some systematists on philosophical grounds. Maximum Parsimony uses an age-old principle in science–Occam's razor–whereby the tree that requires the least number of steps, that is, the fewest evolutionary changes, is the preferred phylogeny. Whereas MP makes fewer assumptions than other, probability-based methods that followed, it has been shown to have limitations in certain circumstances. Currently, probabilistic methods, such as ML and BI, are more commonly used to infer phylogenies. Of the several books available, Hall's (2018) book makes phylogenetic analyses accessible to all biologists by combining the basic theory of the methods mentioned above with a stepwise guide to doing basic analyses with a user friendly and popular phylogenetics software package, MEGA 7.

Reading a Phylogenetic Tree

Consider the phylogenetic tree in Figure 3 that shows the relationships of the 2 species of human lice *Pediculus humanus* and *Pthirus pubis* (modified from Reed et al., 2007).

This tree was generated by Reed and his colleagues (2004; 2007) who analyzed a combined dataset of DNA sequences

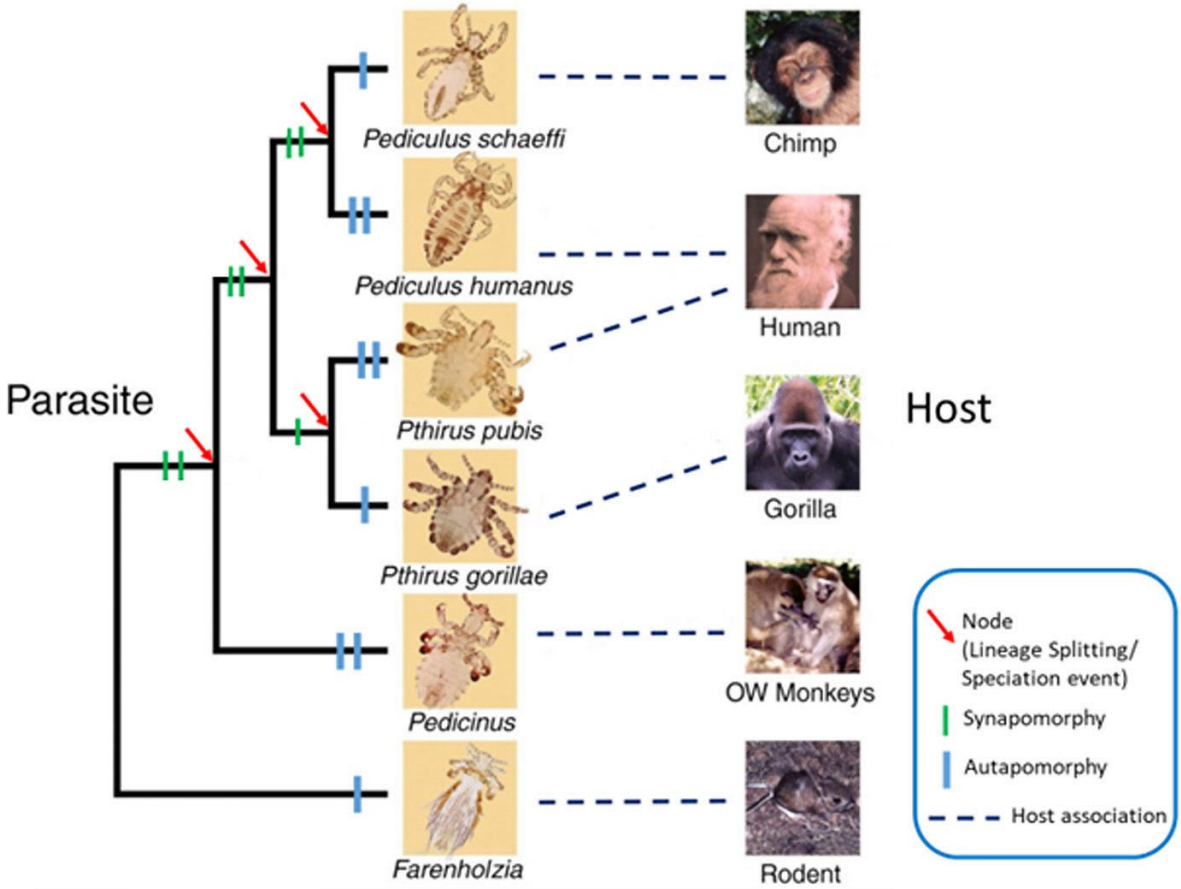

Figure 3. Phylogenetic tree for primate lice and their vertebrate hosts showing nodes, synapomorphies, autapomorphies, and host associations. The number of lines shows the number of synapomorphies and autapomorphies. Source: Adapted from Reed at al. 2004; 2007. Photo credits: J. W. Demastes, T. Choe, and V. Smith, 2004. License: CC BY 2.0.

of genes for cytochrome *c* oxidase subunit 1 (*cox1*) and cytochrome *b* (*cytb*). What do the various parts of the tree mean? First, locate the taxa (in this case species of lice) placed terminally at the end of the branches. The branching pattern reveals the relationships among these lice species. *Pediculus humanus* is most closely related to *Pe. schaeffi* from chimpanzees. Because *Pe. humanus* and *Pe. schaeffi* are each other's closest relatives, they are called **sister species**. *Pthirus pubis* is most closely related to *Pt. gorillae* from gorillas, so they are sister species as well. The red arrows point to the **nodes** of the tree. Nodes signify the splitting of the ancestral lineage into 2 daughter lineages, and in this tree denote the **speciation events** that produced the daughter species. The green bars on the internodes denote the **synapomorphies** based on which the relationships are established. Blue bars on the branches denote apomorphic features unique to each species; such traits are called **autapomorphies** (plural; singular autapomorphy) and are useful for diagnosing or identifying individual species but are not useful for uncovering relationships (recall that only synapomorphies can reveal relationships; see Figure 4). The letters **A, B**, **C**, and **D** are the ancestors of their daughter lineages or species. This is where we have to be cautious in our interpretation. **C** is the ancestor of *Pediculus schaeffi* and *Pe. humanus*. **D** is the ancestor of *Pt. pubis* and *Pt. gorillae*. **B** is the common ancestor of **C** and **D**. Going down to the base of the tree, one finds **A**, the common ancestor of **B** and the lineage that produced the genus *Pedicinus* in Old World monkeys. Another genus of lice, *Farenholzia*, found in rodents, serves as the **outgroup** to the group of lice being analyzed (the **ingroup**). The outgroup is used to root the tree, which is used to establish the order of change in the characters used in the analysis. The relative position of the different branches of the tree produce the tree's **topology** or shape. Note that this phylogenetic analysis indicates that *Pe. humanus* and *Pt. pubis*, are *not* each other's closest relatives, even though they are both found in humans.

Further Applications of Phylogenetic Systematics in Parasitology: Some Examples

Phylogenetic systematics can change our understanding of parasite relationships. Consider the case of the parasitic flatworms; they are grouped into 3 classes: Trematoda, Monogenea, and Cestoda. For much of the 20th century, and despite some opinions to the contrary, the monogeneans were considered trematodes. However, molecular phylogenetics indicated that the monogeneans are actually more closely related to cestodes than to trematodes, which in retrospect was suggested by the presence of the cercomer, a larval structure that some considered homologous to the monogenean haptor (see Figure 5).

A multi-gene phylogenetic analysis (Laumer et al., 2015) corroborates the inference that all parasitic trematodes had a common ancestor and that monogeneans are likely more

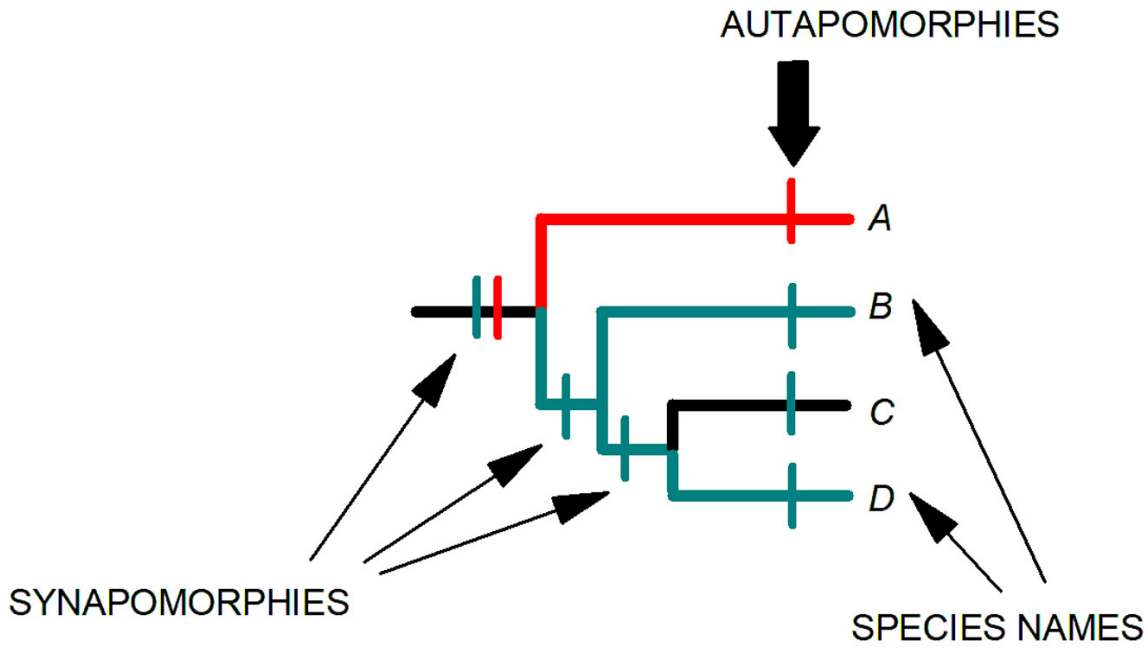

Figure 4. A phylogenetic tree showing distribution of characters. Characters that are shared by species are called **synapomorphies** (meaning, shared derived characters). A character that occurs only in 1 species is called an **autapomorphy**, which, more generally speaking, is a trait that is unique to a taxon. Source: S. L. Gardner, HWML. License: CC BY.

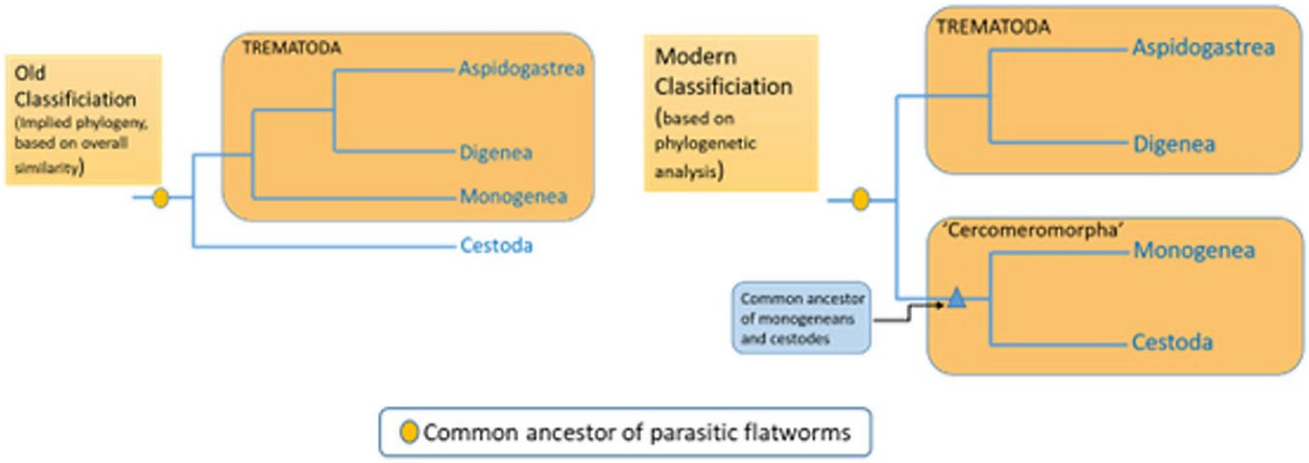

Figure 5. Cladograms showing the common ancestor of parasitic trematodes (flatworms) under an old classification and under a more modern classification. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

closely related to cestodes, but they found weaker support for the Cercomeromorpha than previous analyses suggested (see Figure 6). Laumer et al. (2015) also found that, as was previously proposed, parasitism evolved once in the flatworms and that all parasitic flatworms had a common ancestor.

Several conclusions can be drawn from this tree: 1) The parasitic flatworms form a strongly supported clade called the Neodermata, that is, the 3 groups of parasitic flatworms had a common ancestor in the distant past, 2) the Neodermata is a relatively late branching (recently evolved) clade of flatworms and sister to the free living Bothrioplanida, 3) the tapeworms and monogeneans form a clade called the Cercomeromorpha, and are therefore are more closely related to each other than either of them is to the Trematoda.

As methods improve and become more rigorous and sophisticated, phylogenetic reconstructions/hypotheses change and become arguably more robust. Let us examine how this happened using the case of a group of blood cell infecting parasitic organisms, the haemosporidians, that includes the human malarial parasites. In other words, a question that arises is: What are the relationships of the human malarial parasites, *Plasmodium falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*, and has that understanding changed over time?

One of the early studies on phylogenetic relationships of *Plasmodium* spp. was by Escalante et al. (1998) who used the Neighbor Joining (NJ) method to analyze sequences of the cytochrome *b* gene. They found that the 5 human infecting malarial species *did not* form a clade by themselves; instead, these species were in different parts of the tree (Figure 7). This suggested that humans became hosts of *Plasmodium* at different times in the evolutionary history of these parasites. In addition, there is strong nodal (statistical) support for the relationship of *P. falciparum* and *P. reichenowi*, statistically unsupported evidence of a sister relationship between *P. malariae* and *P. ovale*, and for a well-supported clade that contains *P. knowlesi* and *P. vivax*, as well as with 8 other species that infect a variety of other animals. The analysis also shows that an unknown species of *Hepatocystis* falls within a clade of *Plasmodium* spp.

The tree generated by Escalante et al. (1998) may be compared with a more recent, large, multigene study of human malarial species, using a maximum likelihood (ML) approach (Rutledge et al., 2017; see Figure 8). First, note that there is a difference in the number of species used in the 2 studies. Several species present in the earlier study by Escalante et al. (1998) are absent in this more recent analysis. Having different species in various analyses is not unusual when different datasets are used; not all species may have been available and the focus of the studies are different. Nevertheless, all of the human malarial species and several other species are present, which allows us to compare the interrelationships of the human *Plasmodium* species in the two studies.

Two clades are highlighted with colors, the *Plasmodium malariae* clade in red and the *P. ovale* clade in blue. The *P. ovale* clade contains the 2 subspecies of *P. ovale* and the *P. malariae* clade contains an additional form (possibly species) that the researchers uncovered in their analysis. Note that the human malarial species are in different clades. In several aspects this tree is similar in topology to the one by Escalante et al. (1998): 1) The human malarial species don't form an exclusive clade by themselves but are spread across the tree in different clades, 2) *P. falciparum* is closely related to *P. reichenowi*, and 3) *P. vivax* and *P. knowlesi* are in the same clade. A notable difference is the relationship between *P. malariae* and *P. ovale*, although note that the node showing the

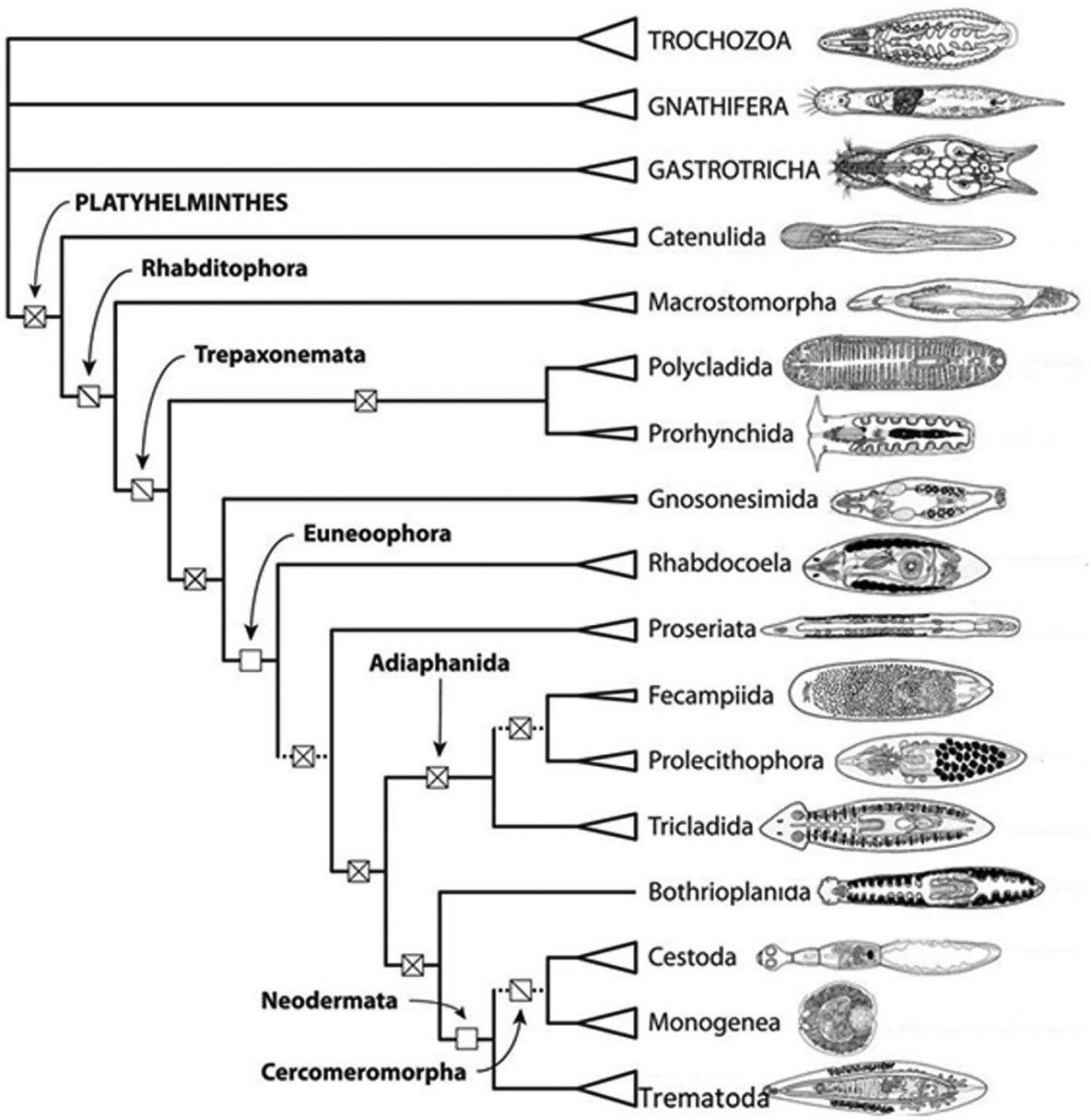

Figure 6. A multi-gene phylogenetic analysis by Laumer et al. (2015) corroborates the inference that all parasitic trematodes had a common ancestor and that monogeneans are likely more closely related to cestodes, but they found weaker support for the Cercomeromorpha than previous analyses suggested. Source: Laumer et al., 2015. License: CC BY.

relationship of these 2 species in the NJ tree by Escalante et al. (1998) has no statistical nodal support. The more sophisticated phylogenetic method used by Rutledge et al. (2017) has resulted in a better (meaning, more robust) tree with very high nodal support for the clades.

Rutledge et al. (2017) also used a molecular clock model to estimate the divergence levels of the species as calibrated to the *Plasmodium falciparum* and *P. reichenowi* split (×). They used a previously published date of 3.0–5.5 Ma (= million years ago) for the *P. falciparum* and *P. reichenow*i split. Calibrating the other splits to this date, they dated the *P. ovale* split to 20.3 Ma and the *P. malariae* split to 3.5 Ma. Cartoon silhouettes show the typical hosts of the different species.

Galen et al. (2018) improved upon previous studies. They analyzed a combined dataset of sequences from 21 protein coding nuclear genes and produced a comprehensive phylogenetic analysis of haemosporidians (see Figure 9). How should the tree be interpreted? Does it change the relationships of human malaria causing *Plasmodium* spp. inferred from previous analyses?

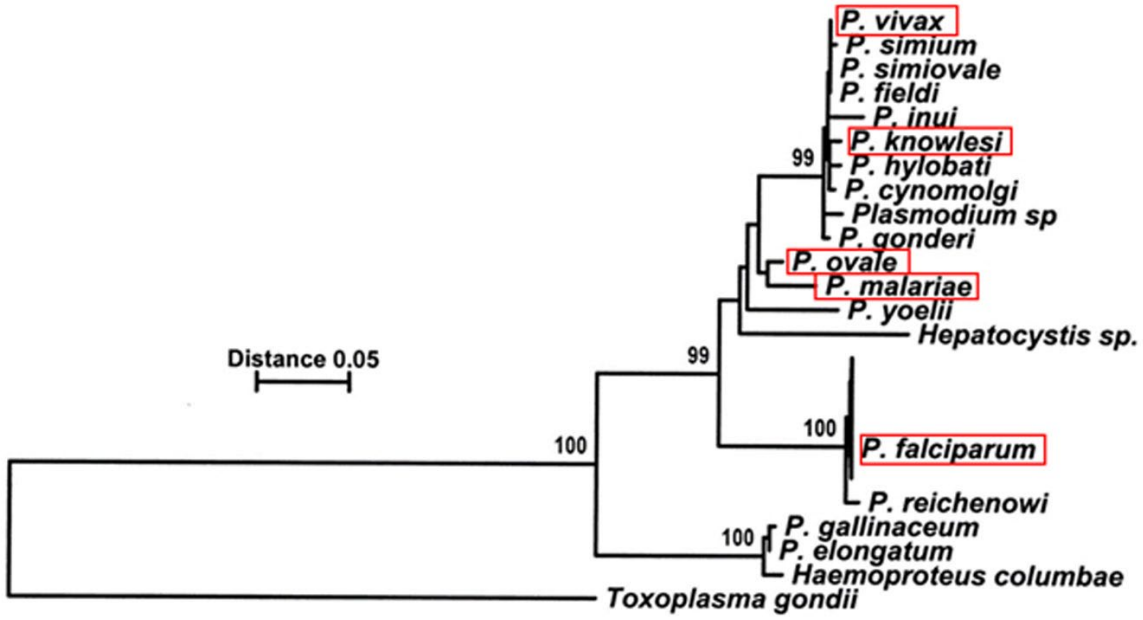

Figure 7. Relationships of the different *Plasmodium* spp., including the human malarial species *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*. Neighbor joining (NJ) analysis. Source: Escalante et al., 1998. Public domain.

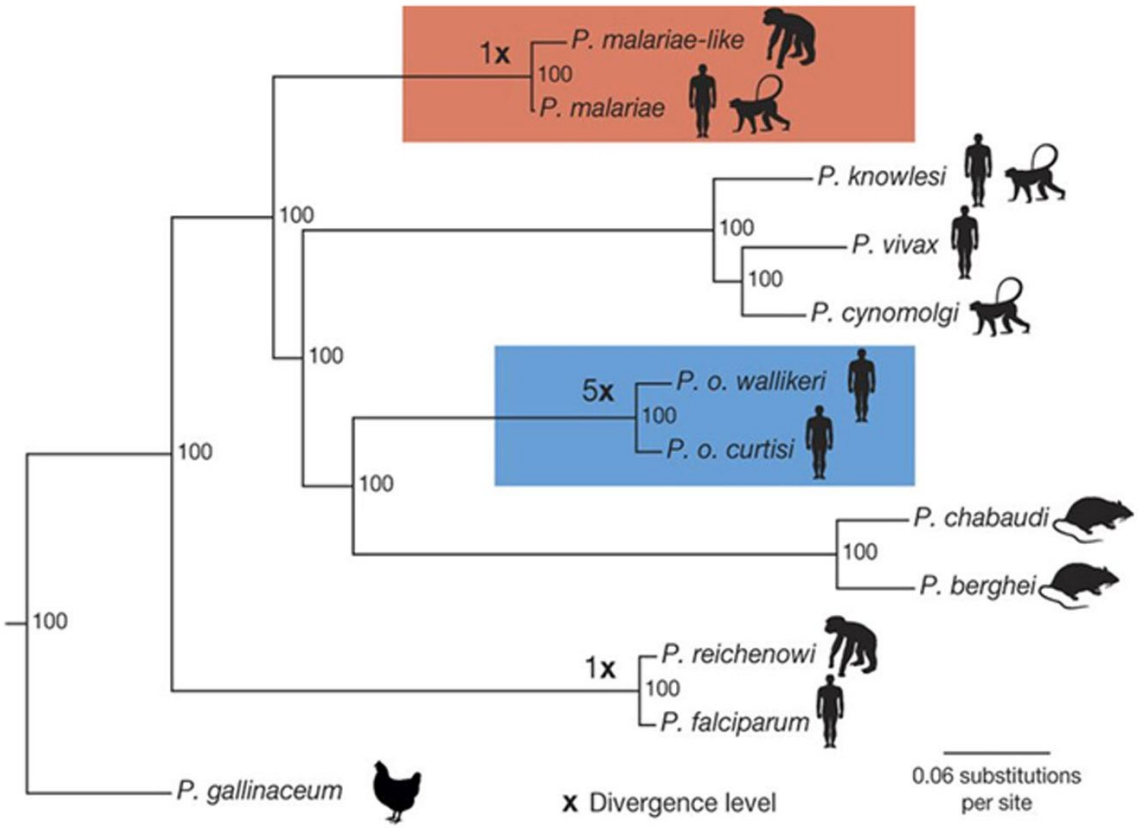

Figure 8. The more sophisticated phylogenetic method used by Rutledge et al. (2017) compared to the one employed in the study by Escalante et al. (1998) has resulted in a better (meaning, more robust) tree with very high nodal support for the clades. Source: Rutledge et al., 1998. License: CC BY 4.0.

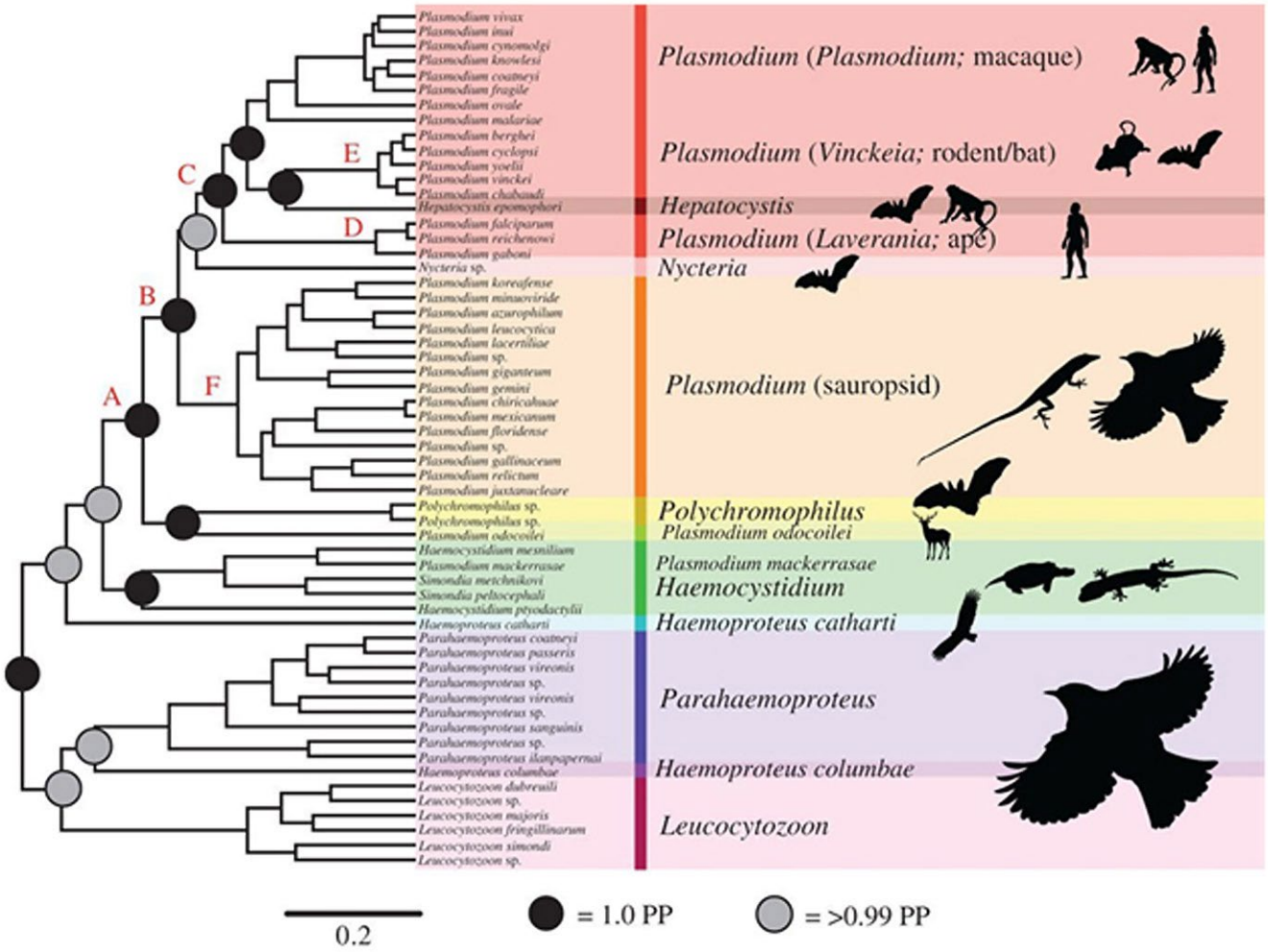

Figure 9. This is the favored haemosporidian phylogeny according to Galen at al. (2018). Shown as silhouettes are representatives of the vertebrate host group for each haemosporidian lineage. Source: Galen et al., 2018. License: CC BY 4.0.

If the tree generated by Galen et al. (2018) is compared with the tree of Rutledge et al. (2017), it is evident that the branching relationships of the human *Plasmodium* spp. are generally consistent. *Plasmodium falciparum* is related to *P. reichenowi*, a relationship that appears in both Escalante et al. (1998) and Rutledge et al. (2017). Thus, it appears that *P. falciparum* had a very separate origin than the other human *Plasmodium* species. *Plasmodium vivax* and *P. knowlesi* still belong to the same clade, albeit without strong support, which corroborates both previous studies. However, this tree goes far beyond analyzing the relationships of the human malarial species. By analyzing all the known haemosporidians, the authors have provided a tantalizing deep historical view of these parasites. It appears that the original ancestral hosts of the haemosporidians are birds (and other sauropsids) of the past.

Coevolution and Host Shifting (Host Switching)

One of the fundamental questions that parasitologists often ask is: How did a particular species of parasite come to be associated with a particular species of host? For example, how did humans become hosts of their 2 louse species, *Pediculus humanus* and *Pthirus pubis*? Comparing the phylogenies of the lice and humans allows exploration of that question. The example shown in Figure 10 is taken from the work of Reed and colleagues (2007). Note that Janzen (1985) considered a more strict definition of coevolution to be reciprocal evolution of host and parasite

When comparing the phylogeny of the lice (on the left) with the phylogeny of their primate hosts (on the right), there is a congruence (topological similarity) between portions of the louse phylogeny and the primate host phylogeny. This suggests that the parasites evolved along with their hosts; this is considered by some researchers to represent

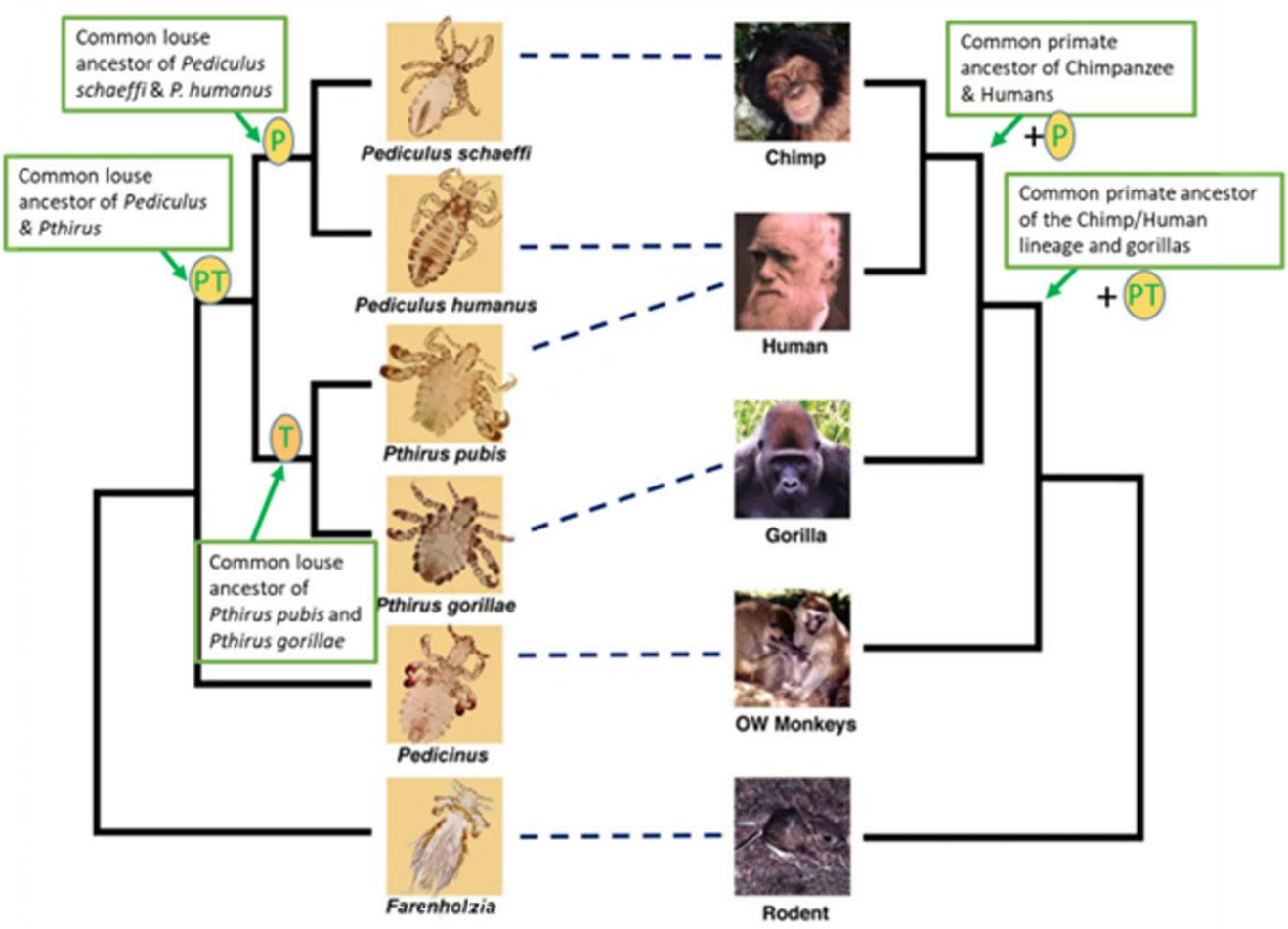

Figure 10. Phylogenetic trees for primate lice and their vertebrate hosts. Trees shown as a cladogram with no branch length information and based on molecular and morphological data. Dashed lines represent host-parasite associations. Humans are unique in being parasitized by 2 genera (*Pediculus* and *Pthirus*). Source: Adapted from Reed at al., 2007. Photo credits: J. W. Demastes, T. Choe, and V. Smith, 2004. License: CC BY 2.0.

coevolution. For example, the 2 sister species of *Pediculus* occur on hosts (chimps and humans) that are also each other's closest relatives. Logically, it may be inferred that the common ancestor **P** of the 2 *Pediculus* sister species was present in the common ancestor of chimps and humans. This type of coevolution, where there is a tight congruence between parasite and host phylogeny, that is, where the parasite phylogeny mirrors the host phylogeny, is called **cospeciation**. Similarly, by further comparing the phylogenies of the louse and primate hosts, we can infer that because the genus *Pediculus* is sister to the genus *Pthirus,* the common louse ancestor **PT** of *Pediculus* and *Pthirus* must have occurred in the common primate ancestor of the chimp-human lineage *and* gorillas. However, upon further scrutiny, it becomes evident that there is an incongruence between the phylogeny of the 2 species of *Pthirus* and their hosts. *Pt. gorillae* is a gorilla parasite and *Pt. pubis* is a human parasite, but the gorilla and humans are not sister host species, while chimps and humans are. So, while the 2 species of *Pediculus* show cospeciation, the 2 species of *Pthirus* do not. How could this have happened? What is the explanation for the current associations of the 2 species of *Pthirus* in gorillas and humans?

There are 2 explanations for the association of *Pthirus* lice. In order to understand the alternate explanations, it will help to first simplify the trees and superimpose the louse and primate host phylogenies (Figure 11).

The incongruence between the phylogeny of the hosts and *Pthirus* (dashed lines, Figure 10) becomes apparent. One explanation for this is that the ancestral *Pthirus* and the ancestral *Pediculus* both originated on the common ancestor of the chimps, humans, and gorillas, that is, there was **duplication** of lineages in that ancestor (see coevolutionary hypothesis 1, Figure 12). Our human hominid ancestors retained

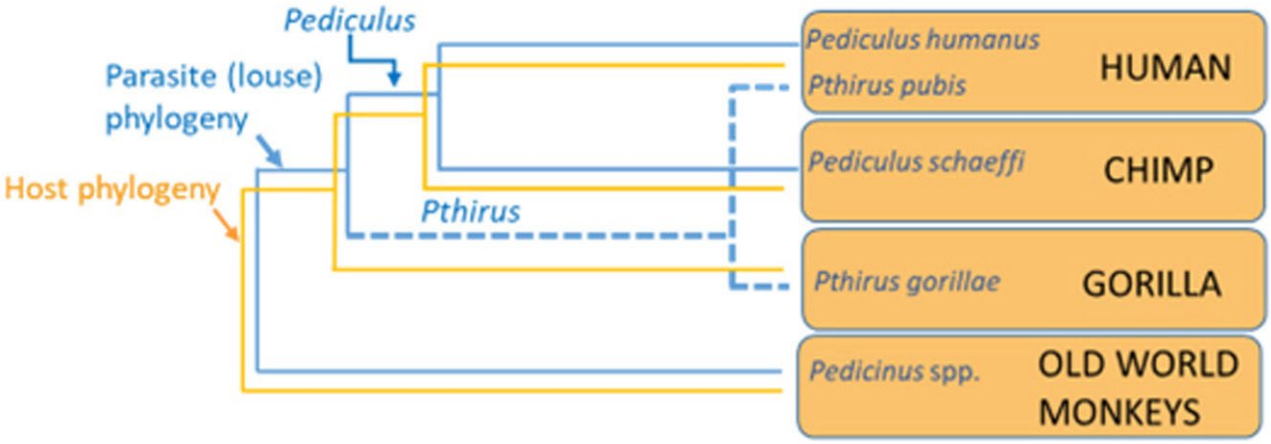

Figure 11. A simplification of the trees with the superimposition of the louse and primate host phylogenies. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

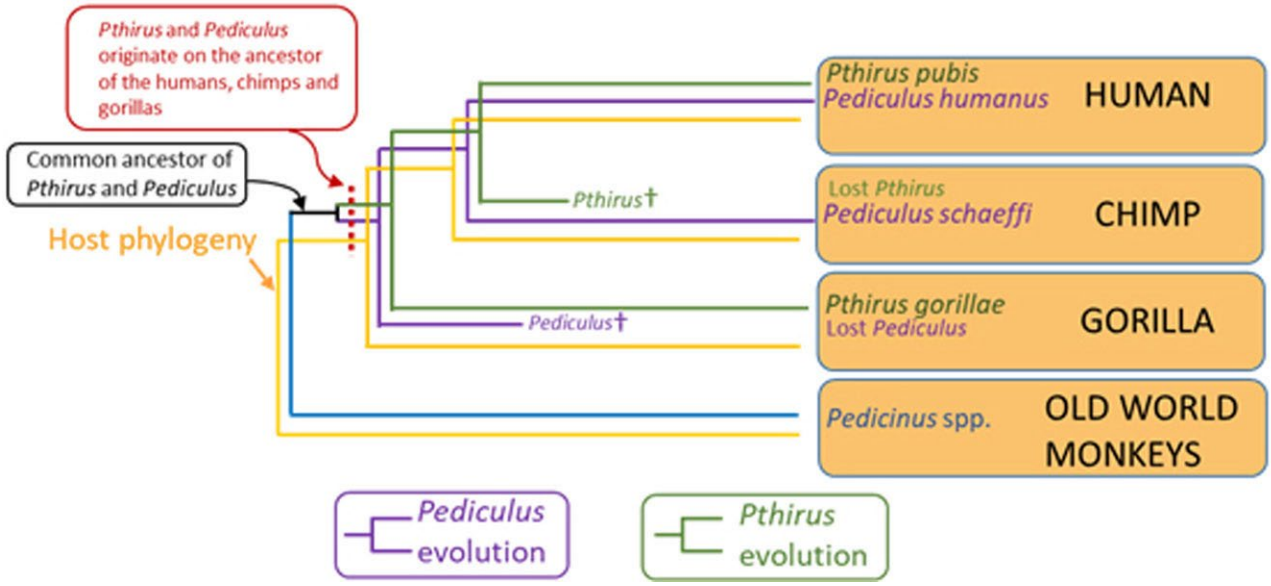

Figure 12. Coevolutionary hypothesis 1. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

both lineages (*Pediculus* and *Pthirus*) but the chimpanzee lineage lost *Pthirus,* while the gorilla lineage lost *Pediculus*. In other words, in this reconstruction, a neat pattern of co-speciation is altered by extinction events in 2 host lineages (chimps and gorillas) that resulted in the louse-host associations seen today.

There is, however, a simpler explanation that does not require the elaborate extinction events proposed in the previous hypothesis. Instead, it may be proposed that human hominid ancestors acquired the louse ancestor of humans' *Pthirus pubis* from some ancient ape of the gorilla lineage, that is, by **host-shifting**, also known as **host-switching** (see coevolutionary hypothesis 2, Figure 13) or **ecological fitting** (see Janzen, 1985).

How to choose between these 2 alternate reconstructions or hypotheses? The principle of parsimony may be applied and then it may be argued that the second hypothesis requires only **1** step, 1 instance of host-shifting, to explain the incongruence between the louse and primate phylogenies. In contrast, the first hypothesis required a lineage duplication, followed by 2 separate, independent, instances of lineage extinction. Therefore, hypothesis 2 is the more parsimonious explanation and in the absence of the any other evidence to the contrary, is the preferred working hypothesis. In this

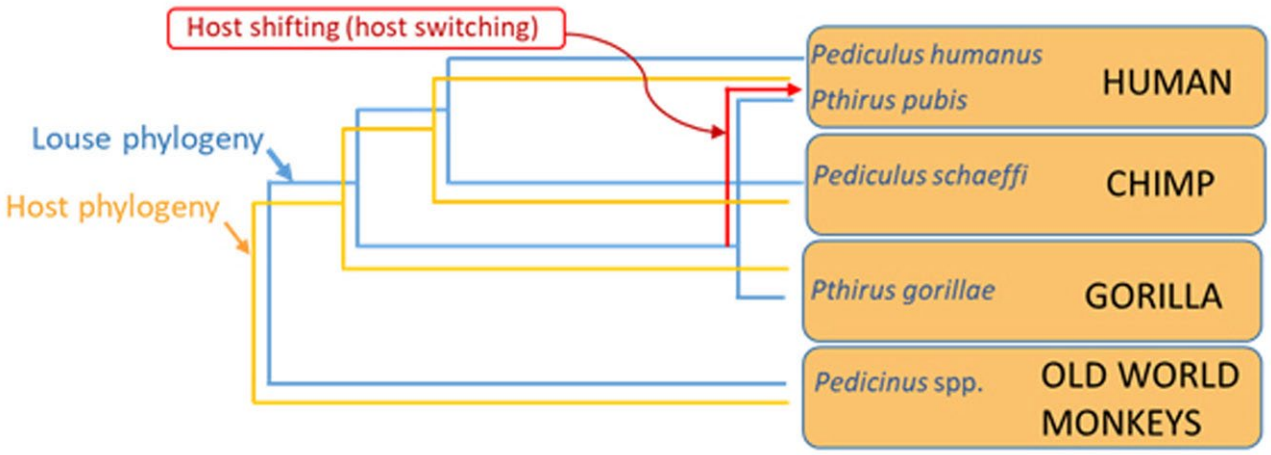

Figure 13. Coevolutionary hypothesis 2. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

particular case, however, the authors were able to apply evidence from estimated divergence times of the primate host lineages to show that *neither* hypothesis on its own was consistent with the known evolutionary history of the hosts. Their final analysis indicated that both duplication and extinction, followed by host-shifting likely occurred to produce the present-day associations between the lice and their primate hosts.

Phylogenetic Systematics, Coevolution, and Biogeography

Phylogenetic systematics not only allows the examination and exploration of the coevolution of parasites and hosts but also their historical biogeography, that is, how and where they came to be associated with their hosts. Here is a simple example that illustrates this application. The trematode genus *Bunodera* comprises species that are found in fishes belonging to the family Percidae (perches) and Gasterosteidae (sticklebacks). Three of the species occur in sticklebacks. By mapping the hosts and their distribution on the phylogeny of these trematodes (Figure 14), both the coevolutionary history as well as the history of the host-parasite associations

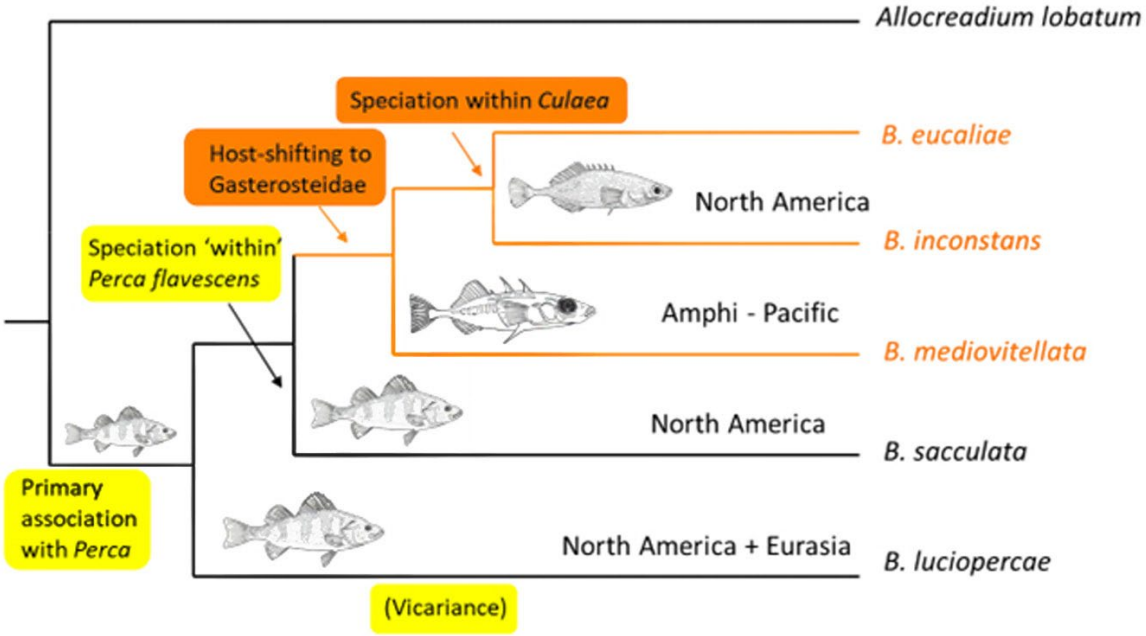

Figure 14. The trematode genus *Bunodera* likely originated in percid fishes in the northern latitudes and became associated with sticklebacks in North America via ecological fitting in the distant past. There appears to have been further speciation in the freshwater brook stickleback, *Culaea inconstans*, a stickleback species endemic to the freshwaters of North America. Source: A. Choudhury and V. León-Règagnon, 2005. License: CC BY-NC-SA 4.0.

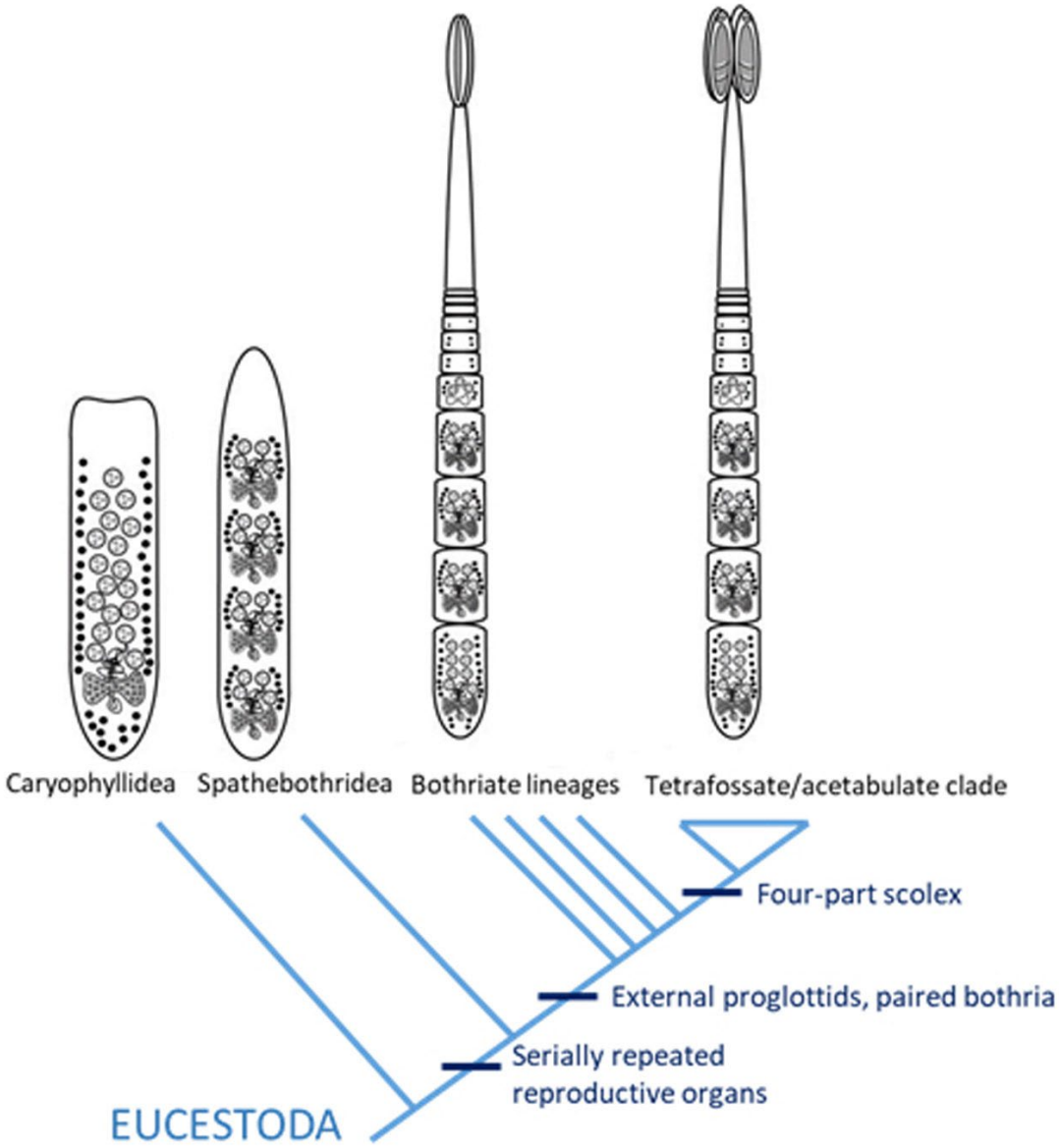

Figure 15. A phylogenetic representation of the evolution of strobilization as a derived character in some cestodes. Image source: A. Choudhury modified after Olson et al. (2001), 2019. License: CC BY-NC-SA 4.0.

may be deduced. Doing so reveals that the genus likely originated in percid fishes in the northern latitudes and became associated with sticklebacks in North America via ecological fitting in the distant past. There appears to have been further speciation in the freshwater brook stickleback, *Culaea inconstans*, a stickleback species endemic to the freshwaters of North America.

Phylogenetic Systematics and Mapping Traits

Phylogenetic trees also can help elucidate the evolution of body plans and a variety of morphological, biological, and behavioral traits. Consider, for example, the bewildering diversity of tapeworms, the Cestoda. The vast majority of tapeworms belong to a subgroup called the Eucestoda. Among the eucestodes are an order of unsegmented tapeworms with a single set of reproductive organs, Caryophyllidea. Another order, Spathebothridea, also comprises unsegmented tapeworms, but they possess serially-repeating sets of reproductive structures. The vast majority of the remaining eucestodes have a strobila with externally-visible segments called proglottids. Is the unsegmented condition with a single set of reproductive structures as seen in Caryophyllidea a primitive feature? Are the caryophyllideans an early branching lineage of tapeworms or is their morphology highly

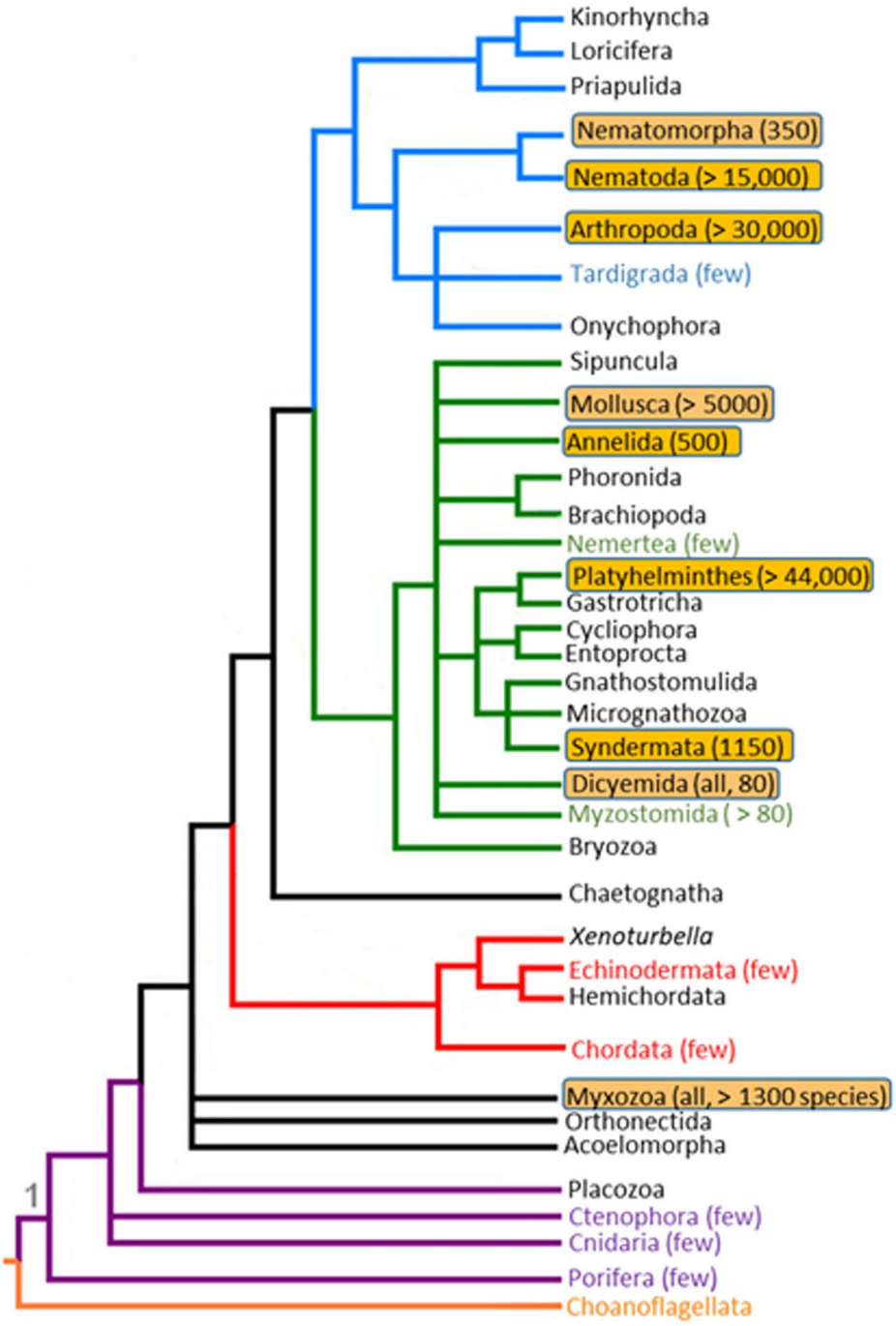

Figure 16. Metazoan phylogeny showing the wide-ranging polyphyly of parasites. Source: A. Choudhury modified after Wlodzimierz (2006), 2019. Public domain.

modified from strobilate segmented cestodes? Mapping the morphology of tapeworms on their phylogenetic tree allows us to address these questions.

A phylogenetic analysis of the Eucestoda by Olson and his colleagues (Olson et al., 2001; see Figure 15) shows that Caryophyllidea is an early-branching group and further reveals that the condition seen in Caryophyllidea is primitive and not highly modified and reduced from strobilate ancestors. The phylogenetic tree also reveals that the superficial external segmentation (proglottisation) of cestodes is a more derived condition and that a scolex with 4 attachment structures (plural bothridia, singular bothridium) may have evolved from a scolex with 2 attachment structures (plural bothria, singular bothrium).

Parasites Are a Polyphyletic Assemblage with a Common Lifestyle

Parasitology is unique in the field of organismal biology since most other subjects in organismal biology are developed and organized around monophyletic organismal groups; ornithology is the study of birds, entomology the study of insects, acarology the study of mites and ticks, mammalogy the study of mammals, and so on. Unlike these other subjects that deal with monophyletic groups of organisms, parasitology is the study of certain organisms, in this case, parasites—all of which share a common **lifestyle** (parasitism), rather than a unique common ancestry as a group. In other words, there is no unique common ancestor only for all parasites. If the phylogenetic tree of animals is examined, parasitic species will be found in a wide range of phyla, highlighted in the tree above (Figure 16), along with their free-living relatives. Parasitic nematodes are related to free-living nematodes, parasitic trematodes to free-living trematodes, parasitic annelids to free-living annelids, and so on. The approximate number of parasitic species in each phylum is in parentheses. This clearly shows that parasitism evolved independently many times in the evolution of life on Earth, and that parasites evolved from pre-existing, closely related, free-living ancestors.

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3

Helminth Identification and Diagnostics: Basic Molecular Techniques

Anindo Choudhury and Scott L. Gardner

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Chapter 3

Helminth Identification and Diagnostics: Basic Molecular Techniques

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Introduction

Molecular systematics, that is, the use of DNA sequences to address a variety of questions on the identity, species boundaries, and relationships of organisms has now become a powerful and useful approach that complements traditional systematics based on morphology. A perusal of the literature on parasite systematics suggests that much but not all recent understanding and hypotheses of parasite identification and phylogenetic relationships have been obtained through the application of molecular methods (for example, Olson et al., 2003; Nadler et al., 2010). This review summarizes some key protocols in molecular systematics as are used for studying helminth parasites.

Collection of Specimens

The first step in doing molecular systematics is the proper recovery of helminths from the host. Although the specimens used for DNA extraction and subsequent processing need not be handled in the same gentle manner as specimens for morphological studies, they should be collected live, cleaned in 0.6% saline or PBS (phosphate buffered saline) by gentle pipetting or agitation in a petri dish to wash off adhering debris, and then preserved and stored for subsequent processing. Specimens that are to be used for DNA work should be stored directly in 95% or 100% ethanol, making sure that the ethanol does not contain denaturing agents such as ketones, aldehydes, methanol, or kerosene, which are harmful to DNA. A careful reading of the label on the ethanol bottle will indicate what denaturing agents were used. Often, commercially available 95% ethanol is preferred because it may not contain any denaturing agents. Isopropanol can be allowed as a denaturing agent. The sample should be stored in ethanol in a cryovial or in a similar suitable vial and should be kept chilled in a regular freezer (at –20 °C) if possible or in a regular refrigerator (approximately 4 to 8 °C) until use. As a cautionary note, formalin is very harmful for DNA work and the worms being used for DNA analysis should never be brought in contact with formalin. See Gardner and Jiménez-Ruiz (2009) for details on collection methods.

Note that each time a sample of worms is collected with the intention of doing molecular work, a small subsample of worms from the same batch should also be separately fixed for a corresponding voucher sample to confirm the identity of the worms being studied using morphological examination. These specimens should be fixed by the proper techniques that will allow good stained whole mounts to be produced and be suitable for histology or scanning electron microscopy (SEM). For certain helminths (cestodes, trematodes, nematodes), using hot (steaming) 5% or 10% neutral buffered formalin is an easy way of producing relaxed and well-fixed specimens for subsequent stained whole-mounts. If a fume hood or proper ventilation is not available, killing helminths with hot PBS (or saline) and then placing them in unheated fixatives (formalin alcohol acetic acid (FAA) and so on) will suffice for producing adequate stained whole mounts, but worms fixed in this way are not suitable for histology and not ideal for SEM work.

In certain cases, for example, in the case of cestodes, a piece of the worm may be collected in ethanol for DNA analysis and the rest of the worm fixed for morphology, which now allows the specimen to be treated as a **hologenophore** (meaning, a vouchered specimen for which there is corresponding DNA sequenced data) (Pleijel et al., 2008). Occasionally, acanthocephalans, nematodes, monogeneans, and larger trematodes can also be treated in this manner (Gardner and Jiménez-Ruiz, 2009).

Another technique that is now often used is killing the worms in hot water or hot PBS and immediately placing them in 95% ethanol. This saves time and desired portions of the worms can be later excised in the lab for DNA extraction. The disadvantage of this method is that ethanol is only a preservative and is not a fixative, and 95% ethanol can cause worms to shrink, become rubbery, and collapse.

While collecting and fixing specimens for morphological and molecular studies, it is important that vials, Petri dishes, and pipettes that have come in contact with formalin or other fixatives such as Bouin's or FAA (AFA) be kept separate from instruments and glassware used for handling worms being collected for DNA work.

Several specimens should be collected for molecular analysis but even 1 specimen is better than none. For worms that are less than 0.5 mm in length, 2–5 specimens are usually enough to guarantee sufficient DNA on extraction. For specimens 3–5 mm in length, 1 or 2 specimens is/are usually sufficient. DNA can be even extracted from single worms as small as 0.2 mm. Specimens can be stored in 100% molecular grade ethanol in a refrigerator or freezer for years but the quality of the DNA does decline with length of storage time unless the sample is stored at less than −85º C.

Another important aspect is the proper recording of data and the proper labeling of tubes. Tubes or vials that contain specimens for DNA work should be labeled on the outside with paint markers or in other ways that will not be erased by freezing and thawing. Paper labels are often used for labeling specimens inside the vial but should not be used for specimens being stored for DNA analysis because the labels may introduce contaminants.

DNA Extraction

DNA can be extracted from collected worms using standard techniques, such as phenol-cholorform extraction or a variety of commercially available kits. The phenol-chloroform extraction is a standard extraction technique, but phenol is a harmful chemical and the procedures have to be conducted with the proper precautions. As a result, scientists have switched to less toxic methods or easier and less toxic alternatives such as commercially available and fast extraction kits such as Qiagen's DNEasy DNA extraction kit. Other companies, such as Invitrogen, Promega, and others, also manufacture extraction kits. Such kits combine extraction with a subsequent cleaning step and each company provides a booklet with its kit that outlines the protocol. The extracted DNA can be stored in the freezer at −20 °C or at colder temperatures of −85 °C (or even lower).

DNA Amplification

The next step in the process is the amplification of the desired genes of the specimens from which the DNA is extracted. In helminth systematics, the ribosomal RNA gene array (sometimes referred to as rRNA) and the cytochrome *c* oxidase subunit 1 gene (*CO1*) are commonly targeted for obtaining sequences. The most common regions of the rRNA gene array are usually parts of the small subunit (18S) and large subunit (28S) but also portions of the internal transcribed spacers (ITS-1 and ITS-2) as well as the 5.8S region. In the absence of full-length sequences, partial sequences of certain regions of these genomes are still useful. The method that is most widely used for amplifying portions of the target genes is the polymerase chain reaction (PCR). The **PCR reaction** requires several key ingredients:

1) A **polymerase enzyme** that will not denature at high temperatures. The successful isolation and commercial production of a polymerase from thermophilic prokaryotes allowed such enzymes to be used in the high temperature conditions encountered in the reaction. Several types of polymerase enzymes are available of which the polymerase isolated from the hot springs bacterium *Thermophilus aquaticus* (Taq polymerase) is the most common. This enzyme can be purchased from a variety of biotech companies.
2) **Primers**: These are small (usually 20–30 bp long; bp = base pairs) strands of DNA with sequences that are identical to portions of the genes that are being targeted for amplification. In a PCR reaction, primers are used in pairs (a forward primer and a reverse primer), and prescribed quantities of each primer are used. The forward primer binds upstream on the target gene and the reverse primer binds downstream and they work in opposite directions on each of the 2 complementary single strands of the double stranded DNA (ds DNA); the denaturing of DNA is part of the PCR reaction. Primers are usually made to order by supplying the biotechnology company that manufactures primers the letter sequences needed. There are several standard primer sequences that have been published in the literature.
3) **Magnesium buffer**: A special buffer that contains the required amount of magnesium for the enzyme to work adequately is supplied by the company that supplies the polymerase enzyme.
4) **DNA substrate**: This is the DNA that was extracted from the parasites using the protocol outlined before.

The reagents listed above are mixed in prescribed amounts in special PCR tubes and the reaction mixture is placed in a thermocycler. Numerous models of thermocyclers are commercially available from biotech companies, such as the ones made by Perkin-Elmer. Thermocyclers can be programmed and users have to specify the reaction conditions. Most published papers specify the PCR conditions. The PCR method, once standardized for a certain pair of primers, can be repeatedly used with success. Once amplification is completed, the PCR tube is removed from the thermocycler and the amplified DNA is first tested by running (electrophoresis) a small

aliquot (~ 5 µl) on a mini gel along with a DNA ladder appropriate for PCR products. PCR products can range anywhere between 300 to 2,000 bp, depending on the primers, the gene being targeted for amplification, etc. If the electrophoresis gives positive results and there is no evidence of mispriming (multiple amplified products on the gel), the remaining PCR product is purified by passing it through a membrane or column which binds the amplified DNA, which is then eluted out in a buffer or sterile deinonized water. There are standard kits for purification that are available commercially from biotech companies. This amplified and purified DNA sample can be stored in −20 °C or −80 °C (or lower) and a small amount of this is usually used for sequencing.

When the sample is ready for sequencing, it is thawed and a small aliquot of the purified PCR product is sent along with an aliquot of the primers but separately (unlike the PCR reaction, the sequencing reaction only uses one of the primers at a time). The sequencing reaction usually requires ~ 40 ng of purified amplified DNA and so the purified DNA has to be quantified first. Quantification can be done using DNA quantification ladders in a mini gel electrophoresis.

Sometimes the sequencing primers may be different from the PCR primers but most times the PCR primers are also used for the sequencing reaction. The sequencing can be done manually but this is time-consuming and no longer cost effective. Instead, most sequencing is now done on automated sequencers but due to the high cost of purchasing, maintaining, and operating automated sequencers (both material and personnel costs), many labs send their PCR products and primers in a standardized mixture to biotech labs that offer sequencing services. The turn-around time is usually fast. In the United States, many such sequencing facilities are able to send back the sequences within 2–3 days of receiving the samples.

In summary, here are the steps in PCR-based identification and systematics:

1) DNA extraction
2) PCR-based amplification
3) Purification of PCR product
4) Sequencing
5) Retrieval and evaluation of DNA sequences
6) Alignment of sequences
7) Comparisons and phylogenetic analyses

Working with the Sequences

Sequence data are usually received in 2 formats: As chromatograms and as actual nucleotide (letter) sequences. Each sequence is first manually checked for accuracy by checking the chromatogram, using a viewing or editing software package such as FinchTV (Geospiza, Inc.) or ABI EditView, or any number of other packages for manipulation of molecular sequences. These programs can generally be downloaded from the web. Undetermined nucleotides in the sequences to be examined are either left as "N" or are replaced by the correct nucleotide if this is apparent from the chromatogram. Careful examination and proper judgment are necessary to determine how much of the sequence is usable. The usable portion is extracted and copied and pasted into a sequence manipulation program. Such a program allows the assembly of a database of sequences for further comparison and analysis.

Often, one of the first steps in using any DNA sequence that is generated is finding what that sequence is most similar to among the vast number available in GenBank. GenBank is a repository of sequences deposited by researchers from published and unpublished studies (https://www.ncbi.nlm.nih.gov/genbank/). The search is done using the Basic Local Alignment Search Tool (BLAST) through the NCBI BLAST portal (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This search, which provides results usually in a few seconds to minutes, allows one to see a list of taxa with sequences that match the sequence that has been generated. The BLAST search also shows pairwise comparisons between the sequence submitted and the sequences that match it as well as other details of the comparisons.

One popular program that allows working with the sequences is MEGA (Molecular Evolutionary Genetic Analysis). It is also updated in a timely fashion by the authors, the latest version being MEGA 11.0. This program can be downloaded without cost from https://www.megasoftware.net. In MEGA, sequences from GenBank can be downloaded into an alignment file for additional comparisons. Once an alignment file with the sequences of various species of interest has been compiled, the next step is to align these sequences, that is, to have the nucleotide bases lined up in a homologous corresponding manner (since we do not know the exact position of the sequences in the genome); different sequences may start and end at different base positions in a gene or genome. There are several stand-alone programs that can also be used to align sequences, such as 'ClustalX' (Thompson et al., 1997). In MEGA, the sequence alignment programs 'ClustalW,' and 'Muscle' are embedded within the MEGA software. Alignments are performed on the assembled sequences from the various species using parameters that are set by the program or by manipulating certain parameters depending on the nature of the sequences (Hall, 2001). A copy of the unaligned raw sequences should always be saved and not overwritten by the aligned file because if a new sequence is added to the database, it must be added to the unaligned (meaning, raw) sequence database and the alignment performed again.

Systematic Applications

Once the sequences have been aligned, the unaligned extra overhanging portions on either side are pruned or trimmed and this new dataset can now be used for a variety of purposes, including:

1) The sequences of species can be compared to determine the similarity. This may provide clues as to whether or not 2 samples belong to the same species or can be used to study variation between populations. For example, if consistent molecular differences among isolates from the same geographical area correlate with morphological differences and/or different levels of host range, then a case can be made for different species.
2) The aligned sequences can be used for identification purposes or to determine the evolutionary relationships among the species being studied. There are several programs that can be used for such analyses and several are available in MEGA. There are various settings that can be chosen while doing a phylogenetic analysis, and there are various methods to evaluate how robust the resulting tree of relationships is; the bootstrap analysis is perhaps the most common.

Examples of Explanations about How to Identify Particular Species

Correct application of species names to specimens by biologists is critically important, because species are named according to the agreed-upon rules of scientific naming using the system of **binomial nomenclature** developed by Linnaeus (1758) with the publication of the 10th edition of Systema Naturae. Each species with a unique binomial (**bi** = 2; **nom** = name, from Greek; in this case, **genus** and **species**) provides an instant means to know what species are being referred to anywhere in the world (ICZN, 2024). Following are descriptions of a few sources of methods for species identification.

A useful example of the application of molecular techniques to address questions of helminth systematics is a paper by Hernández-Mena et al. (2019) that examines the relationships of species in the family Allocreadiidae. Pertinent references as well as details of the methods used can be found there.

Methods for collecting and processing mammals for museum collections can be found in Wilson et al. (1996). Specific techniques for collecting parasites from vertebrates can be found in Gardner and Jiménez-Ruiz (2009), which is focused on obtaining and processing parasites from bats; however, the methods can be applied to collections of helminths, ectoparasites, protozoans, and blood parasites from any of the vertebrate classes. Additional methods are found in a book chapter specifically written for reptiles by Gardner et al. (2012), and for mammals in general by Gardner (1996) and Galbreath et al. (2019).

Examples of descriptions of species of *Eimeria* (phylum Apicomplexa: family Eimeriidae) include Jensen et al. (2015) and Tinnin et al. (2012). Some examples of descriptions of nematodes (phylum Nemata) can be found in Drabik and Gardner (2019) and Rodrigues et al. (2020). For descriptions of some of the phylum Platyhelminthes including cestodes, see Caira et al. (2017), and for those in the family Arostrilepididae, see Dursahinhan et al. (2022). For descriptions of trematodes of the family Dicrocoeliidae, see Gardner and Pérez-Ponce de León (2002). This is just a small sampling of available valid descriptive literature.

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4

Parasites in Relation to Other Organisms

Hosts, Reservoirs, and Vectors

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Chapter 4

Hosts, Reservoirs, and Vectors

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Introduction

From the parasite's perspective, a **host** represents a resource and a habitat where the parasite can grow and reproduce. Once produced, reproductive stages of the parasite must find their way back to infect another host. Unlike most free-living organisms, one of the major challenges for a parasite is to continuously encounter and colonize suitable hosts for the propagation of the next generation in the life cycle. From a statistical point of view, any individual parasitic organism has an exceedingly low probability of transferring from one host to another. Indeed, the spatial and temporal difficulties parasites face to complete their life cycle must be overcome by enormous reproductive outputs and/or by exploiting com-plex ecological associations between successive hosts (Tin-sley, 1990).

For any parasite transmission event to occur, an infective stage of a parasite has to first encounter a potential host. This challenge can be considered an **encounter ilter** (Euzet and Combes, 1980). For example, ecological conditions will af-fect the spatial and temporal overlap of host and parasite pop-ulations and species-specific behavioral characteristics can bridge or reduce encounters between parasites and their hosts. Adaptations that increase encounter rates with potential hosts will likely lead to higher infection probabilities (Combes, 2005). Following the encounter; however, another hurdle must be cleared which can be thought of as a **compatibility filter**, and this must be overcome for a parasite infection to become established. In this case, and after encountering a potential host, the compatibility filter determines whether the parasite is able to survive, grow, or reproduce in the host. For example, a parasite might be able to infect a variety of different species of potential hosts, but most of those species would not possess the necessary resources for the parasite to survive. Even when appropriate hosts are encountered, host susceptibility to the parasite is controlled by a variety of host factors such as genetics, immunity, and physiology, among others (Combes, 2005). To overcome these challenges, parasites have evolved various types of life cycles, which include different types and combinations of hosts used for multiplication, growth, reproduction, and/or transmission.

The Role of Hosts in Life Cycles and Transmission of Parasites

Parasitologists differentiate among various types of hosts based on the specific roles those hosts play in the development, reproduction, and transmission of the parasite. In a typical life cycle, a host in which a parasite reaches sexual maturity and reproduces is known as the **definitive host**. In contrast, an **intermediate host** is one that is required for parasite development, but one in which the parasite does not reach sexual maturity. In most cases, the parasite goes through morphological and developmental changes in an intermediate host. In some cases, the parasite increases in numbers within an intermediate host. For example, all species of digenetic trematodes and some species of cestodes increase in number in the intermediate host through an asexual process known as **polyembryony**, the formation of more than one embryo from a single zygote (Craig et al., 1997). As a result of polyembryony, intermediate hosts can play a major role in increasing the probability of parasites encountering the next host in the life cycle.

A **paratenic host**, or **transport host**, is one in which the parasite does not undergo any development. However, in many cases, a paratenic host is essential for the transmission of the parasite and acts as a trophic bridge between the intermediate and definitive host (Baer, 1951). For example, some species of trematodes found as adults in the Eustachian tubes of frogs, use frogs as definitive hosts and aquatic microcrustaceans as intermediate hosts in their life cycles. However, because frogs do not generally consume microcrustaceans, a paratenic host must be involved in bridging the gap in trophic transmission. In this case, aquatic insects that commonly feed on microcrustaceans, such as damselflies and dragonflies, accumulate large numbers of these trematodes in their digestive

tracts, and the trematodes do not develop in the microcrustacean. Frogs then eat the damselfly and dragonfly paratenic hosts and, in the process, become heavily infected (Bolek et al., 2010; Stigge and Bolek, 2015).

Most parasites must complete at least part of their life cycle by infecting 1 or more **obligate** or **required hosts**. In contrast, **facultative** parasites are usually not parasitic, but become so, opportunistically, when they encounter a potential host. For example, when certain species of free-living amoebas, such as *Naegleria fowleri* or species of free-living nematodes in the genus *Halicephalobus* are accidentally ingested or enter an opening of a novel host, they can establish within the host, and in some case cause serious and many times fatal conditions (Anderson et al., 1998; Kinde et al., 2000; Visvesvara et al., 2007). Similarly, when an obligate parasite infects a host which is different from its normal host, that host is called an **accidental** or **incidental host**. A number of cases have been reported of humans serving as accidental hosts for the nematode *Angiostrongylus cantonensis*, a species that normally resides in the lungs of various species of rats. Humans become infected with *A. cantonensis* by ingesting terrestrial gastropod intermediate hosts that are living on raw vegetables, such as lettuce (Pien and Pien, 1999). In humans, the nematodes migrate to the brain where they cause abscesses, brain swellings, and hemorrhages. Eventually, the juvenile nematodes die and degenerate. In this situation, humans can also be considered a **dead-end host** for *A. cantonensis*, because the parasite is not transmitted to functional hosts to continue its life cycle (Pien and Pien, 1999).

It should be noted that most, if not all, free-living species on our planet serve as hosts for many species of parasites. As a result, those free-living animals can serve different roles in the life cycles of different parasite species. One group of free-living animals that commonly serve as intermediate or paratenic hosts for numerous species of parasites are the gastropods (phylum Mollusca: class Gastropoda). Terrestrial, freshwater, and marine snails have been reported as intermediate and/or paratenic hosts for most species of digenetic trematodes, as well as various species of nematodes, tapeworms, and even acanthocephalans (Hopp, 1954; Dollfus, 1974; Rysavý, 1986; Lockyer et al., 2004; Lu et al., 2018). As an example, a single species of freshwater snail, *Physa acuta*, collected from various streams and wetlands across north-central Oklahoma, United States, serves as the first or second intermediate host for at least 9 species of flukes, and as a paratenic or accidental host for 3 species of horsehair worms, 1 species of nematode, and 1 species of thorny-headed worms, all of which infect various insects or vertebrates as definitive hosts (Gustafson and Bolek, 2016; Harkins et al., 2016; Koch, 2018; Figure 1).

Reservoir Hosts and Vectors

Another definition commonly used in the parasitology literature is the concept of **reservoir host**. Broadly defined, a reservoir species maintains a parasite infection in nature and serves as a source of infection for other species of animals. From a medical perspective, the definition of a **reservoir host** is usually restricted to any animal that maintains parasites as a source of infection for humans or domestic animals. In addition, many parasites that infect humans, domestic animals, and wildlife are transmitted by **biological vectors**. The term **vector** has been applied to a diverse group of potential animal hosts, and when used broadly in parasitology, can include any animal that transmits parasites from one host to another (Wilson et al., 2017). However, from a medical, ecological, and evolutionary perspective, a **vector** is defined as a mobile micropredator (for example, mosquito, leech, or vampire bat) that feeds on the blood or other bodily fluids of vertebrates and in some cases invertebrates (Figure 2). (Lafferty and Kuris, 2002; Wilson et al., 2017).

In most sanguinivorous species of animals that can also act as vectors of parasites, blood and/or tissue parasites from an infected animal may be ingested in 2 main ways; 1) Through **telmophagy**, in which the ectoparasitic animal abrades the skin and capillary beds of a vertebrate and a small hemorrhage forms, from which the animal vector then feeds, and 2) via **solenophagy**, in which a vector directly pierces blood vessels of its host to feed. For example, female horse flies and deer flies use telmophagy and when they feed, they lacerate the skin of their host with specialized cutting bladelike maxillae and then suck up the blood with sponge-like labellae (Matheson, 1950). In contrast, female mosquitoes are solenophagic feeders with mouthparts that are adapted to piercing vertebrate skin with their cutting maxillae and then suck blood with the hypopharynx (Choo et al., 2015; Mullen and Durden, 2009). Note that males do the same thing but with plants.

Based on their relationship with the parasite, vector-hosts can be assigned to 2 groups, including either mechanical or biological vectors. **Mechanical vectors** merely transmit the parasite between and among vertebrates, but without any multiplication or development of the parasite within the vector-host. Although not necessary for the multiplication or development of the parasite, mechanical vectors are essential for the **transmission** of various parasite species among its vertebrate hosts. A typical example includes flies (order Diptera) of the family Tabanidae (horse flies and deer flies) which are mechanical vectors for *Trypanosoma evansi* (order Kinetoplastida: Trypanosomatidae) of horses and other vertebrates (Bowman, 2013). Because female tabanids are not subtle and may cause pain when they bite their victim, they are usually quickly dislodged by defensive movements of the

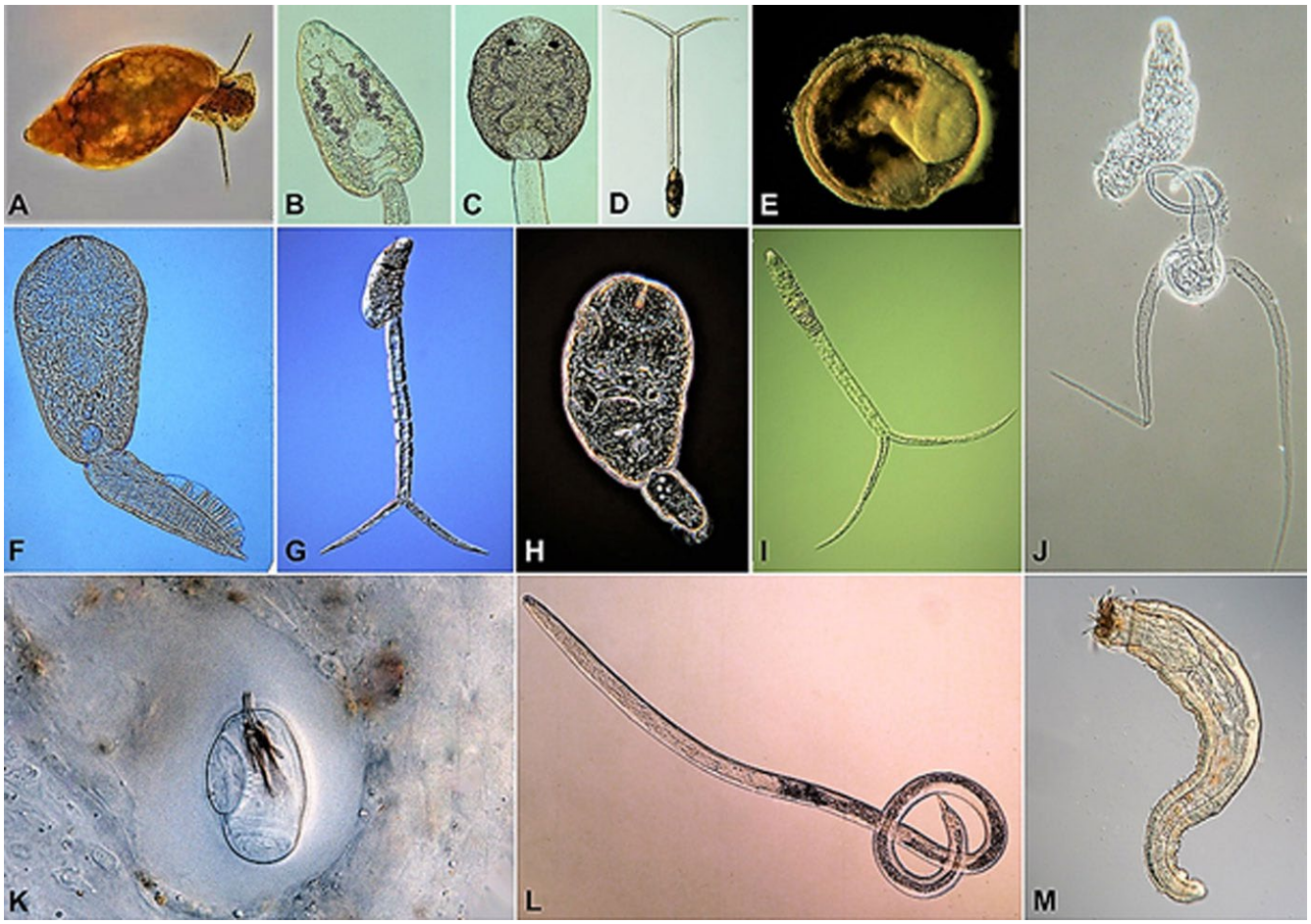

Figure 1. An example of a common North American freshwater snail, *Physa acuta* (A) and 12 species of parasites from 4 phyla representing different types of host associations. B–D, F–J) Show the cercarial stages of 8 species of digenetic trematodes which develop within the snail host and are released into the water column, to infect a second intermediate host. *Physa acuta* serves as the first intermediate host in the life cycles of these parasites. E) A metacercarial stage of the digenetic trematode *Allassostomoides parvus* which is infective to turtle definitive hosts. *Physa acuta* serves as the second intermediate host in the life cycle of this parasite. K) A cyst of a horsehair worm, *Paragoridus varius* in the tissue of *P. acuta*. Horsehair worms infect crickets and other arthropods as definitive hosts and they can use aquatic insects as paratenic hosts. Because crickets do not usually feed on aquatic snails, *Physa acuta* is considered an accidental host for this parasite. L–M) A juvenile *Spiroxys contortus* (nematode) and a juvenile *Neoechinorhynchus emydis* (acanthocephalan). Both of these parasites use microcrustaceans as first intermediate hosts and aquatic turtles as definitive hosts. *Physa acuta* may act as a an accidental/paratenic host for these parasites when individuals ingest infected microcrustacean first intermediate hosts and which are then eaten by the turtle definitive host. Source: M. Bolek. License: CC BY-NC-SA 4.0.

host and rarely remain on a host long enough to become fully engorged with blood. Instead, the tabanid quickly flies off the infected host and lands on another animal to feed again. In essence, it ingests blood frequently from multiple hosts and, in the process, it can mechanically and rapidly transmit *T. evansi* from one horse to another. In contrast to mechanical vectors, a **biological vector** is one in which the parasite multiplies and/or develops within organs and/or tissues of the vector host. Often, there is a time lag between acquisition of the parasite by the biological vector and the ability of the parasite to be transmitted by that vector to a new definitive host. This has been called the **extrinsic incubation period**.

Within biological vectors, and during the extrinsic incubation period, 3 types of multiplication and/or developmental patterns of the parasite can occur (Figure 3). **Propagative transmission**, involves simple amplification of a parasite within the vector-host. In this case, the same form of the parasite taken up by the vector multiplies within the vector and is then transmitted to a new vertebrate host. Examples include various species of bacteria, and some trypanosomatid protozoans, where the parasite multiplies within the vector-host but does not change morphologically. In contrast, **cyclopropagative transmission**, involves asexual and/or sexual multiplication of the parasite, and hence amplification of

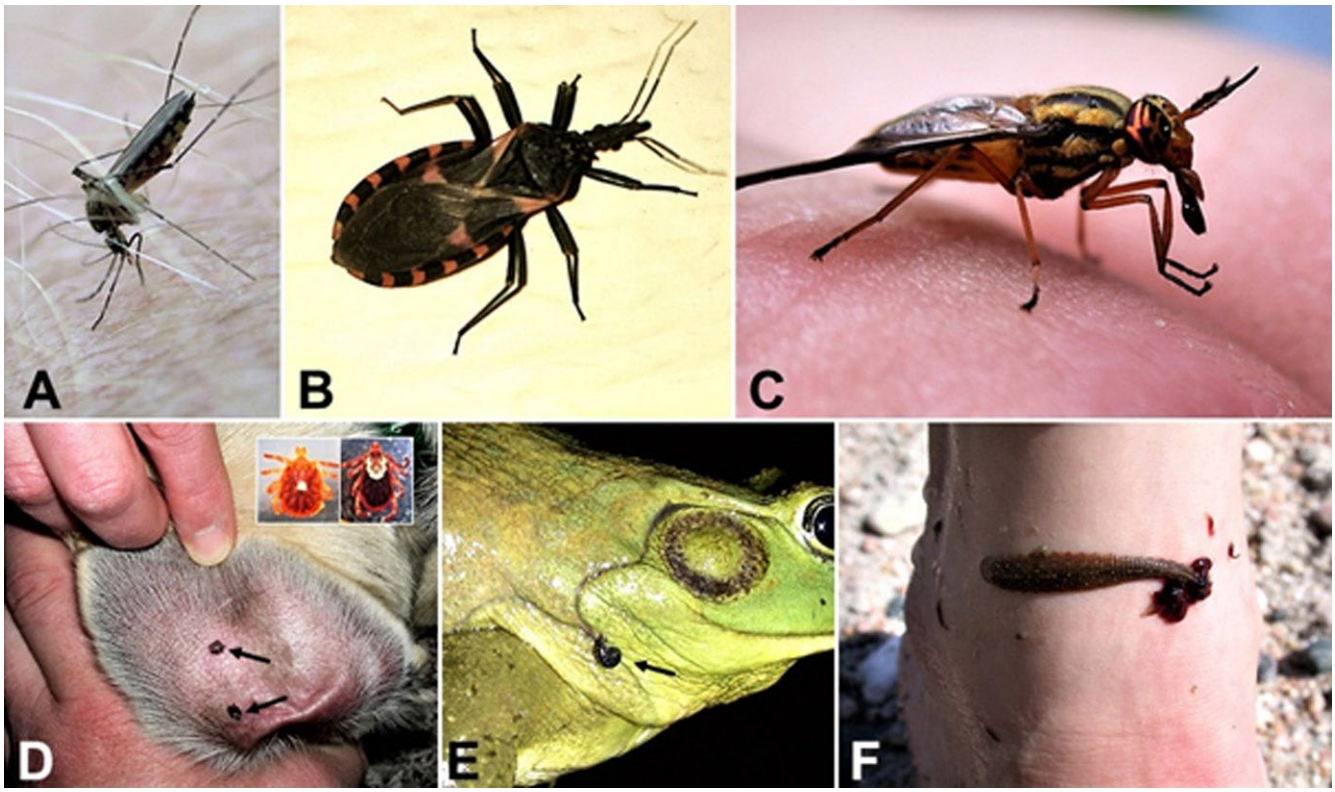

Figure 2. Examples of typical vector hosts. A) Female mosquito in the genus *Aedes* in the process of taking a blood meal. Note the specialized sucking mouth part injected into the skin of author M. Bolek. B) A reduviid bug. This is one of the primary biological vectors of *Trypanosoma cruzi,* the causative agent of Chagas disease. This species of bug can transmit the infective parasite stage to the vertebrate host through its feces. C) A female striped-backed deer fly, *Chrysops vittatus*. Because of their blood feeding habits, many species of deer flies serve as mechanical vectors for parasites. Note the complex mouth parts, used to slice open the skin of the victim, after which the fly sips blood from the pooling blood on the surface of the skin. D) Females of 2 species of hard ticks, *Amblyomma americanum* and *Dermacentor variabilis* (arrows), attached and feeding on the ear of a stray dog, *Canis lupus familiaris*. Ticks are common biological vectors for various parasites including protozoa and various helminths. E–F) Leeches (order Rhynchobdellida: family Glossiphoniidae) *Placobdella picta* (arrow) and *P. rugosa* feeding on a bullfrog, *Lithobates catesbeianus*, and the leg of Melissa Bolek (order Primates: family Hominidae), respectively. Leeches are common biological vectors for protozoan parasites of amphibians and reptiles. Note, in E the numerous young leeches feeding from the same bite wound as the mother leech. Source: M. Bolek. Informed consent obtained from all human subjects. License: CC BY-NC-SA 4.0.

the parasite within the vector-host. Importantly, in cyclopropagative transmission, the form of the parasite transmitted to the next vertebrate host is morphologically distinct from the initial form taken up by the vector-host.

An example of asexual cyclopropagative development occurs in the trypanosomatid *Trypansoma cruzi* within its reduviid bug vector-host; sequential cycles of asexual and sexual reproduction within mosquito and tick vector-hosts occur in various genera of apicomplexans such as *Plasmodium* and *Babesia*, respectively. As a result—and depending on the specific vector-host and parasite reproductive relationship within the vector—some biological vectors can be classified as **definitive** or **intermediate hosts**. In the case of *Plasmodium* in vertebrates and their mosquito host, the **vertebrate is the intermediate host** while the **mosquito is the definitive host because sexual reproduction occurs in the stomach wall of the mosquito**. Finally, **cyclodevelopmental transmission** involves no multiplication of the parasite, but instead, the parasite develops within the vector to the next stage which is infective to the vertebrate host.

In cyclodevelopmental transmission, there is usually mortality and reduction in the number of parasites that are initially ingested by the vector relative to the number that are available when transmitted to the vertebrate host. Hence there is no amplification of the parasite in vector-hosts with cyclodevelopmental transmission. Examples of vector-borne parasites with cyclodevelopmental transmission include filarioid nematodes such as *Litomosoides* spp. (superfamily Filarioidea: family Onchocercidae), which depending on the particular species, reside in various tissues of vertebrate definitive

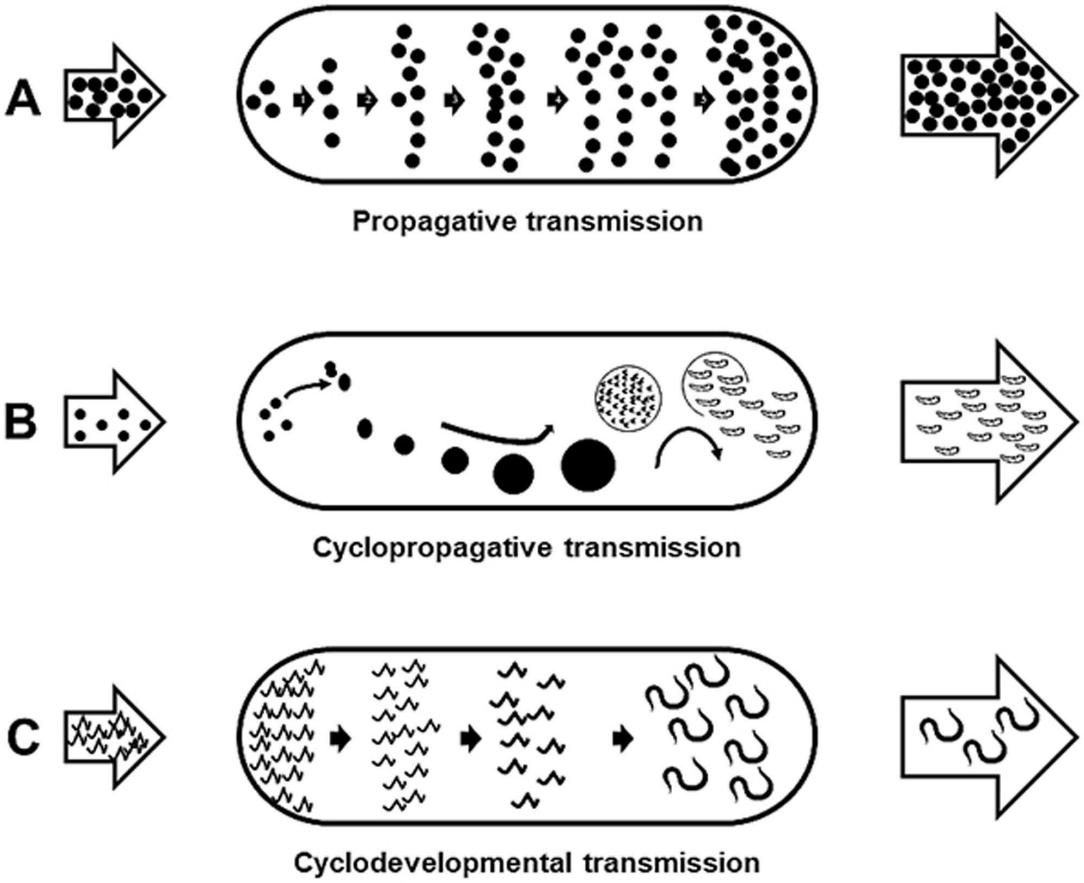

Figure 3. Types of biological associations between parasites and their vector hosts, represented by ovals. The arrows on the left indicate blood ingested by the vector from an infected vertebrate host, and the arrows on the right represent the infective parasite stage transmitted to another vertebrate host after a sufficient incubation period. A) Propagative transmission, the parasite multiplies within the vector, usually by an indefinite number of generations of binary fission. The stages transmitted are the same but far more numerous than originally acquired during the vector's original blood meal. Examples of parasites with propagative transmission include some species of trypanosomatid protozoans. B) Cyclopropagative transmission, the parasite undergoes 1 or more cycles of asexual and/or sexual reproduction where it increases in numbers. The infective stage to the vertebrate host, is morphologically distinct from the form originally acquired during the vector's original blood meal. Examples of parasites with cyclopropagative transmission include the causative agents of malaria and Chagas disease in humans. C) Cyclodevelopmental transmission, the parasite develops from the stage acquired by the vector host to an infective stage to the next vertebrate host, without any multiplication or reproduction. There is usually a loss of parasites from the original number acquired by the vector, and the final number that develop to the infective stage to the next host. Common examples of cyclodevelopmental parasites include filarioid nematodes. Source: Adapted from McClelland (1992), 2019. License: CC BY-NC-SA 4.0.

hosts and release infected stages known as microfilariae into the blood, connective tissues, or skin. Once ingested by their mosquito intermediate vector-host the microfilariae develop to the next stage that is infective to the vertebrate host (Anderson, 2000).

Any parasites within the body of a vector-host must eventually exit the vector to be transmitted to a new host. Many vectors transmit parasites between successive vertebrate hosts during blood feeding. In some mechanical vectors, the parasites may be regurgitated back into the mouthparts and subsequently transmitted to a new vertebrate host during a blood feeding session. Similarly, in many biological vectors, the parasite is transmitted to a vertebrate host through inoculation or contaminated mouthparts during blood feeding. It is important to note, however, that not all vector-hosts transmit parasites between successive vertebrate hosts while taking a blood meal. This is particularly true for parasites that develop to the infective stage within the hindgut, or in the hemocoel of their vector-hosts and, as a result, cannot be transmitted through inoculation via contaminated mouthparts (Figure 2).

The causative agent of Chagas disease *Trypanosoma cruzi* is one such example. *Trypanosoma cruzi* protozoans develop to the infective stage in the hindgut of their kissing bug vector, which includes various species of kissing bugs, such as *Triatoma sanguisuga*, and is then transmitted to the vertebrate host in the feces, when the bug defecates while feeding. Humans become infected when they scratch the bite wound, rub their eyes, or move the feces of the bug into the mucus membranes of the mouth or nose. These actions inadvertently inoculate the infective stages of *T. cruzi* in the bug's feces into the various infection portals. Similarly, the apicomplexan parasite *Hepatozoon americanum* infects dogs as the intermediate host, and the lone star tick, *Amblyomma americanum*, as the vector definitive host. In this case, the parasite develops to the infective stage in the hemocoel of the tick vector and dogs become infected when they ingest infected ticks while grooming (Ewing and Panciera, 2003).

Finally, species-specific interactions between parasites and the type of reservoir and vector-hosts they employ in their life cycles can become extremely convoluted. In some cases, both mechanical and biological vectors can transmit a single parasite species. As mentioned previously, *Trypanosoma evansi* is transmitted to horses through the bite of blood sucking flies *Tabanus* and *Stomoxys* which act as mechanical vectors across Asia and in North Africa (in addition to *Glossina*), where *T. evansi* is endemic. However, *T. evansi* has relatively recently been introduced into Central America and South America, where it can be transmitted to horses by one of the species of vampire bats, *Desmodus rotundus*, which can serve as both vector and reservoir host (Brun et al., 1998). Vampire bats become infected with *T. evansi* by feeding on the blood of infected horses. Parasites enter the bat's bloodstream through the mucus membranes lining the buccal cavity, and some of the infected bats die due to disease caused by the initial phase of infection (Desquesnes et al., 2013). However, some individuals survive the initial infection with the trypanosomes achieving a chronic infection with high blood parasitemia and some individual bats with a chronic infection have trypanosomes in their saliva. These bats then act as biological vectors and can transmit *T. evansi* to horses via their saliva during blood feeding. Additionally, because infected vampire bats commonly groom each other and/or feed other bats in the colony regurgitated blood, these infected vampire bats can propagate the infection among other individuals in the colony (Desquesnes et al., 2013). As a result, vampire bat colonies can maintain *T. evansi* in the absence of infections in horses, and the infected bats can serve as reservoir hosts for infections in horses! Finally, there are reports of canids becoming infected by eating freshly killed mammals that are infected with *T. evansi* (see Woo, 1977).

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5

Parasites in Relation to Other Organisms

Life Cycles

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Chapter 5

Life Cycles

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Introduction

Life cycles of parasites have evolved into complex se-quences of improbable events, with as many as 4 host spe-cies being included in the life cycle of certain parasite spe-cies (Bolek et al., 2010). Relative to their hosts, parasites in their infective stages are rather small and have limited mo-bility in the external environment. As a result, one can make the argument that the most dangerous part of any parasite's life cycle is when the parasite is away from its host. Conse-quentially, adaptive scenarios and evolutionary contingen-cies are both often invoked to explain the complexity of par-asite life cycles and the resulting transmission events (Poulin and Cribb, 2002).

In order for a particular parasite to infect and live in or on an appropriate host, there must be suitable conditions enabling access to the host(s), including: 1) A dependable means of transmission from one host to another, 2) the ability of the parasite to establish itself within that host after reaching it, and 3) specific conditions within that host for the parasite to survive, grow, and reproduce. To accomplish this, parasites have evolved various types of life cycles which enable them to complete the necessary steps (that is, colonize, survive, grow, and mature) among a variety of different but often spe-cific host species. A **parasite life cycle** is defined broadly as including the ontogenetic stages of a specific parasite species, and a set of events, such as growth and reproduction, that must occur before the parasite can survive and reproduce. In the case of parasites, the life cycle also includes all necessary hosts and all transmission events that enable a specific species of parasite to complete its life cycle.

Infection Site

Depending on the species, many parasites occupy a specific infection site and/or location in 1 or more of the hosts infected during their life cycle. Parasites that inhabit the lumen of the intestines, lungs, or other hollow organs of their hosts are said to be **coelozoic**, whereas parasites that live within tissues of their hosts are referred to as **histozoic**. For example, amphibians are commonly infected with 2 distinct genera of myxozoans, a group of parasitic cnidarians (Jirků et al., 2006; 2007; Hartigan et al., 2012). *Cystodiscus serotinum* produces infective spore stages in the gallbladder of amphibians; whereas *Sphaerospora ohlmacheri* produces infective spores in the tubules of the kidneys of frogs and toads. Both species are coelozoic because they infect the lumen of the gallbladder or tubules of the kidneys. However, each species is considered **site specific** in amphibians, such that *C. serotinum* can only develop in the gallbladder and *S. ohlmacheri* can only develop in the tubules of the kidneys (Figure 1). In contrast, many cercaria, which are the larval stages of trematodes, are histozoic and encyst within various tissues of their second intermediate hosts. For example, tadpoles (larvae) of many amphibian species serve as second intermediate hosts for various trematode species (Rhoden and Bolek, 2015). The metacercariae of some trematode species only encyst in specific tissues and organs whereas metacercariae of other species are infection site generalists and can be found in various tissues and organs of tadpoles (Figure 2); thus, some species of trematodes have metacercariae that are generalists and some that are specialists. Studies indicate that cercariae of echinostomes actively seek and enter tadpoles via the cloaca, then migrate to the kidneys where they encyst (Thiemann and Wassersug, 2000; Taylor et al., 2004). In contrast, species of *Telorchis* will penetrate any surface on the body of a tadpole (Schell, 1962). Notably, tadpoles have a greater chance of becoming infected with species of *Telorchis* by mechanically sucking in infective stages of flukes from the water column, whereas cercariae of echinostomatid flukes can only infect tadpoles when they enter through the cloaca (Rhoden and Bolek, 2012).

To a parasite, a host represents multiple microenvironments, and only certain environments meet the parasite's very specific needs. Clearly, not all hosts will be equal, and some

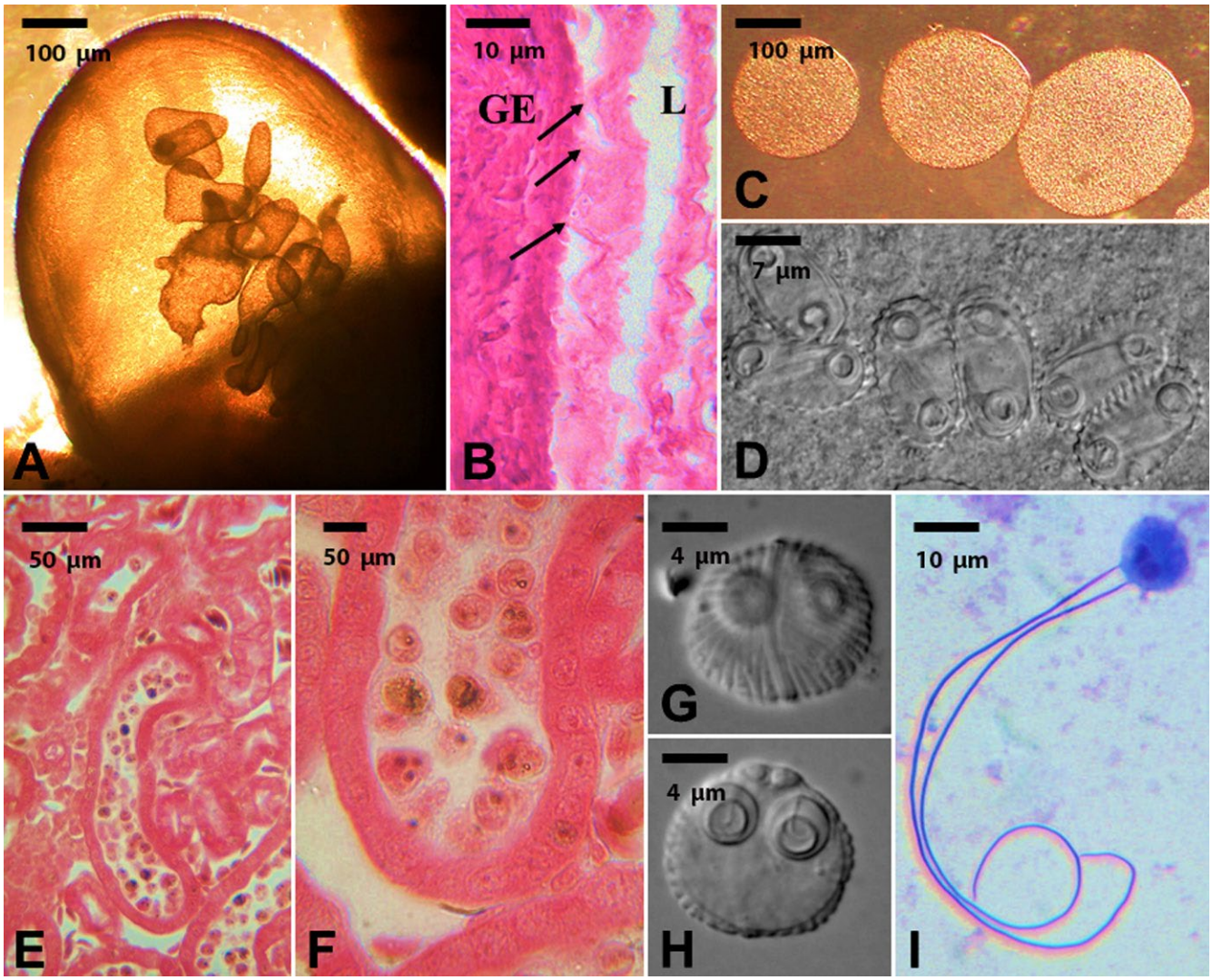

Figure 1. Example of coelozoic parasites with restricted site specificity; showing detailed development of the next infective stage in the life cycle. A–D) Developmental stages of *Cystodiscus serotinum* in the gallbladder of a green frog *Rana clamitans*. A) Gallbladder showing developing plasmodia stages. Scale bar = 100 µm. B) Histological section showing the distribution of plasmodia in the lumen (L) of the gallbladder and their intimate association (arrows) with the epithelial cells of the gallbladder (GE). Scale bar = 10 µm. C) Removed plasmodia from the gallbladder. Scale bar = 100 µm. D) Infective spore stages within the plasmodia. Scale bar = 7 µm. E–F) Developmental stages of *Sphaerospora ohlmacheri* in the kidneys of a Blanchard's cricket frog *Acris blanchardi*. E) Histological section of the kidney showing renal tubule occluded by plasmodia of *Sphaerospora ohlmacheri*. Scale bar = 50 µm. F) Close up of renal tubule occluded with developing spores of *Sphaerospora ohlmacheri*. Scale bar = 50 µm. G–I) Detailed morphology of infective spores of *Sphaerospora ohlmacheri*. Note the detailed surface structures on the spores and the everted extruded polar filaments (I) indicating the spore stages are infective to the next host in the life cycle. Scale bars = 4 and 10 µm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

parasites that infect different host species can behave differently. As a result, the site of infection for some parasites can be influenced by their host species. For example, a recent study on the frog tongue fluke *Halipegus occidualis* showed that these flukes commonly infect 3 species of frogs (Stigge and Bolek, 2016). Anurans (frogs) become infected with *H. occidualis* when they ingest a dragonfly paratenic host that contains encysted metacercariae. However, when green frogs *Lithobates clamitans* and leopard frogs *L. pipiens* ingest an infected dragonfly paratenic host, the worms migrate from the stomach and attach to the lingual vein under the tongue, where they mate and lay eggs. In contrast, when dragonfly paratenic hosts are ingested by bullfrogs *L. catesbeianus*, the worms never attach to the lingual veins under the tongue, but instead reside in the frog's stomach where they mate and lay eggs. It is unclear why *H. occidualis* behaves so differently

in bullfrogs than in green frogs and leopard frogs; nonetheless, the study clearly indicates that different species of host-parasite combinations matter in understanding parasite life cycles and host-parasite interactions.

Host Specificity

To begin, note that host specificity is covered briefly in the introduction to this book. While there is some debate about whether this is the proper framework to consider life cycles, this contention will be left aside for now, since it is referenced extensively in the literature as it has served as the basis for numerous robust studies in the concept of ecology of parasites.

As observed with infection site, parasites can vary in their host-range (also known as specificity) at 1 or more stages in their life cycles. Although most species of parasites are known to develop only in a restricted range of hosts, different parasites exhibit varying degrees of host specificity. For example, some species of cestodes, such as the pork tapeworm *Taenia solium* (Cyclophyllidea: Taeniidae) are only known to mature to egg producing adults in humans *Homo sapiens* definitive hosts and are considered host-specific at the definitive host level. In contrast, and at the other extreme, species of *Trichinella* (class Nemata: family Trichinellidae) can mature in almost any species of mammal. Another example of a parasite with a wide host-range is the coccidian protozoan *Toxoplasma gondii*. This parasite uses cats (order Carnivora: family Felidae) as the definitive host (any cat species will do) but it can use almost any vertebrate as the intermediate host.

These examples exhibit the variety of host-range shown by parasites across 3 phyla of phylogenetically unrelated parasites. A parasite that is specific for a single host species is said to be **oioxenous**, a parasite that infects closely-related hosts is considered **stenoxenous**, whereas a parasite that infects unrelated hosts is considered **euryxenous**. Finally, some parasites exhibit **stadium specificity** where hosts are only susceptible to infection by a particular parasite at a specific developmental stage. Some protozoans such as gregarines (apicomplexans) that infect holometabolous insects and some species of nematodes and acanthocephalans that occur in amphibian definitive hosts, can only infect either the larva or adult stage of their host (Nickol and Heard, 1973; Clopton et al., 1992; Rhoden and Bolek, 2011; Childress et al., 2017). For example, tadpole pinworms, *Gyrinicola batrachiensis* are constrained to the large intestine of tadpole stages of anurans (Adamson, 1981). One explanation for this dramatic difference in host specificity between tadpoles and frogs is differences in their diets and digestive tracts. In general, pinworms feed on the bacteria found in the hindgut of animals that consume plants as a significant portion of their diet. As tadpoles metamorphose to the adult anuran stages, their feeding and correlated digestive tract changes dramatically from a predominantly herbivorous diet to a strictly carnivorous diet, and all *G. batrachiensis* are lost from their intestines (Adamson, 1981). As a result, separate and distinct parasite niches corresponding to distinct life cycle stages of free-living animals can affect parasite host range and measured specificity.

To understand the nature of host range, some parasitologists contend that experimental cross infections should be conducted to determine whether host-parasite associations may be established by true host-parasite incompatibility (Janovy et al., 2007). However, potential host species may simply not be infected with a particular parasite species because they never encounter the infective stage of the parasite in nature due to various environmental factors. With most systems involving parasites of vertebrates, logistical burdens make studying cross infection very difficult, especially when the species are not routinely reared in captivity. There are a number of studies on protozoa, trematodes, nematodes, and annelids testing host compatibility in insect, amphibian, and reptile host-parasite systems (Bolek and Janovy, 2007a; 2007b; 2008; Janovy et al., 2007; Bolek et al., 2009; 2010; Langford and Janovy, 2009; 2013; Childress et al., 2017; Andrews et al., 2015; Stigge and Bolek, 2016). In general, what these studies suggest is that host specificity has a strong ecological component, such that many potential and competent hosts never come in contact with the infective stages of a particular parasite species in nature, undoubtedly affecting host-parasite patterns of associations. Additionally, these studies indicate that it is difficult to predict the range of compatible hosts a particular parasite can infect. For example, Langford and Janovy (2013) tested the host specificity of 7 species of lungworms which infect snakes and anuran definitive hosts. Their field studies and experimental infections indicated that both species of snake lungworms were generalist snake parasites, and in nature and the laboratory they could infect up to 5 species of snakes. However, their laboratory experiments also suggested that lizards can be infected under some environmental conditions. In contrast, lungworms from anurans were found not to infect salamanders or reptiles in nature or in the laboratory. Additionally, amphibian lungworm species ranged from being strictly host specific, infecting only 1 species of frog or toad, to relative generalists, able to infect multiple species of distantly related frog and toad species. Overall, these studies indicate that for many parasite species, host specificity or host-range in nature appears to be limited by both ecological and physiological factors, which vary among parasite species and their hosts.

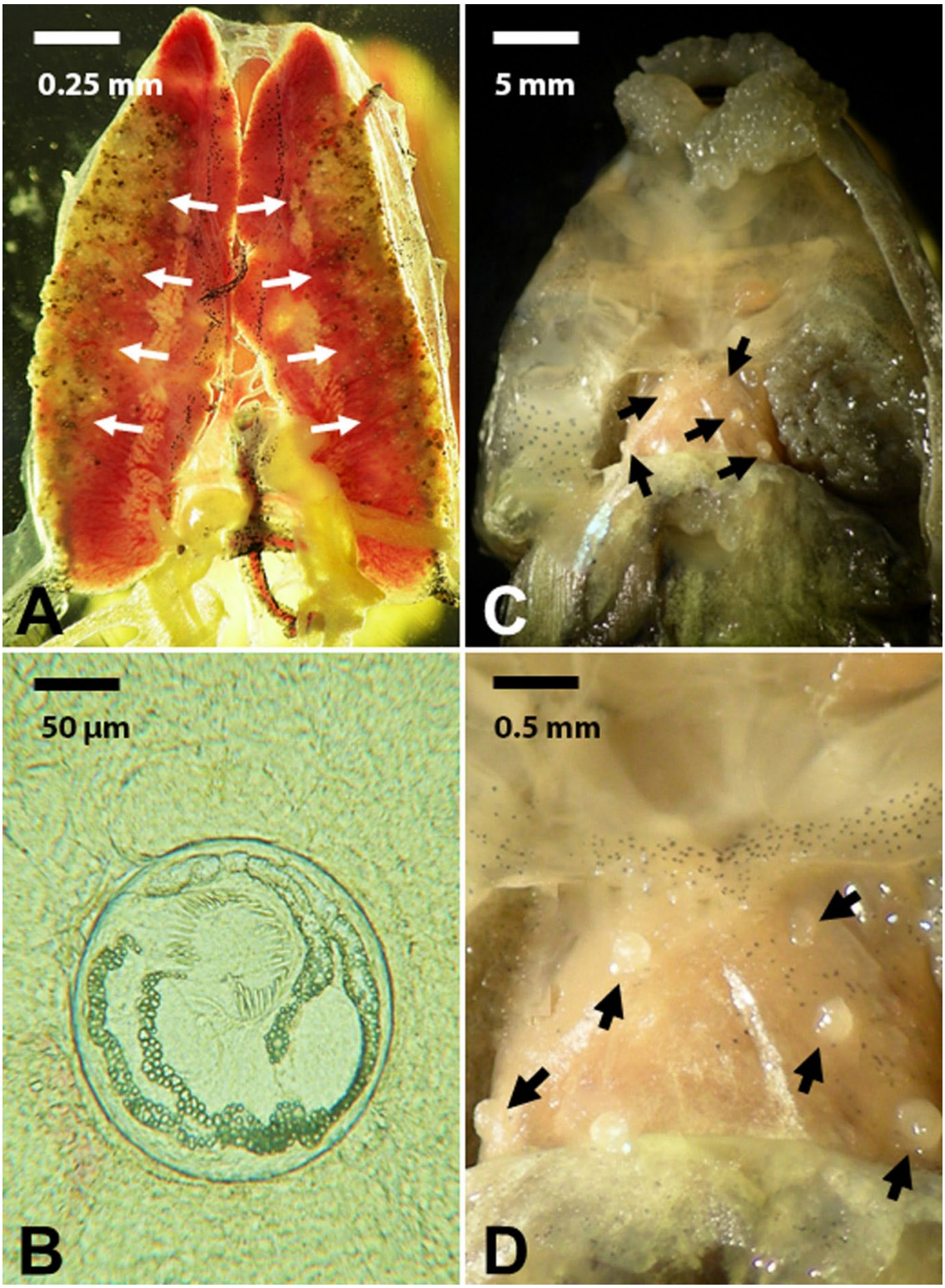

Figure 2. Example of histozoic parasites with variable site specificity in a bullfrog tadpole. A) Removed kidneys from a bullfrog tadpole showing hundreds of echinostomatid metacercarial stages encysted on the lateral sides of each kidney (arrows). Scale bar = 0.25 mm. B) A single echinostomatid metacercaria encysted in kidney tissue of bullfrog. Scale bar = 50 µm. C) Ventral body region of a bullfrog tadpole with the musculature removed showing encysted metacercarial stages of *Telorchis* sp. (arrows). Scale bar = 5 mm. D) Higher magnification of the heart showing the distribution of encysted metacercariae of *Telorchis* sp. (arrows) on the heart. Scale bar = 0.5 mm. Source: M. Bolek.

Parasite Development and Types of Parasite Life Cycles

Parasite development can be categorized as **monoxenous** where the parasite lives and develops within a single host during its life cycle, or **heteroxenous** where a parasite lives and develops within more than 1 host during its life cycle. Additionally, life cycles can be categorized as **simple** or **direct** where a parasite only infects a single host in its life cycle, or as **complex** or **indirect life cycles**, where a parasite uses 2 or more hosts in its life cycle. However, some parasites with direct or indirect life cycles also go through complex reproductive events within their hosts where they alternate sexual and asexual generations in 1 or multiple hosts. As a result, distinct sets of terms are used to differentiate between parasite reproductive events within their hosts and life cycle complexity. For example, many coccidian species in the genus *Eimeria* have direct or simple life cycles and infect their definitive vertebrate host when the host ingests the infective oocyst stages. However, once inside the intestinal epithelial cells of its host, the coccidian goes through a complex set of multiple asexual multiplication events, followed by the production of male and female gametes and eventually sexual reproduction (Figure 3). Parasites that have alternations of sexual and asexual generations in their life cycle are commonly referred to as **heterogenetic parasites**. In contrast to *Eimeria*, all acanthocephalan species (phylum Acanthocephala) have indirect or complex life cycles, including a definitive, intermediate, and commonly an additional paratenic (transport) host. However, except for sexual reproduction in the definitive host, no other complex asexual multiplication or alternations of generations occurs in the intermediate or paratenic hosts in the life cycle (Figure 4). Parasites that have no alternation of sexual and asexual generations in their life cycles, are sometimes referred to as **monogenetic parasites**. As a result of the enormous diversity of parasite species, different combinations of direct or indirect and heterogenetic or monogenetic development can occur in different groups of parasites during their life cycles.

In addition to the examples above, there are other life cycle variations, particularly in parasite species that must exit their host into the external environment and develop into free-living adults and/or to find mates and reproduce in the external environment. For example, life cycles of some species of flies which cause **myiasis** (a term for an infestation of tissues, wounds, or body cavities of living animal by fly maggots) fall into this category (Zumpt, 1965). Many species of flies causing myiasis are **obligate** parasites and their maggots must develop within their hosts to complete the life cycle. For example, flies in the subgenus *Bufolucilia* commonly

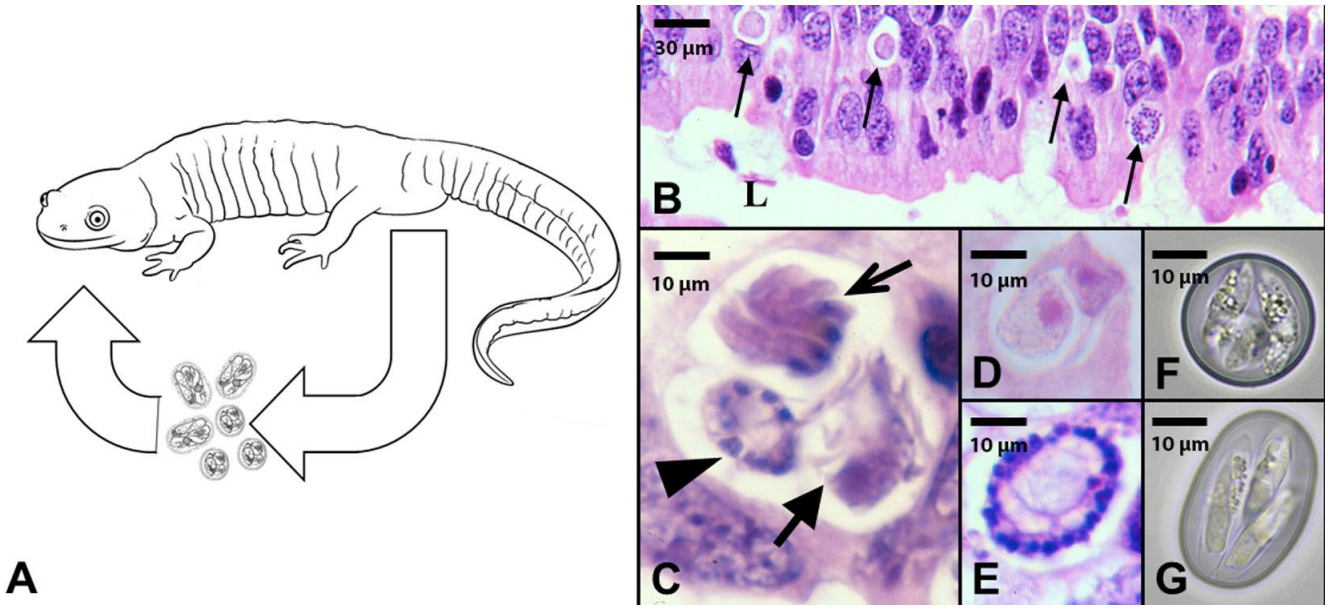

Figure 3. An example of a direct, monoxenous, but heterogenetic life cycle of salamander *Eimeria* spp. A) A tiger salamander *Ambystoma tigrinum* showing the routes of transmission of *Eimeria* species. Salamanders defecate infective stages (oocysts) into the external environment and become infected when they accidently ingest oocysts. B) Histological section of the small intestine of a tiger salamander showing different developmental stages of *Eimeria* species (arrows) in the epithelial cells of the small intestine. Scale bar = 30 µm. C) Higher magnification of an epithelial cell showing asexual multiplication (thin arrow) and development of microgametes (sperm; middle arrow) and macrogametes (ova; thick arrow). Scale bar = 10 µm. D–E) Epithelial cells showing developing oocysts (zygotes) after fertilization. Scale bar = 10 µm. F–G) Fully developed and infective oocysts recovered from the feces of *Eimeria urodela* and *E. ambystomae*. Scale bar = 10 µm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

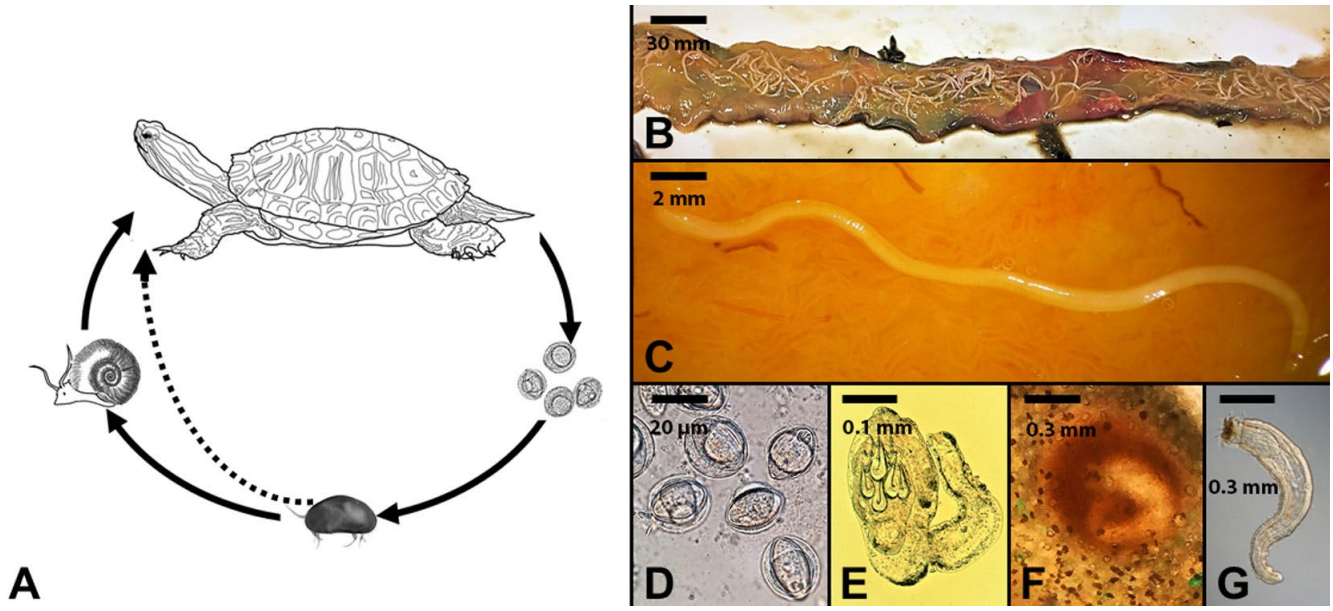

Figure 4. An example of an indirect, heteroxenous, but monogenetic life cycle of the turtle acanthocephalan *Neoechinorhynchus emydis*. A) Turtle definitive hosts release eggs into the external environment where they are ingested by ostracod intermediate hosts. Once the parasite develops to the next infective stages, the infected ostracod can be ingested by a snail paratenic host where no development of the parasite occurs or a turtle definitive host where sexual reproduction occurs. Additionally, turtles can become infected when they ingest snail paratenic hosts. B) The small intestine of a turtle showing hundreds of adult acanthocephalan parasites attached to the intestine. Scale bar = 30 mm. C) Higher magnification of a single adult female worm attached to the intestine mucosa. Scale bar = 2 mm. D) Eggs of an acanthocephalan. Scale bar = 20 µm. E) Developing larval stage recovered from the body cavity of an ostracod intermediate host. Scale bar = 0.1 mm. F) Encysted juvenile acanthocephalan in a snail paratenic host. Scale bar = 0.3 mm. G) Infected juvenile acanthocephalan removed from a snail paratenic host. Scale bar = 0.3 mm. Note the dramatic morphological changes among the different stages in the life cycle. Source: M. Bolek. License: CC BY-NC-SA 4.0.

infect amphibian hosts throughout Europe and North America (Bolek and Coggins, 2002; Bolek and Janovy, 2004; Tantawi and Whitworth, 2014; Arias-Robledo et al., 2019). Female flies locate amphibian hosts visually and deposit eggs on the back and flanks of their unsuspecting frog or toad victims (Figure 5). The larvae hatch, migrate through the skin, and eventually disappear into the frog's tissues. Within 2 to 3 days of infection, an open wound appears and displays the posterior spiracles of the maggots, which allows the maggots to breathe (Figure 5). Within these wounds, maggots develop to mature third instar larvae within 5 to 7 days of hatching, migrate out of the amphibian host, burrow into the soil, turn into pupae, metamorphose into adult flies, mate, and start the process all over again.

Other variations on parasite life cycles include the alternation of free-living and parasitic generations known as **heterogonic reproduction**. For example, lung nematodes in the genus *Rhabdias* alternate between parasitic and free-living generations. Parasitic individuals within the lungs of their amphibian hosts are **protandrous hermaphrodites**, a term for individuals that are functional males before becoming females. The spermatozoa are used to fertilize the eggs, and the eggs are then transported from the host's lungs into the gastrointestinal tract, and defecated into the soil (Runey et al., 1978). The released eggs hatch and begin a free-living generation resulting in adult free-living males and females which undergo sexual reproduction in the external environment (Langford and Janovy, 2009). Next, the free-living female nematode's progeny hatch within her body, where they feed on her internal organs, killing their mother in the process, and exiting her body as infective stages, a process known as **matricidal endotoky**. Finally, the infective juveniles enter the anuran host body cavity orally and/or via skin penetration and eventually migrate to the lungs to begin egg production to continue the life cycle (Baker, 1979).

The Role of Parasite Life Cycles in Transmission

Arguably, some of the most complex parasite life cycles belong to the digenetic trematodes, also known as flukes. During their life cycle, trematodes undergo sexual reproduction in the definitive host, followed by asexual reproduction in the first intermediate host in a process known as **polyembryony**, the formation of more than 1 embryo from a single fertilized ovum. Hundreds to thousands of free-living stages

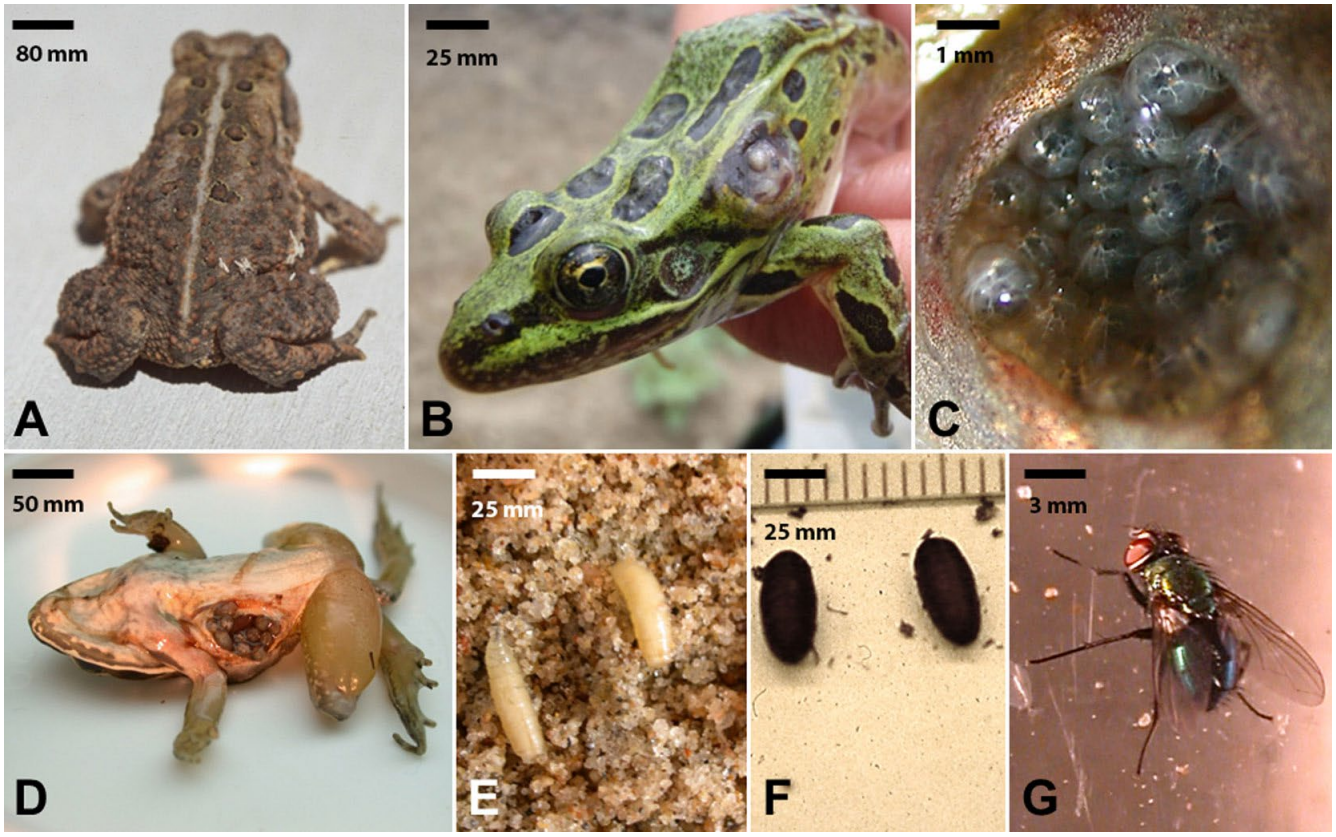

Figure 5. An example of a direct life cycle parasite where the parasites must exit the host and develop into a free-living adult and reproduce. A) Eggs of *Bufolucilia silvarum* glued to the back of an American toad *Bufo americanus*. Scale bar = 80 mm. B) Opened wound on the left lateral side of a northern leopard frog *Rana pipiens*. Note visible third instar maggots of *Bufolucila silvarum* in the wound. Scale bar = 25 mm. C) Third instar maggots of *Bufolucila silvarum* congregating and feeding as a group in an infected wood frog *Rana sylvatica*. Scale bar = 1 mm. D) Third instar maggots of *Bufolucila elongata* in a single wound on the right ventral side of a wood frog *Rana sylvatica*. Scale bar = 50 mm. E) Third instar maggots of *Bufolucila silvarum* searching for a place to pupate after leaving the host. Scale bar = 25 mm. F) Fully formed pupae of *Bufolucila silvarum*. Scale bar = 25 mm. G) An adult male green toad fly *Bufolucila silvarum*. Scale bar = 3 mm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

are then released from the first intermediate host, some of which infect a second intermediate host, which is then ingested by the definitive host (Figure 6).

A typical digenetic trematode life cycle offers a good example of the complexity of the transmission challenges faced by parasites during their life cycles (Lafferty and Kuris, 2002). First, eggs released into the external environment by adult worms in the definitive host hatch into a short-lived miracidium stage, which then must find a suitable first intermediate host, usually a snail. Second, and after asexual reproduction within the snail first intermediate host, the free-living but short lived cercariae emerge from the snail and must locate a suitable second intermediate host where they encyst as metacercariae stages. Third, the metacercaria stage must be ingested along with the second intermediate host by an appropriate definitive host for the life cycle to be completed.

It is hypothesized that parasites with complex life cycles have evolved by either adding or subtracting hosts based on trophic interactions of potential hosts (Poulin and Cribb, 2002). In trophically transmitted parasites with more than 1 host, or in parasites that are transmitted by vectors that take a blood meal from a vertebrate host, there are 2 hypotheses that support the addition of a host. One hypothesis proposes that the original host was preyed upon by other potential hosts higher up in the trophic food chain, and all other hosts have been added over time to the parasite life cycle (Smith-Trail, 1980; Poulin, 2007; Parker et al., 2003). Another hypothesis suggests the opposite. In this case, the original host was a top predator in the food web, and all other hosts with lower positions in the food web than the original host have been added secondarily to the parasite life cycle (Smith-Trail, 1980; Gibson and Bray, 1994; Lafferty, 1999; Parker et al.,

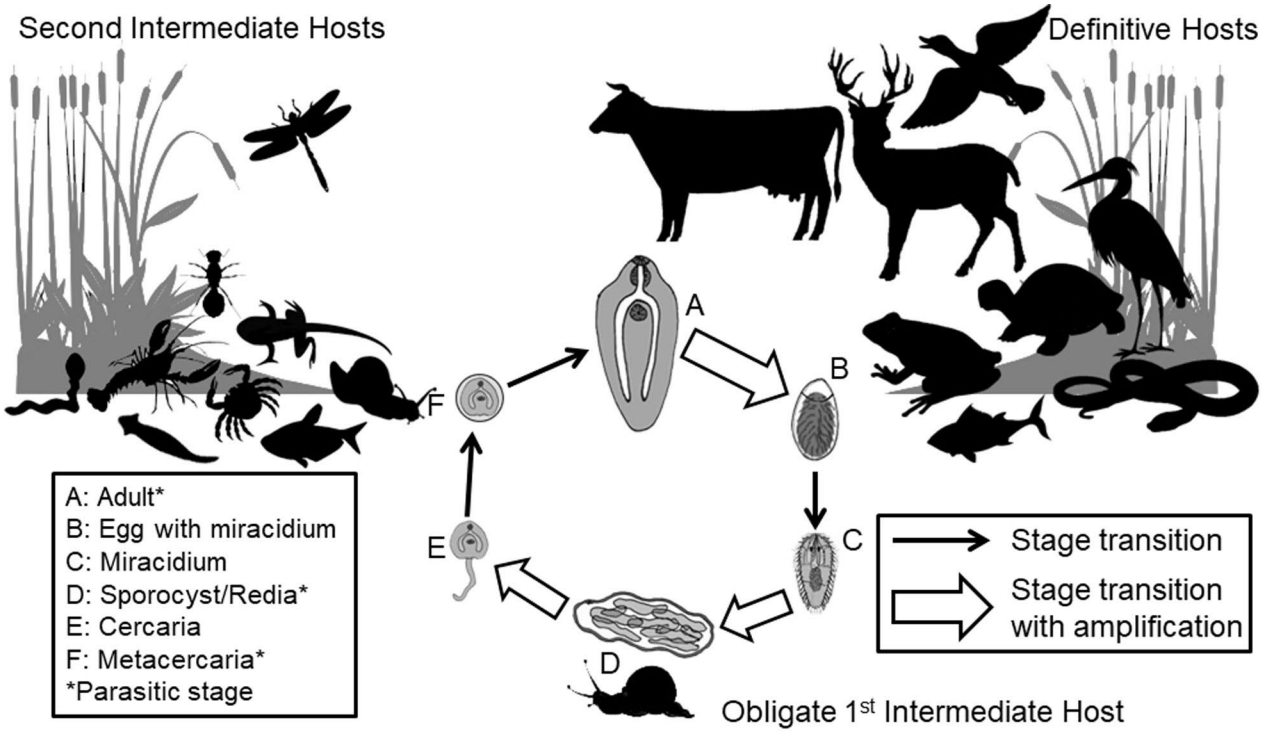

Figure 6. A representative diagram of a typical complex life cycle of a digenetic trematode. Note that most digenetic trematodes are host specific at the snail first intermediate host in the life cycle and much less host specific at the second intermediate and definitive host level. Also note the trematode developmental stages in the life cycle (A–F); including adult worms (A) producing eggs (B) through sexual reproduction in the definitive host (C), asexual reproduction (D) and production of free living cercariae (E) in the obligate snail first intermediate host. Source: M. Bolek. License: CC BY-NC-SA 4.0.

2003). Finally, hosts can also be lost if the life cycle no longer requires a particular host for completion (Poulin and Cribb, 2002; Parker et al., 2003).

A number of studies on parasite life cycles indicate that some species of parasites can survive in the alimentary canal of the predators of their definitive hosts. For example, **post-cyclic transmission** has been reported in a number of acanthocephalan and nematode species (Bolek, 1997; Nickol, 2003). In these cases, when a predator ingests a definitive host, instead of dying or being lost, the parasites simply reattach themselves to the intestine of the predator and resume growth or reproduction. Importantly, the predator may be the same as or a different species than the original definitive host of the parasite. Additionally, the direct life cycle of aspidobothrean trematodes, which parasitize molluscs, commonly promotes their survival and they reproduce in the intestines of turtles and fish that in turn consume infected clams as part of their diet. The aspidobothrean trematodes are considered a basal sister group to the digenetic trematodes which also infect molluscs as first intermediate hosts, but have complex life cycles (Zamparo and Brooks, 2003). As a result, one can imagine the evolution of complex life cycles by the addition of hosts to a direct life cycle.

However, understanding the specific steps of how and why these life cycles have evolved is difficult to decipher due to the lack of a fossil record for most parasites, complex host-parasite associations, and the lack of empirical data on host use for most parasite species in nature (Stigge and Bolek, 2015). For example, it is currently unclear if these processes occur gradually or require less evolutionary time (Stigge and Bolek, 2015). As a result of these difficulties, understanding how life cycles operate in nature and what hosts are used by those parasites can provide empirical data for future hypotheses testing on parasite life cycle evolution.

Parasite Adaptations, and Life Cycle Variation and Plasticity

Reproduction is certainly the most important task that individuals of any species of parasite must accomplish during their lifespan within a definitive host. However, in order for any parasite to reproduce within its host, it must be able to **infect** that host. In combination, these 2 principles (**infection** and **reproduction**) dictate that parasite life cycles have been selected for their ability to increase the probability that individual propagules will infect their hosts and achieve reproductive output (consisting of more propagules).

Understanding parasite life cycles is fundamental for many types of parasitological inquiries because life cycles inform understanding of life history strategies, host-parasite interactions, community and population ecology, life cycle evolution, and the epidemiology of diseases. Yet, the propensity for biologists to portray life cycles as a fixed, invariable unit is a monumental error, as actual real-world life cycles are not captured fully in so-called iron wheel diagrams such as those depicted in textbooks or health agency websites (Bolek et al., 2016). Indeed, understanding life cycle plasticity and variability is crucial to understanding how parasites evolve and function in hosts and the external environment. Despite the importance of this area of investigation, few biologists focus on life cycles of parasites as the center of their research. Furthermore, most parasitologists who have studied life cycles only do so until the life cycle could be completed. Once elucidated, most investigators do not continue to search for alternative hosts to complete the life cycle in nature. It is therefore unsurprising that published life cycles tend to be accepted as absolute truth and their validity is rarely questioned (Krull, 1952; Bolek and Janovy, 2008; Bolek et al., 2009; 2010).

Two examples are given in the following that provide realistic snapshots of how some parasites live in nature, while also highlighting specific life cycle adaptations that may increase both transmission probabilities and reproduction. In addition, these examples demonstrate how unrealistic paradigmatic life cycle diagrams are in deciphering transmission strategies of parasites in nature (Bolek et al., 2016).

The first example considers the life cycles of 2 closely related but host specific species of polystomatid flatworms (phylum Platyhelminthes: family Polystomatidae): *Polystoma nearcticum* and *Pseudodiplorchis americanus* (see Tinsley, 1990). *Polystoma nearcticum* infects the urinary bladders of 2 closely related treefrogs, *Hyla chrysoscelis* and *H. versicolor*, which reside in forests and grassland habitats throughout the eastern United States (Tinsley, 1990; Bolek and Coggins, 1998; Du Preez et al., 2007; Muzzall and Kuczynski, 2017). Interestingly, the life cycle of *Po. nearcticum* is synchronized with the reproductive biology of its treefrog definitive hosts (Figure 7). During the spring, when treefrogs enter permanent ponds to breed, adult forms of *Po. nearcticum* that live in the frog's urinary bladder begin laying unembryonated eggs concurrently with the oviposition activities of their treefrog definitive hosts. The eggs of *Po. nearcticum* are released into the pond in the frog's urine, and over a period of 10 days the eggs develop and hatch into short-lived motile larvae. Once hatched, the larvae of *Po. nearcticum* must find and infect their tadpole hosts within 20 hours of hatching. Interestingly, because tadpoles do not possess a urinary bladder, larvae of the worms enter the gill chamber of their tadpole hosts, where they mature in weeks and begin releasing eggs into the pond. The second generation of eggs produced by the branchial (gill) generation of *Po. nearcticum* develop and hatch coinciding with the metamorphoses of their tadpole hosts. When tadpoles transform into froglets they develop a urinary bladder and the larvae from the second generation of eggs of *Po. nearcticum* enter the froglet's cloaca and migrate into the urinary bladder (Figure 7). Once inside the urinary bladder of their treefrog definitive hosts, *Po. nearcticum* reaches sexual maturity and begins producing eggs when its treefrog hosts return to their breeding ponds the following spring.

In contrast to *Polystoma nearcticum*, *Pseudodiplorchis americanus* infects the urinary bladder of Couch's spadefoot toads, *Scaphiopus couchii*, an amphibian species that lives in deserts and arid habitats throughout the southwestern United States (Tinsley, 1990). Unlike the treefrog hosts of *Po. nearcticum*, Couch's spadefoot toads only enter temporary desert pools to mate and deposit eggs for approximately 21 hours per year (Tinsley, 1990). Since spadefoot toad tadpoles must complete metamorphosis in rapidly drying desert pools, they have one of the shortest developmental periods of any anuran species ranging from 7 to 20 days (Dodd, 2013). However, even with rapid metamorphosis, spadefoot toad tadpole mortality is often quite high in these desert pools, making tadpoles unreliable hosts for *Ps. americanus*. As a result, the transmission of *Ps. americanus* is confined to 1 to 3 nights each summer when the desert-adapted toads spawn.

To overcome this temporal problem, selection has favored a dramatic modification in the life cycle of *Pseudodiplorchis americanus*. Instead of producing eggs that must develop for weeks in the external environment and infect tadpoles, the larvae of *Ps. americanus* complete their development inside the uterus of worms in the urinary bladder of spadefoot toads. Once spadefoot toads enter desert pools to spawn, the larvae hatch within seconds of being released with the toad's urine into freshwater (Figure 7). When the larvae encounter a spadefoot toad in the water, they crawl up the chest of the amphibian and invade the nostrils. The larvae then migrate via the buccal cavity into the lungs where development occurs. Within a few weeks, the juvenile worms then migrate from the lungs by the intestine and cloaca into the urinary bladder. In the bladder, juvenile worms mature and then mate, accumulating new larvae in their uteri that will infect spadefoot toads the following year. Remarkably, the larvae of *Ps. americanus* appear to have specific adaptations for infecting adult spadefoot toads. For example, they are 2 to 4 times the size of larvae of any other species of polystomatid flatworms. Additionally, these giant larvae can swim for twice as long as larvae of *Polystoma nearcticum*, allowing them 2 days to

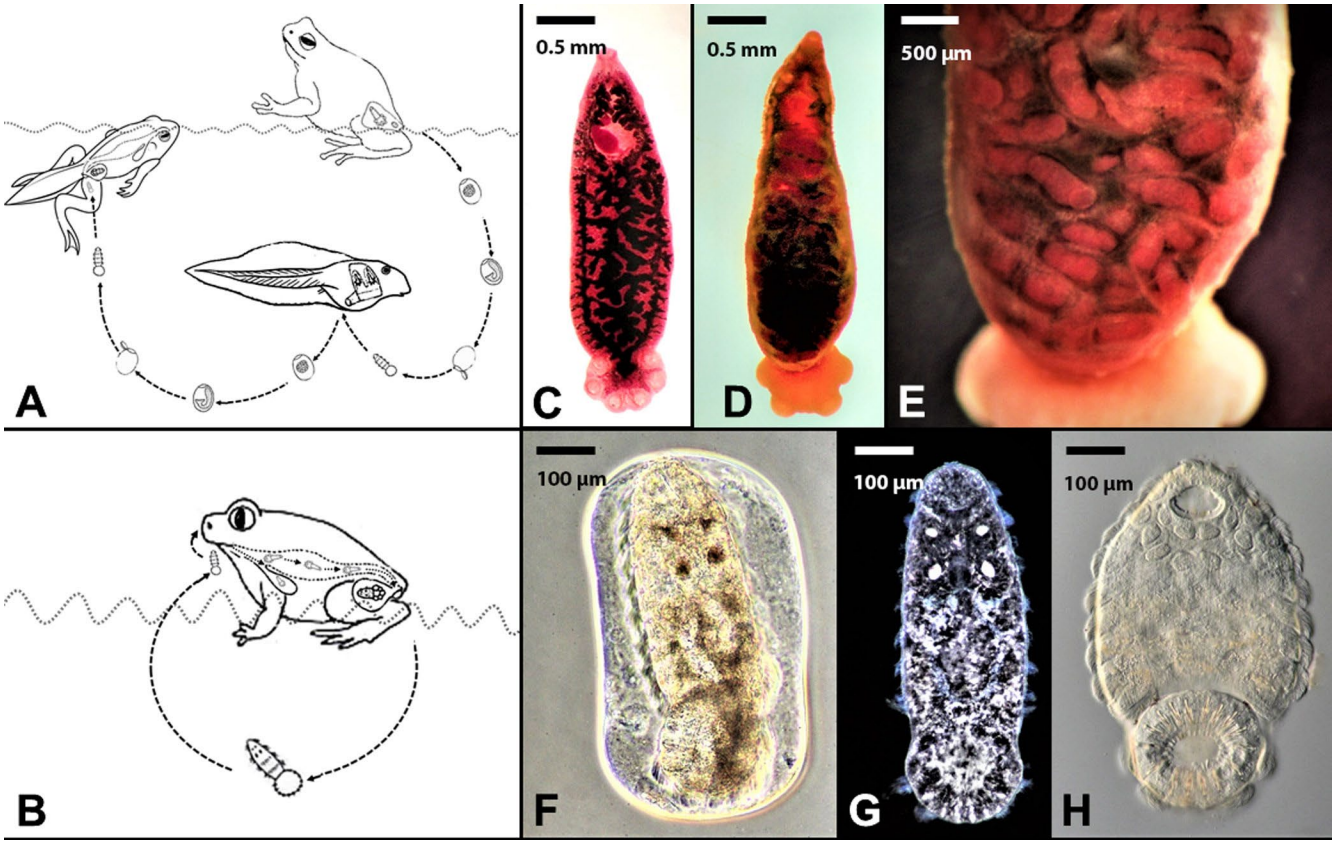

Figure 7. Example of life cycle variation for 2 closely related and host specific polystomatid trematodes. A) Transmission strategies of *Polystoma nearcticum* in the eastern gray treefrog *Hyla versicolor*. Note the egg being released by the urinary bladder generation of worms when their treefrog hosts enter ponds to breed followed by eggs being released from the branchial generation of worms on the gills of tadpoles. In all cases the eggs must develop in the external environment and the larval stage must find and infect metamorphosing froglets by entering their cloaca. B) Transmission strategy of *Pseudodiplorchis americanus* in Couch's spadefoot toad, *Scaphiopus couchii*. Larval stages are released directly from the bladder of spadefoot toads when they enter breeding pools. C) Adult *Po. nearcticum* recovered from the urinary bladder of a Cope's gray treefrog *H. chrysoscelis*. Scale bar = 0.5 mm. D) Adult *Ps. americanus* recovered from the urinary bladder of a Couch's spadefoot toad *S. couchii*. Scale bar = 0.5 mm. E) Higher magnification of *Ps. americanus* showing fully developed larvae in the uterus. Scale bar = 500 µm. F–H) Egg and hatched larvae of *Ps. americanus*. Note the 4 eyespots in (F) and (G) and the ciliated cells containing hundreds of cilia used for swimming in (H). Scale bar = 100 µm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

encounter a spadefoot toad in water. Finally, the larvae of *Ps. americanus* can survive drying for up to an hour, which is likely an adaptation that allows the larvae of *Ps. americanus* to leave the water and crawl up the chest of their spadefoot hosts and enter the nasal passages (Tinsley and Earle, 1983).

The second example demonstrates how a generalist parasite, the tadpole pinworm, *Gyrinicola batrachiensis*, has a modified life cycle that appears to increase its reproductive success in different species of hosts. *Gyrinicola batrachiensis* infects the large intestine of tadpoles and has been reported from 18 species of frogs and toads (Pierce et al., 2018). Adult anurans are resistant to infections and (as noted above) tadpoles lose their pinworm infections when they metamorphose into adults, which in turn gives *G. batrachiensis* limited time for reproduction in its tadpole hosts. To make matters more complex, not all tadpole hosts are equal in terms of pinworm development and reproduction. For example, tadpoles of some anuran species metamorphose in just a few weeks **(short developmental period)** giving limited time for pinworm reproduction, while tadpoles of other anuran species take months to years **(long developmental period)** to metamorphose, giving pinworms more time for reproduction. However, pinworms cannot choose what species of tadpoles they will infect because all tadpoles become infected with *G. batrachiensis* when they accidentally ingest a pinworm egg on the pond bottom.

Investigation has shown that *Gyrinicola batrachiensis* exhibits 2 different but related lifestyles that appear to solve the problem for both short- and long-lived larval anurans. To overcome these constraints, *G. batrachiensis* has evolved 2 different reproductive strategies. The first strategy involves asexual reproduction, by **parthenogenesis**, when unmated female pinworms produce thick-shelled environmentally resistant eggs that are passed in tadpole feces to infect other tadpoles in the pond. The second strategy involves sexual reproduction by female and male pinworms, which results in female *G. batrachiensis* that produce 2 types of eggs: thick-shelled and thin-shelled. The thick-shelled eggs are released into the external environment to infect other tadpoles, which are similar to eggs produced by parthenogenic females. In contrast, thin-shelled eggs never leave the tadpole's intestine and they are **autoinfective**, hatching quickly in the tadpole's gut thus rapidly increasing the number of pinworms in a single tadpole.

Production of thin-shelled autoinfective eggs varies according to the amphibian species and its tadpole developmental time (Figure 8). In tadpoles with short developmental periods that provide limited opportunities for pinworm recruitment and reproduction, pinworms can reproduce **parthenogenetically** (Adamson, 1981). Parthenogenetic pinworms are **monodelphic** and produce thick-shelled environmentally-resistant eggs. While parthenogenetic pinworms do not benefit from sexual recombination, reproduction via parthenogenesis increases the probability that the nematode offspring will infect another tadpole before their current host metamorphoses. Alternatively, in tadpoles with long developmental periods that allow *Gyrinicola batrachiensis* more time for development and reproduction, nematodes reproduce sexually (Adamson, 1981; Rhoden and Bolek, 2011; Childress et al., 2017; Pierce et al., 2018).

Female nematodes in tadpoles with long developmental periods are **didelphic**, producing thick-shelled environmentally resistant eggs in 1 uterine branch and thin-shelled autoinfective eggs in the second branch of the uterus. As a result of the autoinfective reproductive strategy, pinworms in long-developing tadpoles increase their numbers quickly and in the long run, a female worm can produce numerous reproductively active progeny inside a single tadpole host. So, although *Gyrinicola batrachiensis* might not always end up in their ideal host, that is, a long developing tadpole, they always try to make the most of their lot in life!

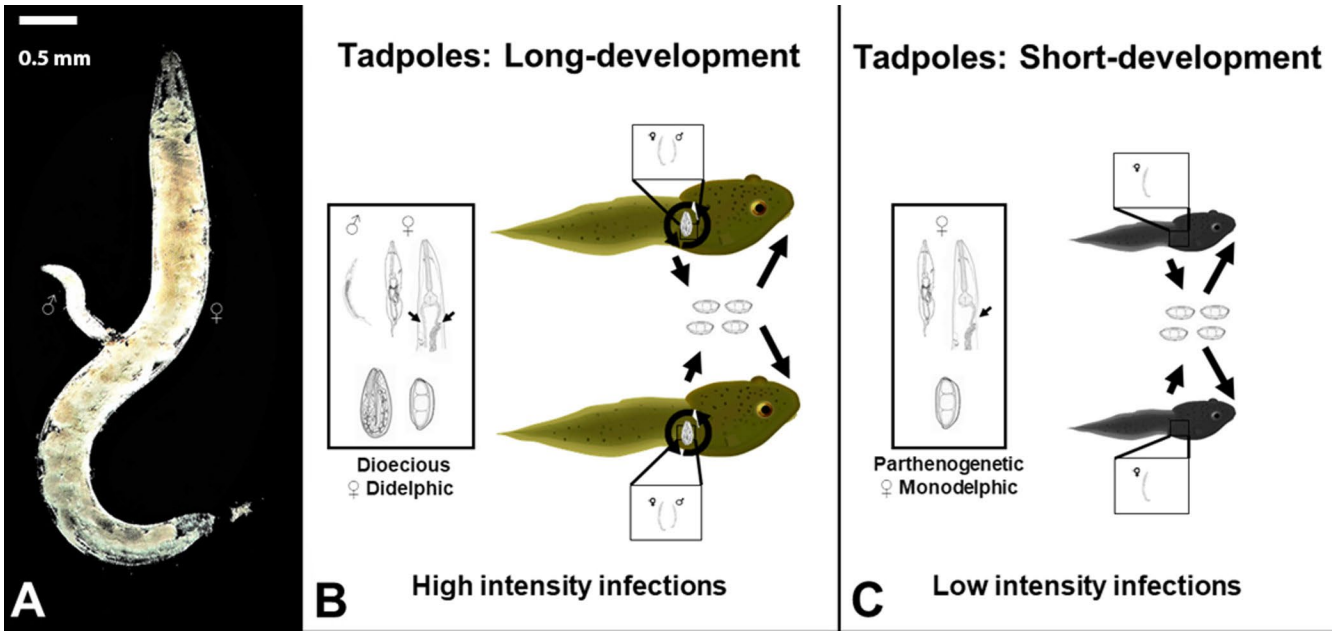

Figure 8. Example of plasticity in a direct life cycle of a generalist parasite *Gyrinicola batrachiensis*. A) A male (♂) in the process of mating with a female *G. batrachiensis* (♀). Scale bar = 0.5 mm. B) Dioecious reproductive strategy of *G. batrachiensis* in tadpoles with long developmental periods. Female worms are didelphic and produce thick-shelled and thin-shelled autoinfective eggs. As a result, tadpoles with long developmental periods have high intensities of *G. batrachiensis*. C) Parthenogenetic reproductive strategy of *G. batrachiensis* in tadpoles with short developmental periods. Female worms are monodelphic and only produce thick-shelled eggs. As a result, tadpoles with short developmental periods usually have much lower intensities of *G. batrachiensis*. Source: M. Bolek. License: CC BY-NC-SA 4.0.

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6

Parasites in Relation to Other Organisms

Behavioral Parasitology

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Chapter 6

Behavioral Parasitology

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Introduction

As pointed out in the previous sections, parasites have an intimate relationship with their hosts and can affect many aspects of their host's biology. By definition, parasites live at the expense of their host, causing some type of physical or physiological damage, but they can also affect host behaviors. Throughout this section, the who, what, when, where, why, and how of parasite manipulation of host behaviors will be investigated.

To categorize before moving on to examples, direct versus indirect effects on host behaviors should be described. Parasites can influence host behaviors **directly** through the physical presence of the parasite within a host or they may **indirectly** influence host behavior when potential hosts exhibit behaviors in order to avoid becoming infected with the parasite. Some examples of these parasite-avoidance behaviors are swatting, moving to a different habitat, feeding/foraging at specific times of day, and grooming (see Moore, 2002 for full review). Although these indirect effects on host behavior are interesting and certainly worthy of study, the direct effects of parasites on host behaviors are most salient. **Parasite-induced behavioral alteration/modification** refers to a behavioral change in a host that is caused by the presence of a parasite; there is no underlying assumption that the behavioral change is advantageous to either the host or the parasite. Note that the word *modification* and *alteration* can be used interchangeably. **Parasite *manipulation* of behavior** implies that the parasite is actively changing a host behavior in order to benefit itself. In the rest of this chapter, the basic principles of parasite-induced behavioral modifications will be established by exhibiting case-studies from the scientific literature to help answer 3 basic questions:

Question 1) **Why** are there parasite-induced behavioral modifications of hosts?
Question 2) **Which** host behaviors or traits are likely to be altered?
Question 3) **When** are host behaviors altered?

At the end of the chapter, there is a set of more advanced questions for those students who may want to delve deeper into the complexity of this aspect of host-parasite relationships.

Learning Objectives

1) Apply the scientific method to address questions about parasite-induced modification of host behaviors.
2) Analyze examples in the scientific literature to learn how scientists have experimentally addressed questions about parasite manipulation of host behaviors.
3) Be able to provide some classic examples of parasite-induced modification of host behaviors.
4) Understand the evolutionary principles of parasite manipulation of host behaviors.
5) Understand the types of host behaviors likely to be altered in relation to the parasites' life cycles.
6) Think critically about host-parasite relationships yet to be investigated from a behavioral standpoint.

Why Are There Parasite-Induced Behavioral Modifications of Hosts?

There is no simple answer to this question, but there are 3 primary hypotheses: 1) The altered behavior is a side-effect of infection, 2) the host benefits from the altered behavior (host-adaptation), and 3) the parasite benefits from the altered behavior (parasite-adaptation) (Poulin, 2010; Moore, 2013). Each will be discussed in turn.

Behavioral Changes as Side-Effects of Infection

Behavioral alterations as side-effects of infection appears to be the simplest answer because an infected host is expected to act sick, especially if the behavioral changes appear to be of no obvious advantage to either host or parasite. However, unless the hypothesis is tested it should not be used as the default explanation. Wise de Valdez (2007) conducted a study to determine whether parasite-induced behavioral changes were a side effect of infection or if they were advantageous to the parasite. The host-parasite system used was mermithid-mosquito system. Mermithids are nematode worms that can use aquatic mosquito larvae for development where they then emerge to a free-living state. During their development

Box 1. Notes on the Scientific Method

No matter in what stage someone is in their scientific career, all employ the scientific method, or at least parts of it, when embarking on new areas of study. In fact, most scientists have internalized the process as they use it on a daily basis. It is helpful to periodically revisit the formal nature of the scientific method. Thus, because for many students, this will be the first time considering parasite manipulation of host behaviors, you may approach it as new scientists using the scientific method. It all starts with an observation followed by a question (or many). Then use your previous scientific knowledge, or read a bit more, to come up with an educated answer to that question: The hypothesis. All good hypotheses must be testable. Now, the hypothesis may or may not be the right answer to the question; it is only a best guess based on previous understanding of a system. Therefore, to determine if the hypothesis is correct, the hypothesis must be tested, and data must be gathered through observational or experimental studies. Through data analysis, it can then be confirmed whether the hypothesis may be supported or rejected.

they grow and eventually take over much of the space inside the mosquito larvae after which they exit the mosquito larvae, killing it. Wise de Valdez (2006) found that mermithid nematodes made their mosquito larvae hosts less active and it is tempting to hypothesize that the change in activity levels is simply a side effect of the worm filling up the entire space of the mosquito larvae. An alternative hypothesis would be that the behavioral changes benefit the parasite by making the mosquito less likely to be eaten by a predator and thus survive long enough to emerge to its free-living stage. To test these hypotheses, Wise de Valdez (2007) experimentally infected *Aedes aegypti* mosquito larvae with mermithid nematodes and confirmed that their activity levels were lower than those without an infection. Predation experiments were then conducted using the predatory mosquito larva *Toxorhynchites rutilus* and it was found that the predator consumed both infected and uninfected larvae at equal rates. Therefore, the experiment supported the hypothesis that the behaviors are a side effect of infection, and the reduction in activity levels did not appear to benefit the parasite because they were eaten just as often as uninfected mosquitoes.

However, a singular set of experiments supporting a hypothesis does not necessarily make the hypothesis definitive. The important thing is that data were gathered and allowed the investigators to begin to make more educated assumptions about a system. Future scientists could use this study to develop new hypotheses that might lead to other conclusions after testing. This is what is so great about science, new hypotheses can always be tested! When the host's behaviors don't necessarily fit the classic sick behavior or when entirely new and unexpected behaviors are observed, other explanations may be sought.

Host Adaptation: The Host Benefits from Altered Behaviors

Another hypothesis to consider how to answer the why of behavioral alterations is that the host could benefit in some way from a change in behavior. The altered behavior would then be considered a host adaptation. An **adaptation** is a character that increases the fitness of an organism and **fitness** is the ability of an organism to survive long enough to successfully reproduce. Adaptations arise through **natural selection**; individuals that exhibit a particular trait survive and reproduce more than individuals that do not exhibit that trait. The parasite-induced behavioral changes of an infected host would be a host adaptation if they help to reduce or rid the host of parasites and thereby increase host survival and reproductive capacity (its fitness).

Unusual foraging habits that are a form of self-medication have been observed as a behavioral change that benefits the host. For instance, chimpanzees will eat medicinal plants that are not part of its normal diet (Moore, 2013, citing Huffman, 1997). Caterpillars infected with a parasitoid fly will switch plant food source and increase its survival (Moore, 2013, citing Karban and English-Loeb, 1997). Two other classic behavioral strategies that have evolved in some infected hosts in response to parasitism are known as **behavioral fever** and **behavioral chills** which are characterized by movement of infected hosts to a higher or lower than normal temperature to rid themselves or reduce the impact of a pathogen (see Moore, 2002 for review). Both of these are most likely to occur in organisms that cannot regulate their temperature metabolically. Metabolic fever in endotherms is well documented. It induces a behavioral change that brings the afflicted individuals to a habitat with a particular temperature. Müller and

Schmid-Hempel (1993) found that bumblebees infected with parasitoid fly larvae remained outside the hive where it was colder and when given a choice they spent more time in cold areas than uninfected bumble bees (behavioral chills). By altering their behavior to choose colder temperatures, these infected bumblebees lived longer and had fewer fully-developed parasitoids that the infected bumblebees that were kept at normal temperatures. A study by Watson et al. (1993) showed that house flies infected with a fungal pathogen that spent at least 8 hours in 40 ºC temperature shortly after infection survived longer than those that did not. Interestingly, this behavior did not benefit the house fly if the infection was more advanced (after 5 days post infection). Even more interesting, and evidence that parasite-induced behavioral alterations are complex, was that the flies that did not successfully employ behavioral fever moved to cooler temperatures, a behavior that benefited the fungal parasite; cooler temperatures enhanced the propagation of the parasitic fungus!

Parasite Adaptation: The Parasite Benefits from Altered Behaviors

In the first half of the 20th century, several researchers proposed that parasites may be able to alter the behaviors of their hosts in ways that increase their transmission success (Lefèvre et al., 2009 citing Cram, 1931; Van Dobben, 1952). Later, in the 1970s and 1980s, researchers provided some of the first empirical evidence that intermediate hosts infected with parasites exhibit different behaviors than those that were uninfected. Furthermore, the infected hosts were more likely to be consumed by the next host in their life cycle, thereby increasing transmission success (Hindsbo, 1972; Bethel and Holmes, 1973; 1974; 1977; Moore, 1983). These studies involved acanthocephalan parasites and their crustacean intermediate hosts. Bethel and Holmes (1973; 1977) demonstrated that small aquatic crustaceans, *Hyalella azteca* and *Gammarus lacustrus*, infected with 1 of 2 different species of acanthocephalans, *Polymorphus paradoxus* or *Corynosoma constrictum*, exhibit behaviors that move them to areas where their habitat overlaps with the feeding area of the parasites' definitive host and may make them more conspicuous. Through predation experiments using birds and muskrats, they found that infected crustaceans were more vulnerable to predation by mallard ducks and accidental ingestion by muskrats (both definitive hosts) than uninfected crustaceans. A study by Moore (1983) showed that the juvenile stage of *Plagiorhynchus cylindraceus* induces risky behavior of its isopod pill bug host, thereby causing it to be more conspicuous to its definitive host predator, the European starling (the details of this study will be discussed later in the chapter).

These initial studies kick-started research on parasite manipulate of host behaviors in earnest and since then researchers have found examples across all parasite taxa: protozoan parasites, Plathyhelminthes parasites in the classes Trematoda (flukes) and Cestoda (tapeworms), Acanthocephalans, Nematodes, Nematomorphs, and parasitic arthropods (see reviews by Adamo, 1997; Moore, 2002; Lefèvre et al., 2009; Hughes et al., 2012). Discovering the adaptive nature of these behavioral alterations in a scientifically sound way became a main area of discussion (Poulin, 1995; 2010). Furthermore, the types of questions being asked about parasite-induced behavioral alterations have expanded to include more complex questions (see end of chapter). For the remainder of the chapter the primary focus will be on the hypothesis of parasite manipulation of behaviors as parasite adaptations. The next question is: Which host behaviors or traits are likely to be altered, and when?

Which Host Behaviors or Traits are Likely to Be Altered, and When?

Life Cycles and Transmission Routes

In order to understand the adaptive nature of a parasite-induced behavioral change, the life cycle of the parasite in question must be understood. The parasite life cycle plays a major role in which host is likely to be manipulated and which behaviors are manipulated. Parasites with **complex life cycles** have multiple hosts; 1 or more **intermediate hosts** which are infected with an immature stage of the parasite, and a **definitive host** in which the parasite reaches sexual maturity. Parasites with **simple life cycles** have only 1 host, and **parasitoid life cycles** are unique in that 1 host is always killed by the parasite as it emerges to a free-living stage. Each life cycle has different requirements for how the parasite moves within the environment to reach a reproductive stage. Parasites with complex life cycles require movement from 1 host to the next. This movement can be up the food chain where 1 host lower on the food chain is consumed by the next host in the life cycle that is higher in the food chain (trophic transmission; Figure 1A). Movement can be through a vector, where 1 host (the vector) transmits the parasite to the next host (often via a bite) without being killed (vector-borne transmission; Figure 1B). Additionally, some parasites with complex or simple life cycles might require a host to bring them to a specific habitat where their eggs or larvae might be deposited (Figure 1C). Parasites with simple life cycles (1 host) are interesting because they may live their entire life within the single host or they may have 1 or more free-living stages, spending only part of their life cycle in the host. Some of these single-host parasites may use their host as

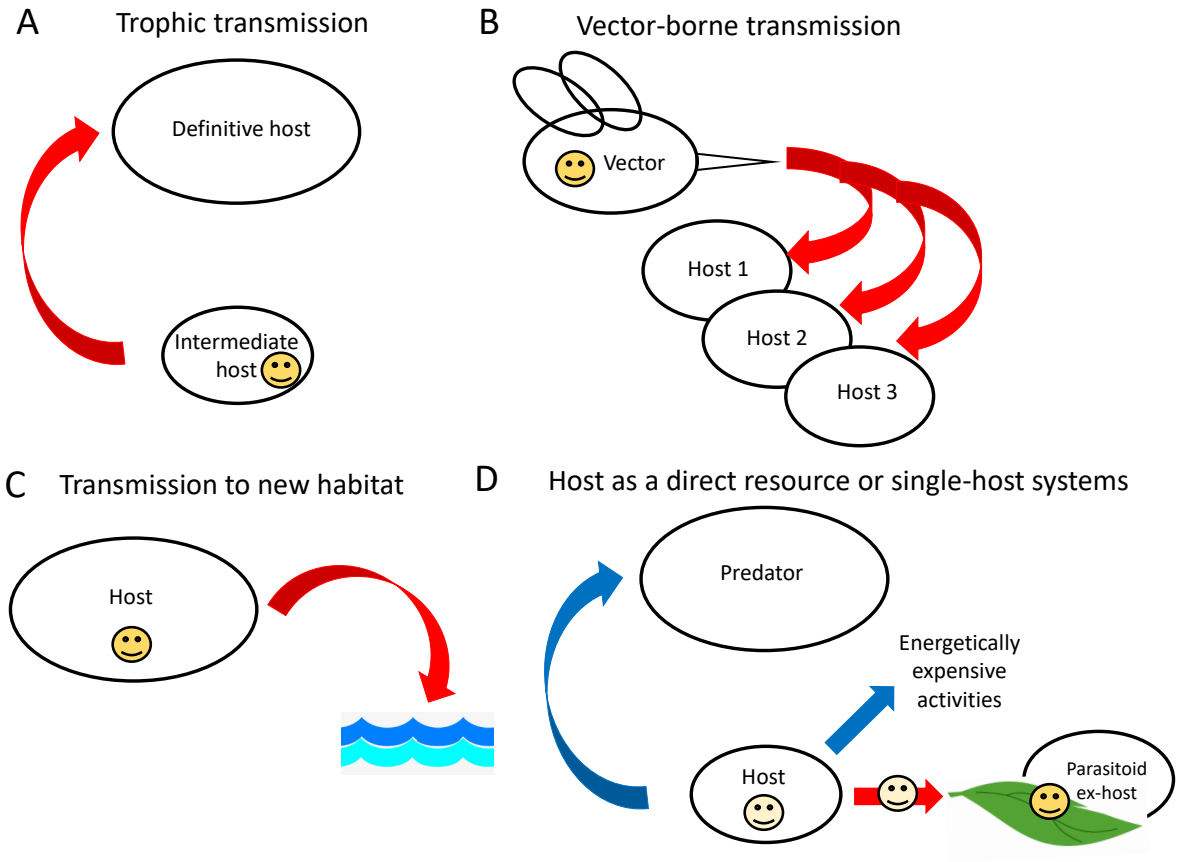


Figure 1. Presented are 4 main scenarios in which behavioral alterations have been seen. The smiley face is the parasite and the arrows indicate the stage in the life cycle where behavioral alterations are likely to occur. Red arrows indicate behaviors that increase the likelihood and blue arrows indicate behaviors that decrease the likelihood. A) Trophic transmission: Trophically-transmitted parasite behaviors of the intermediate host should be altered to increase transmission to the next host. B) Vector-borne transmission: In vector-borne parasite transmission, behaviors of the vector should be altered to increase transmission to multiple hosts or to increase the parasite load delivered. C) Transmission to a new habitat: Parasites that require delivery to a new environment, either themselves or their propagules, should manipulate the host to bring it to the appropriate habitat. D) Hosts as a direct resource: Parasites that use a host as a direct nutritional resource, usually parasitoids, should modify host behaviors to increase nutritional access or to prolong its survival and in some cases to elicit post-emergence protection. Note: These scenarios are not mutually exclusive. Source: Adapted from Poulin, 2010. License: CC BY-NC-SA 4.0.

a direct nutritional resource (Figure 1D), especially parasitoids, that usually consume much of their host in order to develop to their free-living stage. All of these different life cycles and transmission requirements open the door for some interesting ways in which parasite-induced behavioral modifications are manifested.

Trophic Transmission

In complex life cycles where trophic transmission is required, it would be expected that the host likely to be manipulated would be the intermediate host and the altered behaviors should result in an increase in consumption of that intermediate host by the next host in the life cycle (Figure 1A). Even these expectations have their nuances; the behaviors manipulated will be different if the next host is a natural predator or if the next host is not a natural predator of the intermediate host. If the intermediate host is a natural food source of the next host in the life cycle, it would be expected that the parasite would alter its normal predator avoidance behaviors. For example, the intermediate hosts of *Toxoplasma gondii* are rats and the definitive hosts, cats, are a natural predator. Normally, rats find cat urine odor repulsive. This is a natural defense mechanism that elicits an avoidance behavior. However, when infected with *T. gondii* rats are attracted to cat urine and might even seek out the cat which should theoretically increase the rate of predation on infected rats and thereby promote trophic transmission (Berdoy et al., 2000). On the other hand, if the intermediate host is not a regular food source of

the next host in the life cycle, parasites might manipulate behaviors that increase the contact these hosts have with their non-natural predator. For example, the trematode parasite *Dicrocoelium dendriticum* uses an ant as its intermediate host. In order for the life cycle to be completed, the ant harboring the juvenile trematode must be consumed by a grazing herbivore (usually a sheep or cow) which does not intentionally consume ants. The parasite manipulates the behavior of the ant in order to increase contact with the grazing definitive host. Ants infected with *D. dendriticum* act normally during the day but when the temperature drops, they climb to the top of blades of grass and clamp down with their mandibles. The ants are unable to release until the temperature rises again, thus positioning themselves to be eaten by grazing definitive hosts (Anokhin, 1966). Another extraordinary example that is quite evolutionarily complex is a nematode that not only causes the posterior end of an ant to turn red, but also manipulates the ant to hang out near a cluster of red berries. Yanoviak and fellow researchers (2008) conducted predation experiments and found that this manipulation of the phenotype and climbing near berries increased predation by the definitive host, frugivorous (fruit-eating) birds, that do not normally consume ants.

Included above are brief descriptions of just a few of the many studies that support the hypothesis that infected intermediate hosts behave differently than uninfected hosts and that these behavioral changes may be adaptive by increasing trophic transmission to the next host. However, many studies reported in the scientific literature (see review in Moore, 2002) have not provided experimental evidence that definitively supports that hypothesis. The reason these studies are less frequent in the literature is that they are simply hard to do. Pick any life cycle illustrated in this book and imagine what it would take to study the primary questions of parasite-induced behavioral changes. Not only would it first need to be established that the behaviors of infected and uninfected hosts differ, but then the next host in the life cycle would need to be included to determine if they became infected more often due to this behavior. Sometimes that next host in the life cycle is an animal that simply can't be used in experiments (think humans, large carnivores) or may be uncooperative in experimental arenas. Despite this difficulty there are studies out there. Following is a detailed description of one of the seminal works that provides experimental evidence of parasite manipulation of hosts in a trophically transmitted parasite system.

Moore (1983) investigated the acanthocephalan parasite *Plagiorhynchus cylindraceus* and the behavioral manipulation of its intermediate host *Armadillidium vulgare* (common pillbug). The life cycle of *P. cylindraceus* requires that the intermediate host, the pillbug, be eaten by the definitive host, the European starling (*Sturnus vulgarus*; Figure 2).

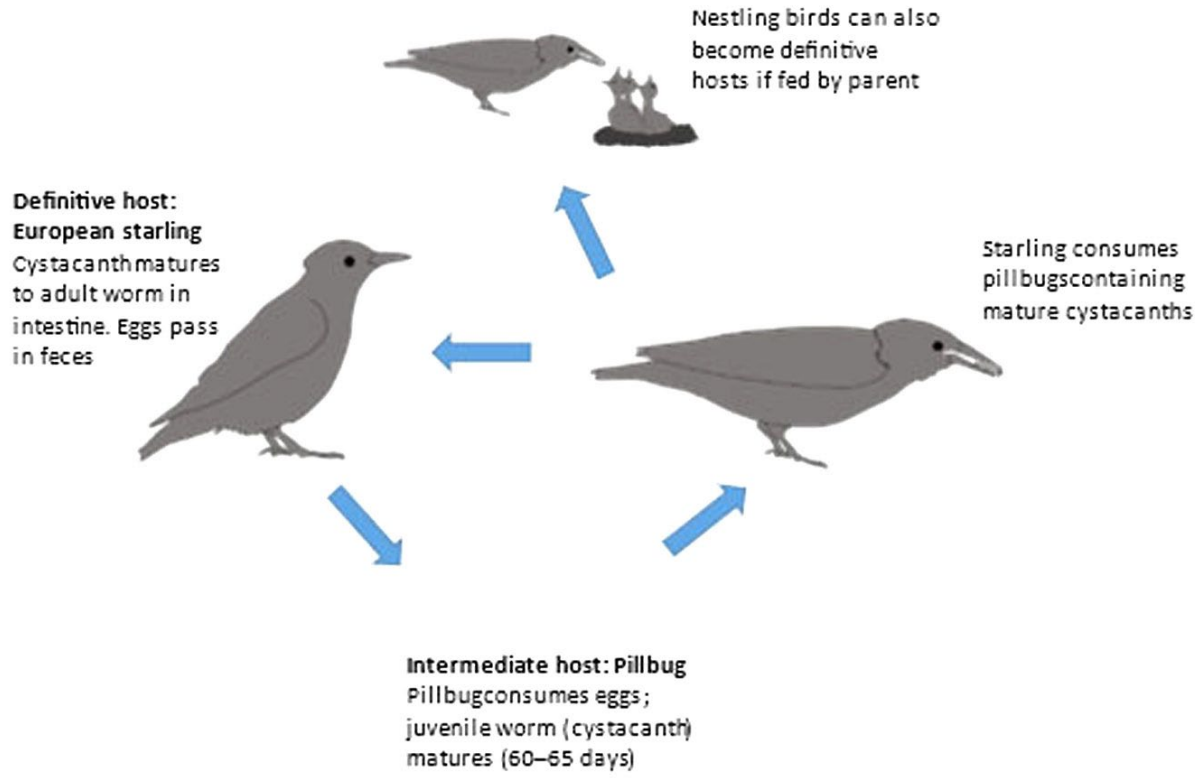


Figure 2. Life cycle of the acanthocephalan parasite *Plagiorhynchus cylindraceus*. Source: M. Wise de Valdez. License: CC BY-NC-SA 4.0.

Box 2: Stop and Think

Before reading further, take a look at the life cycle (Figure 2) and think about what you already know about pillbugs and birds. Where do they live? How do they behave? What behaviors might be targeted by the parasite that might help it reach the starling? By doing this you are starting to formulate one or more hypotheses. How might these hypotheses be tested?

Moore conducted both laboratory and field experiments to investigate this host-parasite system. For this example, it is interesting to consider how 2 primary questions were answered: 1) Do infected pillbugs behave differently from uninfected pillbugs, and 2) Are infected pillbugs more likely to be eaten by starlings?

In order to answer the first question, "Do infected pillbugs behave differently from uninfected pillbugs?" Moore experimentally infected pillbugs and sham-infected others (Figure 3A). **Sham infection** is when the researcher treats the control animals similarly during the infection experiments but does not include the actual parasite. In behavioral experiments it is

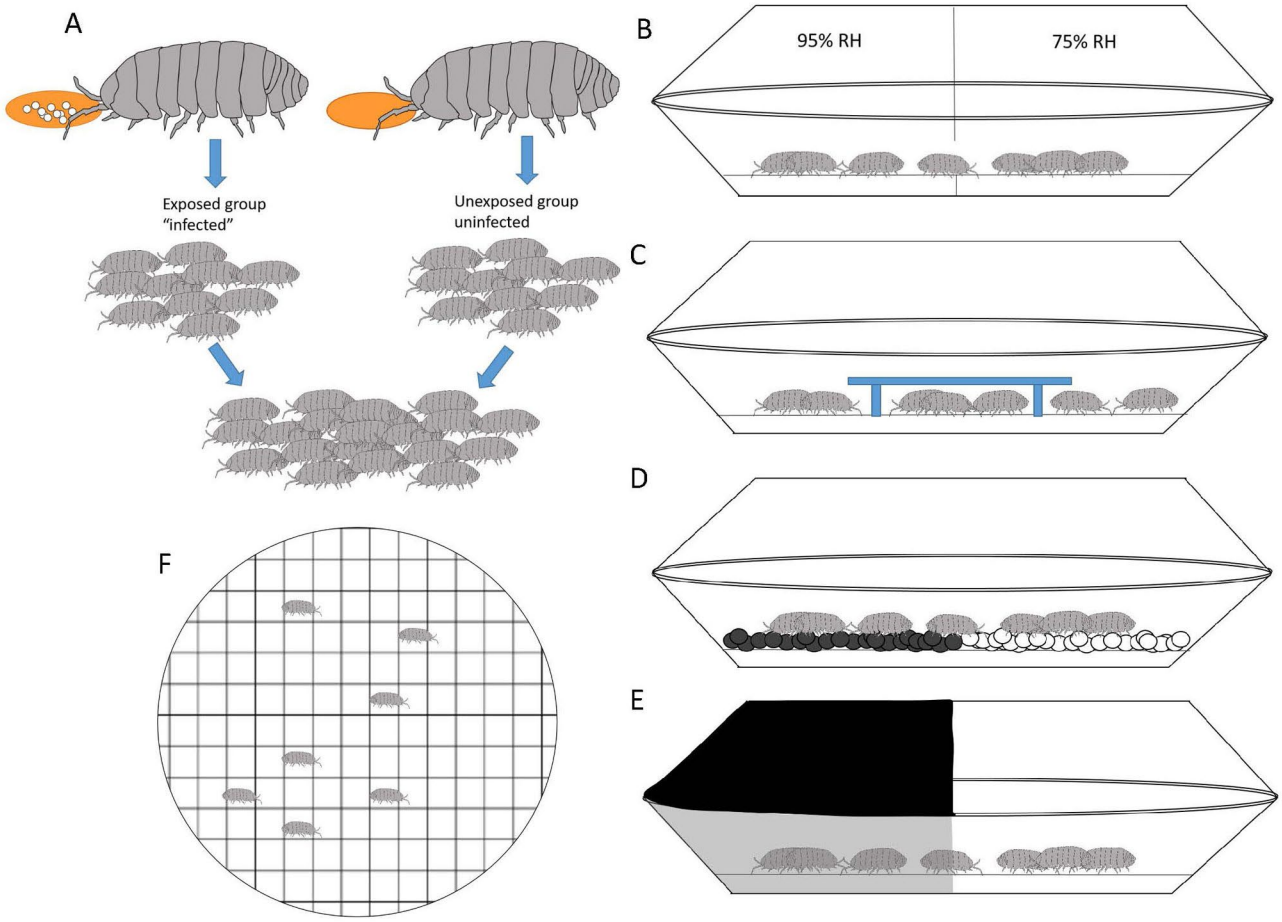


Figure 3. Experimental design to test behavioral differences between uninfected pillbugs and those infected with *Plagiorhynchus cylindraceus.* A) Experimental infections: Pillbugs were fed carrots with (exposed) or without (unexposed) *P. cylandraceus* eggs. Pillbugs were maintained for 3 months to ensure the cysticanth had reached the stage when it could be infectious to birds. Prior to placement in the arenas (B–F), an equal number of exposed and unexposed pillbugs were combined into a group, then each was uniquely marked. At the end of each trial all pillbugs were dissected to look for cysticanths. Each behavior trial was thus **blind** (the observer did not know infectious status during behavioral observation). All arenas consisted of 2 pie plates, one on top of the other. A wire mesh bottom was placed as a platform for pillbugs. Different aspects were manipulated to test the behavioral response. B) Humidity choice: High relative humidity (RH) or low RH. C) Shelter seeking: Under a shelter or exposed. D) Substrate preference: White or black. E) Phototaxis: Light or dark. F) Locomotion: Distance moved and resting behaviors. Source: Adapted from Moore, 1983.

important to institute multiple controls in order to ensure that behavioral differences observed are the result of the parasitic infection and not a difference in treatment of the organisms. Another important thing to note is that the pillbugs in the intentionally-infected group may not always become infected. Exposure to parasite eggs does not ensure that the infection will take. For this reason, the 2 groups are referred to as exposed and unexposed (Figure 3A).

Because pillbugs are normally found in areas of high moisture and under leaf litter, bark, or rocks, and because these habitats also provide protection from potential visual predators, Moore chose to look at behaviors associated with habitat preference (humidity, shelter, substrate, and light) and overall activity level of the pillbugs. Moore set up several arenas to test habitat preference of infected and uninfected pillbugs (Figure 3B-E) and one to determine activity level (distance moved and time resting; Figure 3F).

Before adding the pillbugs to the arenas, 5 exposed and 5 unexposed pillbugs were mixed together and were then marked with a unique identifier. By mixing them before the study, it enabled Moore to conduct **blind assays** in which she did not know which pillbugs were exposed and which were unexposed. In this way she controlled for observational bias. The trials consisted of placing the 10 pillbugs in the arena, allowing them to acclimate, and then recording the location of each pillbug every minute for 30 minutes. At the end of each trial, the pillbugs were dissected to determine infection status. Moore did this for each of the different arenas: humidity choice (95% relative humidity:75% relative humidity; Figure 3B), shelter seeking (under a shelter:exposed; Figure 3C), substrate choice (white:black; Figure 3D), and phototaxis (movement to or away from light; Figure 3E).

Moore found that infected pillbugs spent significantly more time in less humid and unsheltered areas and spent

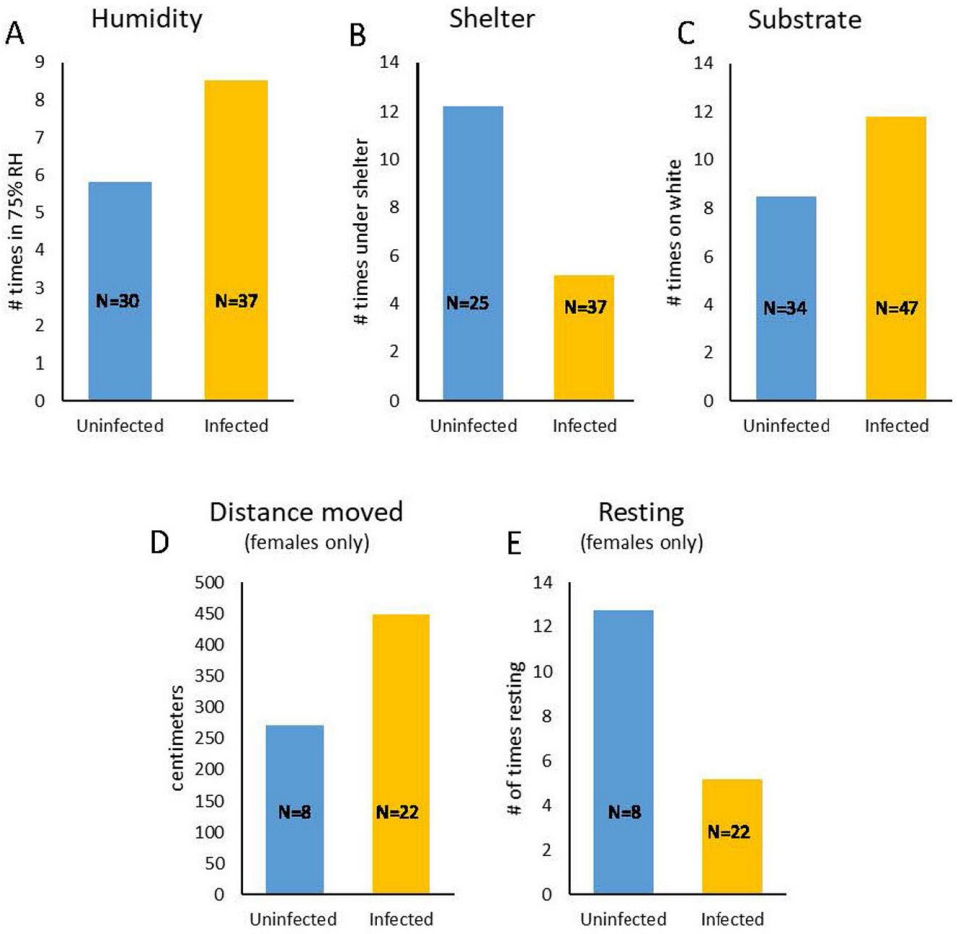


Figure 4. Infected pillbugs were found more frequently in less humid areas, in unsheltered areas, and on white substrate than uninfected pillbugs. There was no difference in phototaxis between infected and uninfected groups, both were negatively phototactic. Infected females were more active than uninfected females; infected females moved a greater distance in a set time period and rested less than did uninfected females. Males did not show differences between infection groups. Error bars not shown (but see Moore, 1983). Source: Adapted from Moore, 1983. License: CC BY-NC-SA 4.0.

more time on white substrate than uninfected pillbugs (Figure 4A-C), however there was no difference in preference for darkness (all preferred dark). Activity levels differed between infected and uninfected female pill bugs; infected females traveled further and rested less than uninfected females (Figure 4 D-E).

The second aspect of Moore's study was to establish whether starlings fed preferentially on infected pillbugs. Moore used experimental data from outdoor cage studies as well as observational data from the field. In the outdoor cage trials Moore used 5 individual wild-caught adult starlings and provided each individual bird with 10 infected and 10 uninfected pillbugs (pillbugs were unmarked; Figure 5). Pillbugs being presented to the birds were on a pan where they were offered a choice of black/humid or white/less-humid substrate. After the bird had eaten 10 pillbugs, the uneaten pillbugs were dissected in order to determine which pillbugs the starling had eaten (infected or uninfected). Moore found that 71% of the infected pillbugs were eaten and only 44% of the uninfected pillbugs were eaten (Figure 5), indicating that behavioral differences in the pillbugs led to an increase in predation rates on infected pillbugs.

In the field, Moore used the infection rate of nestling starlings to establish if parents were foraging randomly or preferentially on infected pillbugs. Note that nestlings can become infected by being fed infected pillbugs by their parents (Figure 2). Moore collected data from wild starlings to determine how often pillbugs were fed to nestlings and the natural infection rate of pillbugs in the field area. With these data, she calculated the probability of nestlings receiving infected pillbugs from their parents if the parents chose pill bugs randomly in the field arena. She then compared this probability to the actual infection rate of nestlings in the field. She found that more nestlings were infected than would be expected if parents were choosing pillbugs randomly. Which means that adult starlings were feeding their nestlings infected pillbugs more often than they were feeding them uninfected pillbugs because the adults are more likely to capture infected pillbugs due to the risky behavior exhibited by the infected pillbugs. These field observations corresponded with what she saw in the lab predation experiments. In conclusion, Moore provided experimental and field-based evidence that the behavioral manipulation of pillbugs by *Plagiorrhynchs cylindraceus* is a parasite adaptation to increase the chance of being consumed by the next host in the life cycle.

Vector-Borne Transmission

Not all parasites that have a complex life cycle involve trophic transmission. Parasites that use a vector to transmit parasites to multiple hosts are also exhibiting a complex life cycle (Figure 1B), but in this case, 1 host transmits the parasite to the other without being consumed. A vector-parasite life cycle often involves an arthropod that is capable of blood-feeding (think mosquitoes, ticks, kissing bugs, sand

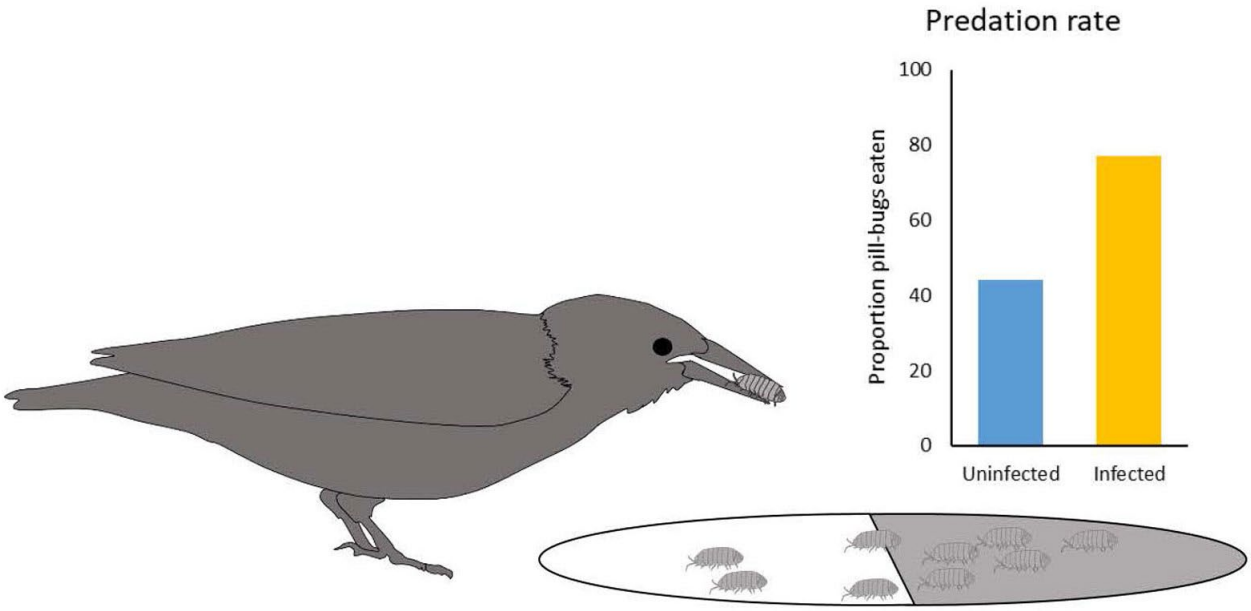


Figure 5. Field-cage predation experiment. Starlings were offered an equal number of infected and uninfected pillbugs in a pan that allowed pillbugs to choose their habitat. The habitat provided was either white dry sand or dark humid sand. Over 5 trials, 71% of infected pillbugs were eaten and only 44% of uninfected pillbugs were eaten. Source: Adapted from Moore, 1983. License: CC BY-NC-SA 4.0.

flies) on a vertebrate host. Through the act of blood-feeding, parasites are transmitted to the other host in the life cycle, often a vertebrate.

Box 3: Stop and Think

Before reading further, think about times when you have been bitten by a mosquito. You hear and see them and you likely swat or slap them or you just give up and go inside. What if that mosquito was infected with a parasite that could be transmitted to you? What mosquito behaviors might the parasite manipulate to ensure transmission to you? What behaviors might be manipulated to ensure it was also transmitted to the other people hanging around outside with you? How would you formulate these questions into hypotheses that you could test?

Behaviors that are likely to be altered in this type of host-parasite relationship are those that increase the transmission rate or the parasite load delivered upon transmission. The vector behaviors that are most often targeted are the feeding behaviors, although host-seeking/finding behaviors have also been shown to be altered by parasites (see reviews: Molyneux and Jefferies, 1986; Hurd, 2003; Lefèvre et al., 2006). One of the first accounts of modified feeding behavior of a vector was by Bacot and Martin, 1914 (referenced in Moore, 2013), where they observed that fleas carrying the plague bacteria (*Yersinia pestis*) were less successful at feeding due to blockage of their feeding apparatus by *Y. pestis* and that the blockage led to plague transmission. *Plasmodium*, the parasite that causes malaria, is vectored by mosquitoes and multiple studies have shown that *Plasmodium* can alter mosquito host-seeking and blood-feeding behavior in ways that can potentially increase transmission rates (Cator et al., 2012).

Another well-studied vector-borne parasite that has been studied in light of its behavioral manipulations is the protozoan parasite *Leishmania* (Killick-Kendrick et al., 1977; Beach et al., 1985, citing Chung et al. 1951; Rogers et al., 2002). *Leishmania* are single-celled parasites that are transmitted to humans or other mammals by the bite of a sand fly and in humans can cause various debilitating pathologies and symptoms (see Chapter 12 for detailed descriptions). The most common form is cutaneous leishmaniasis which is characterized by painful open sores on the skin that have difficulty healing. Rogers and Bates (2007) investigated whether 2 species of *Leishmania* that cause cutaneous leishmaniasis, *L. mexicana* and *L. infantum*, manipulate the behavior of their sand fly hosts (*Lutzomyia longipalpis*) in ways that increase transmission efficiency in a mouse model (use of humans in experimental infections is reasonably restricted). An elegant multi-dimensional study provides evidence that *Leishmania* can manipulate host behavior to increase transmission and infectivity, described below.

In order to interpret when and how behavioral alterations are likely to occur in the sand fly-*Leishmania* system, the life cycle of *Leishmania* must be understood (see Chapter 12 for more on *Leishmania*). In short, the life cycle of *Leishmania* involves an infected sand fly biting an uninfected mammalian host and injecting the motile promastigote stage. The promastigotes invade white blood cells and develop into amastigotes. An uninfected sand fly becomes infected when it bites an infected mammal and ingests blood containing the amastigote stage. In the sand fly, the amastigote stage transforms into the promastigote stage over the course of 7–10 days (extrinsic incubation period). Thus, it is important to remember that the promastigote stage is the stage that is infective to the mammal and that the amastigote stage is infective to the sand fly.

Some of the previous work on this system must be understood before delving into the study by Rogers and Bates (2007). Several research teams established that *Leishmania* damage the stomodeal valve and physically block the gut with a matrix made by a gel they secrete (Schlein et al., 1992; Stierhof et al., 1999; Rogers et al., 2004). This blockage interferes with sand fly feeding and limits the amount of blood it can take in. As a result, they take longer to feed and probe the skin more often (Rogers et al., 2002). A different group studying the rodent malaria-mosquito model of *Plasmodium yoelli* and *Anopheles stephensi* found that feeding persistence increased in infected mosquitoes but only after *Plasmodium* had reached the stage in which it was infective to humans (Anderson et al., 1999).

Box 4: Stop and Think

How is it advantageous to the parasite to alter vector behavior only during certain times? Think about what a vector has to go through when it needs to feed? What are the risks?

Hypotheses	Assay	Experiment	Result	Interpretation
1) *Leishmania* manipulate sand flies to persist with blood feeding only after they become infective to the mammalian host	Biting persistence assay (Figure 7)	Compared biting persistence of uninfected sand flies with those infected with either *L. mexicana* or *L. infantum* after interruption over the course of 11 days as the *Leishmania* developed from amastigote to the infective promastigote	Infected sand flies persisted longer than uninfected (Fig.ure8A) and the persistence increased as the infection matured	**Hypothesis supported** Infection with either species of *Leishmania* leads to a change in biting persistence and that the change is stage-specific, occurring to a higher degree when the parasite is infective to the mammalian host
2) *Leishmania*-infected sand flies feed on multiple hosts	Second host choice assay. Similar to Figure 7 but with 2 mice	Compared probability of a host-switch after repeated disturbance of uninfected sand flies and those infected with either *L. mexicana* or *L. infantum*	Infected sand flies were more likely to feed on multiple hosts whereas uninfected flies often gave up on feeding after repeated interruption (Figure 8B)	**Hypothesis supported** Infection with *Leishmania* increases the number of hosts on which sand flies feed and thus can be considered a mechanism for increased transmission success
3) *Leishmania*-infected sand flies that have been behaviorally manipulated will deliver a higher parasite inoculum per host than non-manipulated infected sand flies	Biting persistence assay (Figure 7) and uninterrupted feeding assay using only infected sand flies	Compared the lesion thickness of mice bitten by sand flies exhibiting feeding persistence and lesion thickness of mice bitten by non-persistent feeders after the biting persistence assay and after an assay where they were allowed to feed uninterrupted	Sand flies that were more persistent delivered a greater inoculum of *Leishmania* (as measured by lesion thickness) than sand flies that were less persistent (Figure 8C) when interrupted, but when uninterrupted there was no difference (Figure 8D)	**Hypothesis supported** The behavior manipulation by *Leishmania* to cause greater biting persistence can lead to a greater parasite load delivered than if the behavior did not occur. This indicates that the behavioral manipulation is indeed an adaptation to increase transmission

Table 1. Summary of experimental design to establish that *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. Source: Adapted from Rogers and Bates (2007), 2019. License: CC BY-NC-SA 4.0.

Armed with that background information, it can be understood how Rogers and Bates (2007) developed their hypotheses: 1) *Leishmania* manipulate sand flies to persist in blood-feeding only after they become infective to mammals (when the parasite reaches the promastigote stage), 2) *Leishmania*-infected sand flies feed on multiple hosts, and 3) *Leishmania*-infected sand flies that have been behaviorally manipulated will deliver a higher parasite inoculum per host than non-manipulated infected sand flies.

In order to answer these questions, Rogers and Bates used a biting persistence assay in which individual sand flies were allowed to land and attempt feeding on an anesthetized mouse for 1 minute, after which they were disturbed by brushing the leg or antennae every 10 seconds until they stopped trying to feed (Figure 6).

The time it took for the sand fly to stop attempting to feed was considered their feeding persistence. The biting assay was modified to address each of the hypotheses. In order to test the first hypothesis, Rogers and Bates experimentally infected sand flies by feeding them rabbit blood with *Leishmania* amastigotes (they used 2 species of *Leishmania*; *L. mexicana* and *L. infantum*), or rabbit blood alone (the uninfected group; Figure 7).

They then used the biting persistence assay as described; testing both infected and uninfected sand flies. Recall that the first hypothesis also stated that the parasite should alter the behavior only when it becomes infective to the next host. Therefore, they conducted this assay daily over the course of the infection: Four days post-infection (non-infective stages) through 11 days post-infection (highly-infectious stages). They found that sand flies infected with either *L. mexicana* or *L. infantum* exhibited greater feeding persistence than uninfected sand flies and that this occurred later in infection when the parasite could be effectively transmitted to a mammalian host (Figure 8A).

The second hypothesis, which stated that infected sand flies are more likely to feed on multiple hosts, required modifying the biting persistence assay to include a second mouse. The sand fly was allowed to locate and begin feeding on a mouse for 1 minute and then disturbed every 10 seconds until the sand fly switched to the other mouse or stopped

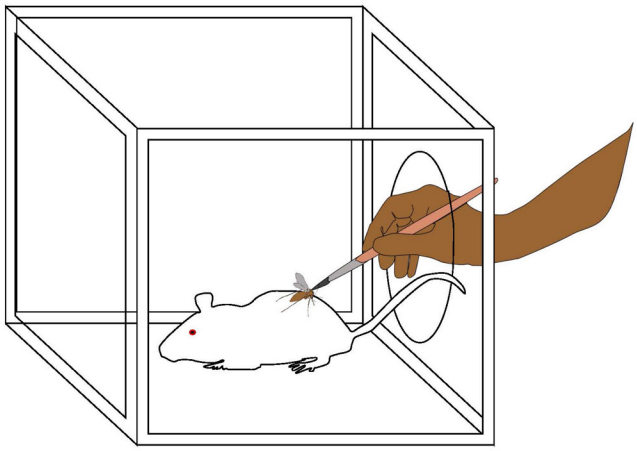


Figure 6. Biting persistence assay. One sand fly at a time was placed in a cage with a single anesthetized mouse. The sand fly was allowed to feed for 1 minute, after which it was disturbed every 10 seconds by brushing its hind legs until the sand fly stopped trying to feed. The total time it took to stop attempting to feed was known as its feeding persistence. After the trial sand flies that were experimentally infected with *Leishmania* were dissected and parasite load determined. Source: M. Wise de Valdez, 2019. License: CC BY-NC-SA 4.0.

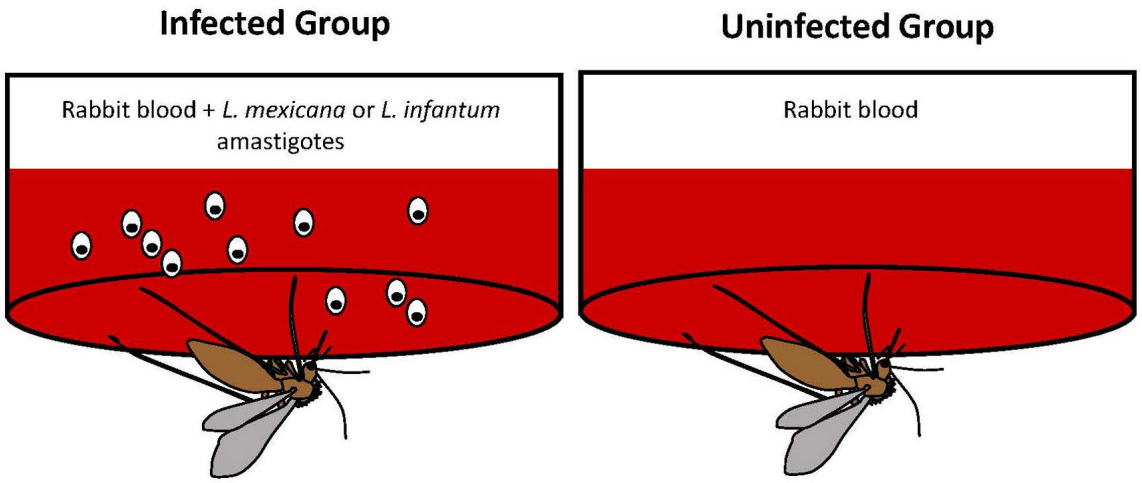


Figure 7. Experimental infection of sand flies was carried out using an artificial membrane system. Each feeder held fresh rabbit blood with either *Leishmania* amastigotes (*L. mexicana* or *L. infantum*) or rabbit blood alone. Source: M. Wise de Valdez. License: CC BY-NC-SA 4.0.

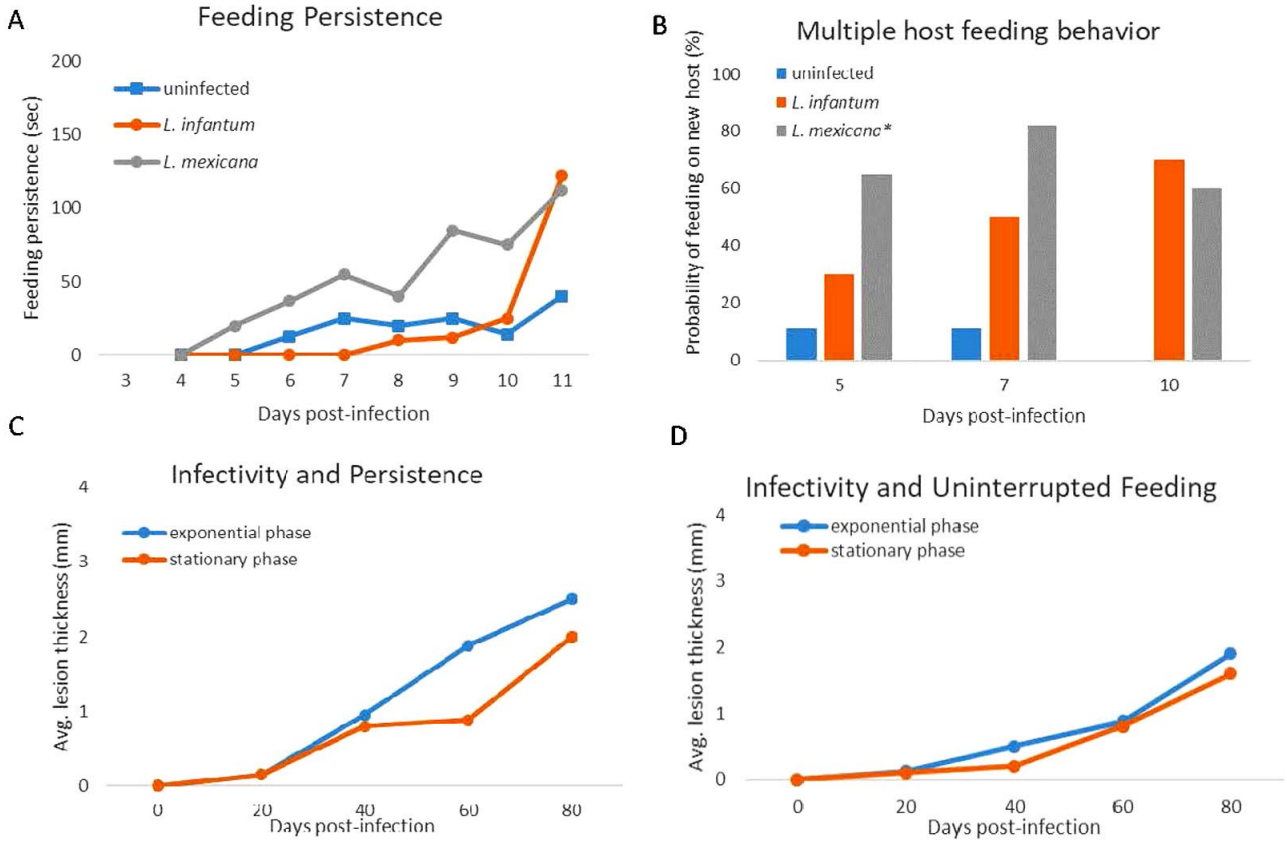


Figure 8. Results from the biting persistence assays. A) Feeding persistence of infected and uninfected sand flies. Infected sand flies exhibited greater feeding persistent than uninfected sand flies. For each day post infection, 16 infected and 16 uninfected sand flies were assayed to establish an average feeding persistence. Error bars not shown. B) Proportion of sand flies assayed that switched to a novel host after repeated interruption. On days 5, 7, and 10 post-infection 12 sand flies from each group were assayed. Error bars not shown. C) Average lesion thickness of mice bitten by persistent sand flies (blue) or non-persistent sand flies (orange). Error bars not shown. Persistent sand flies produced greater lesions and thus delivered a greater inoculum of parasite than non-persistent sand flies. D) Average lesion thickness of mice after being bitten by uninterrupted sand flies. Error bars not shown. There was no difference in lesion thickness between the exponential and the stationary stage infected sand flies when they were allowed to feed without interruption. Source: Adapted from Rogers and Bates, 2007. License: CC BY-NC-SA 4.0.

attempting to feed. The researchers observed sand flies on days 5, 7, and 10 post-infection. They found that sand flies infected with *L. mexicana* or *L. infantum* were more likely to switch to a new host than uninfected sand flies (Figure 8B).

The third hypothesis required a more elaborate set-up. The hypothesis was: An increase in biting persistence leads to a greater parasite load delivered to the mammalian host. In order to test this, they had to be able to compare a group of infected sand flies that exhibited increased feeding persistence and infected sand flies that did not. Rogers and Bates were able to isolate different phenotypes of *L. mexicana*: One that elicited an early increase in biting persistence (7 days post-infection; exponential phase) and another that did not elicit an increase until closer to day 10 (stationary phase). They experimentally infected sand flies with either the exponential phase or the stationary phase. On day 7 post-infection, they conducted the biting persistence assay and followed the development of the resulting lesions on the mice. They used the thickness of the lesions as a proxy for the inoculum size (the number of parasites injected by the sand fly). They found that the average lesion thickness was greater in mice bitten by more persistent sand flies than less persistent sand flies (Figure 8C). In a parallel experiment to confirm that the biting persistence was the primary mechanisms for an increased inoculum, the authors allowed sand flies of both infection types to feed without interruption. They found that the average lesion thickness on mice did not differ between the 2 groups (Figure 8D). This is further evidence that the modified behavior of increased feeding persistence was the mechanism for an increase in transmission efficacy.

Rogers and Bates's primary conclusions were that, 1) Timing of parasite development is linked to feeding persistence, 2) parasites do not increase risky feeding behavior until the stage that is infective, and 3) that this behavioral manipulation strategy enhances *Leishmania* transmission by increasing transmission to multiple hosts and increasing parasite load during biting. Thus, this set of experiments provided evidence for adaptive parasite manipulation of the vector behavior and the fact that it occurs in more than 1 species lends strength to this conclusion.

Transmission to a New Habitat

Some life cycles require that the parasites be delivered to a new habitat where they emerge themselves or where their propagules (eggs or juveniles) are released (Figure 1C). Delivery to a new habitat can be as simple as the parasite taking advantage of where its host is already going, or it may require the manipulation of a behavior to take a host where it wouldn't normally go. Mermithid nematodes (*Gastromermis*) in adult mayflies (*Baetis bicaudatus*) do both. *Gastromermis* nematodes that infect mayflies use the female mayfly's natural oviposition behavior of laying eggs in streams to reach a water source where they then emerge to mate (Vance, 1996a). However, when the nematodes find themselves in a male mayfly they are a bit stuck because males do not display oviposition behavior. Vance (1996a) showed that mermithids feminize male mayflies which causes them to exhibit oviposition behavior, thus delivering the worms to water where they can emerge. This type of study provides unique evidence that the behavioral manipulation is adaptive because the parasite does not manipulate behavior of all hosts, only those that do not exhibit the behavior necessary for it to complete its life cycle. This selectivity within the same system regarding which hosts are manipulated and which are not is indicative of a phenotype that is a direct result of natural selection. This host-parasite system is also unique because it exhibits host sex-specific manipulation.

Sometimes it is not about the adult stage emerging in a habitat where it can mate, it is also about delivering the immature stages to habitats where they can get to the next host. *Plagiorchis elegans* manipulates its snail intermediate host (*Stagnicola elodes*) to rise to the water surface to release the cercarial stage (Lowenberger and Rau, 1994) and several parasitic fungi alter the behavior of their insect host to find perching areas to better release their fungal spores (Poulin, 2010, citing Andersen et al., 2009; Maitlan, 1994).

One of the most well-known examples of parasite behavioral manipulation is horsehair worms (phylum Nematomorpha) that cause their terrestrial insect host to jump into water. Thomas et al. (2002) carried out field observations and experiments in the field and lab to evaluate this behavior. This study bears highlighting since it 1) includes non-manipulative field observations of multiple host species being manipulated by 2 different species of nematomorphs, and 2) the authors use a y-tube olfactometer which is a tool in studying preference and/or choice (Figure 9). Behavioral biologists across many fields use some form of the y-tube olfactometer regularly.

The field observations made by Thomas et al. (2002) involved recording the number of insects coming from a nearby forested area (with known natural habitats for nematomorphs), moving across a concrete pathway towards a swimming pool, and jumping into a pool. They also recorded how many of those insects were infected. They conducted these observations every night over 2 consecutive summers. They recorded 9 different species that jumped into the swimming pool and all were infected (Figure 10C). The most common species recorded were *Nemobius sylvestris* (Figure 10A), with 70 individuals that committed suicide, and *Meconema thalassinum* (Figure 10B), with 30 individuals taking a dip.

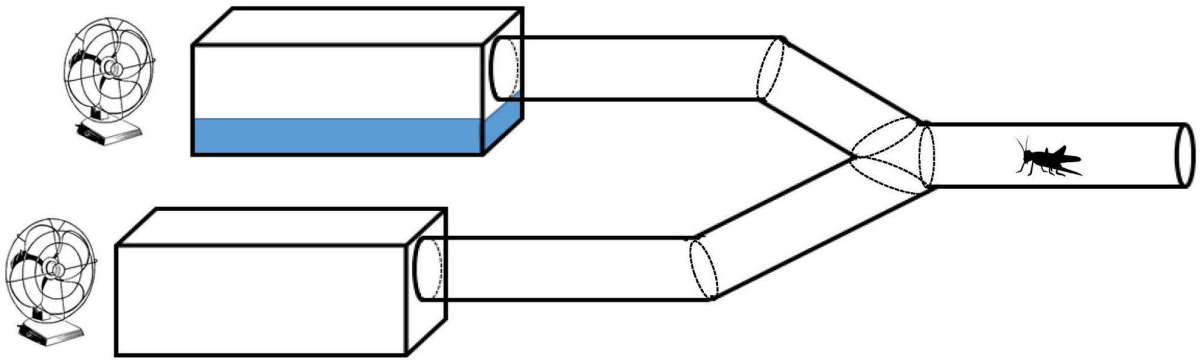
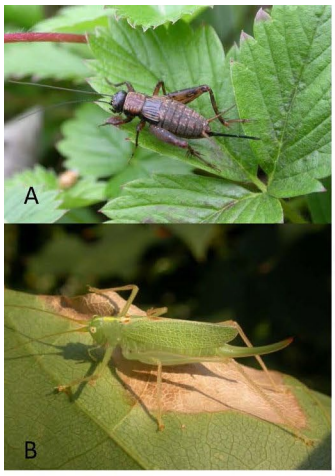


Figure 9. Y-tube olfactometer. A hypothetical design of the y-maze choice assay conducted by Thomas et al. (2002) to assess whether water served as an attractive stimulus. At one end of each arm was a trough, 1 with water and 1 without. A fan was placed at the end of each arm to gently send the "odor" down each arm. Crickets were tested one at a time by placing them at the end of the tube. After 15 minutes their location was recorded. Source: Adapted from Thomas et al. (2002), 2019. License: CC BY-NC-SA 4.0.

In the field-based experiment, Thomas and others used field-caught crickets. They collected 33 *Nemobius sylvestris* crickets from the forested area (presumed uninfected) and 38 from the concrete area around the pool (presumed infected). They then placed the 4 crickets, 2 from the forest and 2 from the pool, under a cup on the concrete near the pool. They studied the crickets' behavior for 15 minutes, recording which individuals jumped into the pool. After the trial, they dissected all crickets to establish their infection status. They found that significantly more infected crickets entered the water than did uninfected crickets (Figure 10D). When they analyzed which of the 33 forest-collected crickets were infected, they found that 15% were infected, while 95% of the poolside-caught crickets were infected. This significant difference between the infection prevalence of poolside versus forest-caught crickets indicates that water-seeking behavior is more common in infected crickets.

The goal of the laboratory experiment was to determine if the presence of water was an attractive stimulus for infected crickets. They used the y-tube olfactometer (Figure 9) to allow crickets (uninfected and infected) to choose an arm with water at the end, or one without water. Again, they used field-caught crickets (forest-caught and poolside-caught). They found that infection status did not affect the arm that the crickets chose. However, of the crickets that chose the arm with water, all infected crickets jumped into the water and only 1 of the 12 uninfected crickets jumped into the water. These data clearly show that nematomorphs manipulate water-seeking behavior but the mechanism by which they alter the behavior is not via an increase in water detection.



C

Cricket host species	Nematomorph species	# of times observed
Nemobius sylvestris (A)	*Paragordius tricuspidatus*	70
Meconema thalassinum (B)	*Spinochordodes tellinii*	30
Pholidoptera griseoptera	*S. tellinii*	11
Uromenus rugosicolis	*S. tellinii*	5
Ephippiger cunii	*S. tellinii*	1
Barbitstes serricauda	*S. tellinii*	1
Leptophyes punctatissima	*S. Tellinii*	1
Antaxius pedestris	*S. Tellinii*	1
Yersinella raymondi	*S. Tellinii*	7

D

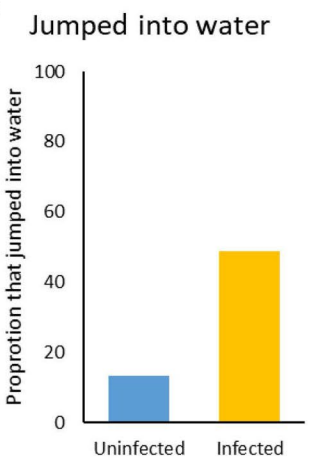


Figure 10. A) The European bush cricket *Nemobius sylvestris*. B) The oak-bush cricket *Meconema thalassinum*. C) Results of the field observations: species of crickets that jumped into water, the species of nematomorph they harbored, and the number of times they observed individuals of each species jumping into the water over the course of 2 summers. D) Results of the field experiment: Proportion of infected and uninfected crickets that jumped into the water. Source: Adapted from Thomas et al. (2002). License: CC BY-NC-SA 4.0.

Box 5: Stop and Think

What might be some other mechanisms for how the nematomorph manipulates the behavior? How would you test this?

Hosts as a Direct Resource or Single-Host Systems

Parasite-host relationships in which the parasite has only a single host for the duration of its life cycle or which relies on the host for its own development offer a unique set of hypotheses on adaptive manipulation of host behaviors (Fritz, 1982). First, it would be expected that these parasites alter host behaviors in ways that decrease the host's risk of predation. The parasite requires the host to stay alive long enough for the parasite to reach maturity and by altering behaviors that reduce predation on the host, the parasite thereby increases its own chance of survival. Second, it would be expected that these parasites would alter host behaviors in ways that ensure that sufficient nutritional reserves are available to the parasite. Parasites that develop to maturity in a host and then emerge usually require vast nutritional resources directly from the host. There are 2 sets of behaviors that might be targeted. Parasites might reduce energetically expensive behaviors in order to reserve nutritional stores or they might increase host foraging behavior to keep up with the nutritional needs of both the parasite and host. There are relatively few studies that experimentally investigate whether these behavioral changes occur (Moore, 2002) and fewer still that provide evidence for adaptation (but see Benton and Pritchard, 1990; Vance, 1996a; 1996b; Vance and Peckarsky, 1997; Wise de Valdez, 2007; Barquin et al., 2015; Soghigian et al., 2017).

One theme that emerges from the literature, however, is that there appears to be a trade-off between reducing predation risk and ensuring that enough nutrition is obtained. Revisiting the mermithid-nematode system helps to explain this point. Mermithid nematodes infect juvenile mayflies (in their aquatic stage) and there they undergo partial development. The mayfly larvae have to stay alive long enough to emerge into flying adults in order for the mermithid to complete its development. Therefore, it might be expected that the mermithids in the larval mayflies would reduce risky behaviors so as not to become fish food. However, they in fact increase their risky behaviors and are preyed upon more often than uninfected mayflies (Benton and Pritchard, 1990; Vance, 1996a; 1996b; Vance and Peckarsky, 1997). The researchers propose that there is a trade-off between maintaining nutritional reserves and predator avoidance. They suggest that the developing mermithid induces a nutritional deficit and therefore increasing feeding behaviors (and thus risky behaviors) may make up for that deficit. Note however, that the study has not continued past the point of establishing that a behavioral difference between infected and uninfected larval mayflies exists.

In 2 larval mosquito-parasite systems researchers have been able to extend the study to answer whether behavioral changes were adaptations or not. The research by Wise de Valdez (2007) described earlier concluded that the reduction of activity levels of mosquito larvae infected with mermithid nematodes was likely not a parasite adaptation because predation rates did not decrease. Soghigian et al. (2017) on the other hand investigated a protozoan gregarine parasite that uses mosquito larvae as its only host. They looked at larval behavior of *Aedes triseriatus* infected with *Ascogregarina* and found that they were less active and these behavioral changes *did* lead to reduced predation rates by the predatory larval mosquito *Toxorhynchites rutilus*. This difference in results is likely due to the evolutionary relationship in these 2 systems. The latter system is common in nature where they are exposed to natural selection pressures and which presumably has a longer evolutionary relationship. The former system however used laboratory-reared colonies of the mosquito, the parasite, and the predator. Laboratory environments can shift the selection pressures these organisms face. Therefore, it is important to acknowledge and consider the source of the test organisms when interpreting the results.

Box 6. Stop and Think

Addressed earlier was how nematomorphs manipulate their hosts to jump into water so that they can emerge. This host-parasite system is also one in which the parasite uses the nutritional stores of the insect in order to complete development and requires that the cricket host stays alive for more than a month. What other types of cricket behaviors might the nematomorph alter while it is developing?

In the cricket-nematomorph parasite system, Barquin et al. (2015) used information from several studies on the impact of insect parasitoids on the calling behavior of infected crickets compared to uninfected crickets (Cade, 1984; Zuk et al., 1993;

Orozco and Bertram, 2004; Kolluru et al., 2002) to hypothesize that calling behaviors of crickets should be manipulated by nematomorphs because calling is both energetically costly and attracts the attention of auditory predators. Although this study addresses only whether behavioral alterations occur and not whether they are adaptive, this study is highlighted because it exemplifies how hosts are handled in a laboratory setting and how some behaviors need to be assessed through means other than visual observation. Next, one of the experiments conducted by Barquin et al. (2015) is summarized.

Box 7. Stop and Think

What might be the next set of experiments someone would want to develop in order to test these remaining questions? Reading papers that have unanswered questions and then coming up with ideas for how someone could answer them is what budding scientists should be doing. So students should find those biological systems that have unanswered questions, or have questions yet to be asked, and find a way to answer them! (Hint: Students should talk to professors and ask if they can do research in their lab.)

Barquin and colleagues (2015) exposed *Acheta domesticus* crickets to *Paragordius varius* nematomorph larvae 2–3 days after wing development (30 exposed, 30 sham-exposed). Crickets were marked with waterproof paint to give them each a unique identity (Figure 11B). Crickets were housed in an insectary with a 12–12 light/dark cycle to keep the circadian rhythms. Individual cricket chirping frequency was recorded for 12 hours (dusk to dawn) on day 5 post-exposure and every 6 days thereafter using individual cages, microphones, and a computer program set up to record sound (Figure 11A).

The computer program allowed them to measure how much time they spent chirping and the intensity of the chirping events (Figure 11C). Note that the same cricket was followed throughout the course of its infection, for this reason it was imperative that each cricket had a unique identifier that would not wear off over the course of a month. After the trials infection status was determined by placing the cricket in a bowl of water and checking for worm emergence (Figure 11D). Note that exposure does not necessarily result in infection, therefore there were fewer infected crickets than uninfected crickets when data were analyzed (Figure 12).

This section would be incomplete without mentioning that some insect parasitoids manipulate the behaviors of their hosts in ways that protect them even after they have emerged. One species of parasitic wasp manipulates its orb-weaving spider host to spin it a specialized protective pouch just before it emerges. The wasp larva is then deposited in this pouch which serves to protect it while it pupates (Poulin, 2010, citing Eberhard, 2000). Another species of parasitic wasp, which uses a caterpillar host, somehow has manipulated the caterpillar to stick around even after it emerges in order to protect it from other predators (Poulin, 2010, citing Brodeur and Vet, 1994; Grosman et al., 2008).

Box 8. What Did All These Studies Have in Common?

- Started with questions and developed hypotheses that could be tested.
- The life cycle of the parasite had to be well understood.
- Needed source of infected individual: experimental infections.
- Hosts were always dissected afterwards to establish infection status.
- All studies required uninfected controls so that behaviors could be compared.
- Required both definitive and intermediate hosts as well as the appropriate habitats in the experimental design.
- Experiments were repeated: scientists used multiple organisms and multiple trials of each assay performed.
- None of them had all the answers.

A Quick Note: How Do Parasites Do It?

The mechanisms by which parasites manipulate host behaviors are elusive but more often than not they can be categorized into direct or indirect mechanisms: A direct mechanism is something produced by the parasite and an indirect mechanism might be physical interference with a biochemical pathway. Often the manipulation passes through neurological

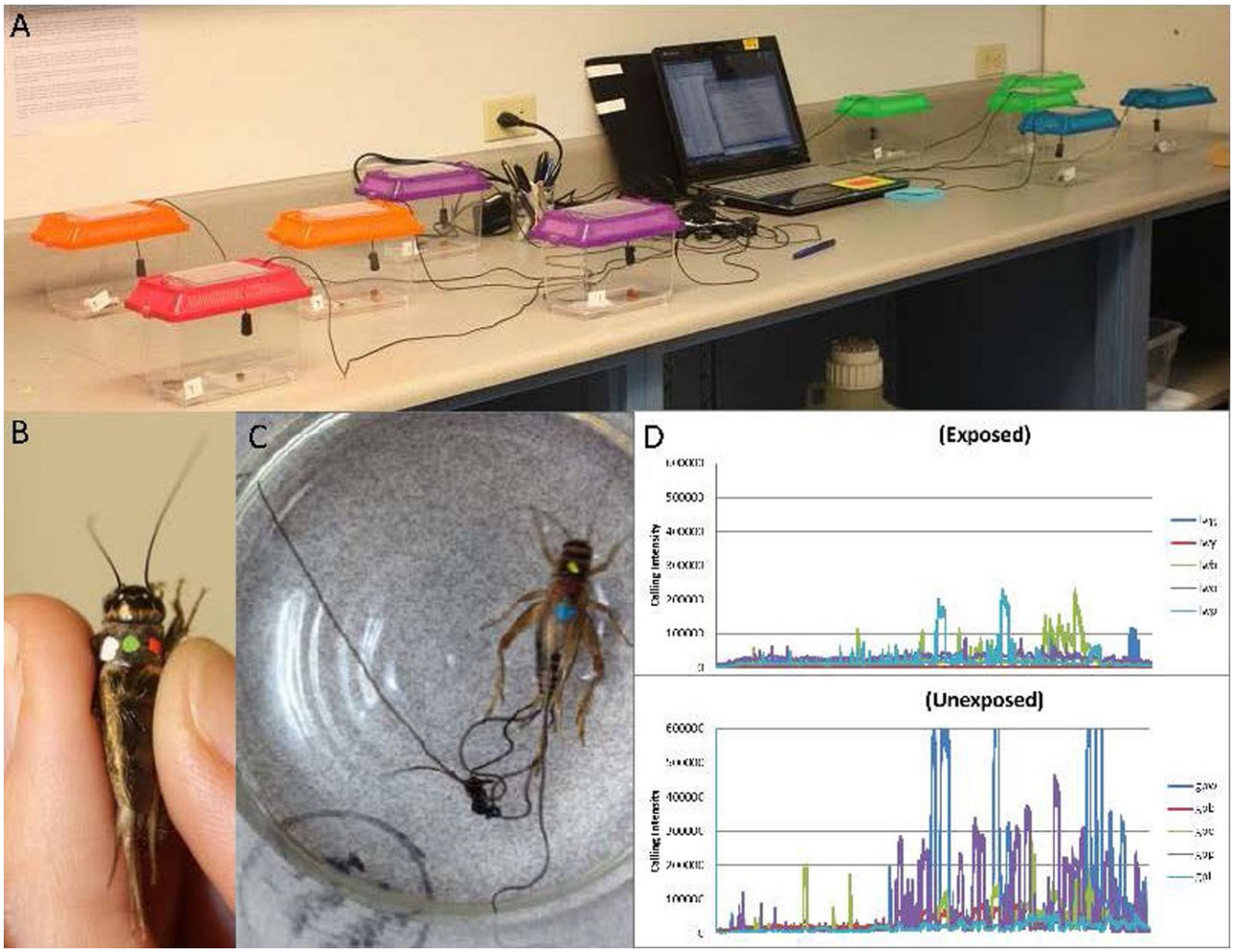


Figure 11. Experimental design used to study the effect of nematomorphs on calling behavior of crickets. A) Set up: Each cage held a microphone attached to a computer that ran a program to record frequency and intensity of calling over 12 hours. A single cricket was housed in the cage with a source of water and food. B) Example of a unique identifier. C) Sample output from a 12-hour recording period. Each different colored line was an individual cricket. Notice that on the sample day when this was recorded (6 days post-infection) the uninfected called more often and with greater frequency than exposed crickets (they did not yet know their infection status). D) Example of how the researchers checked the infection status, the nematomorph is emerging from the posterior end of the cricket. Source of images: M. Wise de Valdez, 2019. License: CC BY-NC-SA 4.0.

routes; some parasites secrete peptides that influence neural function, others can either directly or indirectly alter concentrations of hormones or neurotransmitters of their hosts (Poulin, 2010). A more recent area of study, proteomics, involves seeing which proteins may be manipulated by parasites and the downstream effect of those proteins on behavior (Lefèvre et al., 2009). It has also been suggested that perhaps parasites may alter the expression of host genes in a way that results in a behavioral change but this has yet to be studied (Poulin, 2010). For a more thorough discussion and concrete examples of research on how parasites manipulate behavior check out reviews by Thomas et al. (2005; 2010); Lefèvre et al. (2009); Poulin (2010); and Adamo (2012).

Summary

Review: **Learning objectives 1, 2, and 5:** Apply the scientific method to address questions about parasite manipulation of host behaviors. Analyze examples in the scientific literature to learn how scientists have experimentally addressed questions about parasite manipulation of host behaviors. Understand the types of host behaviors likely to be altered in relation to the parasites' life cycles. The details of 4 experimental studies were described where the researchers first asked questions, formulated hypotheses, tested them, gathered and analyzed data, and interpreted the results to either support or reject their hypotheses. Each study highlighted a specific mode of transmission and the behavioral

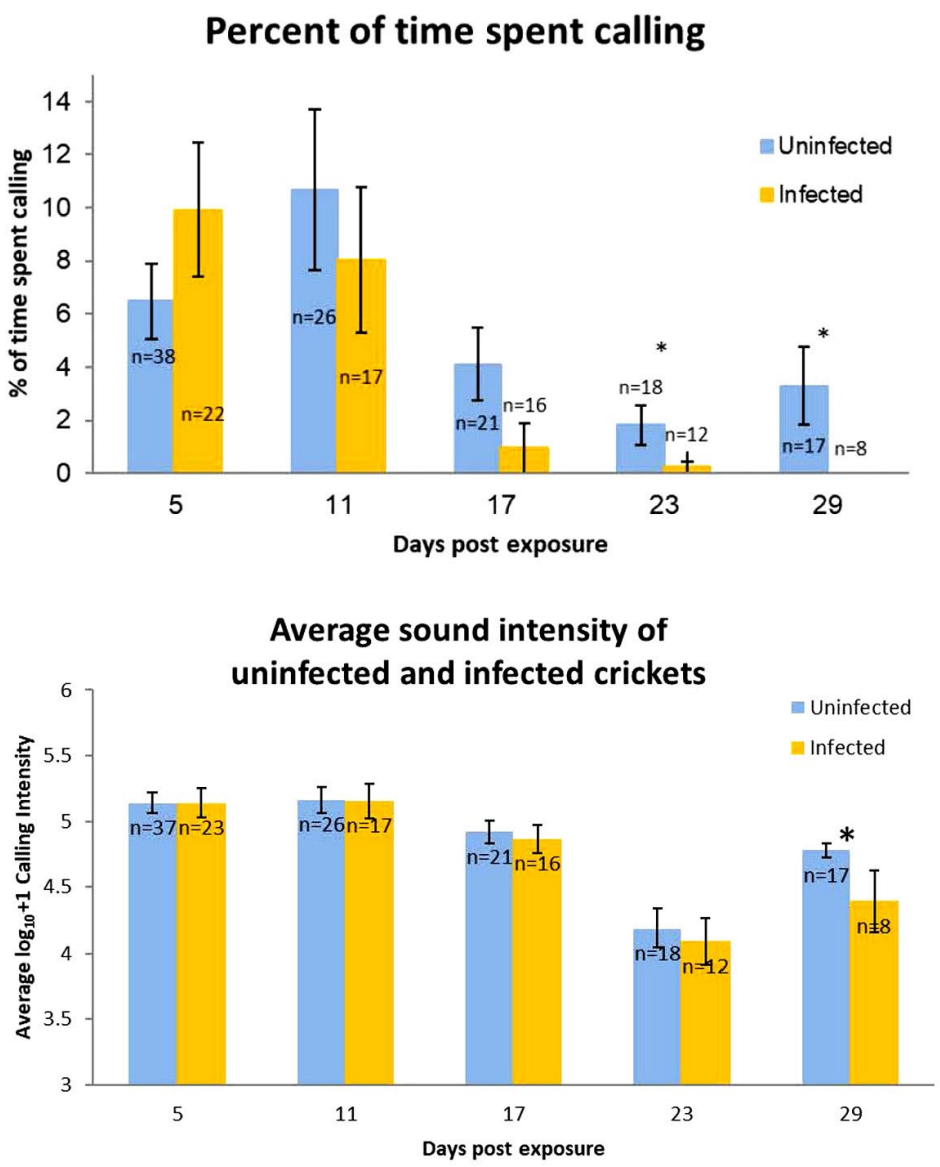


Figure 12. Time spent calling and calling intensity of male *Acheta domesticus* crickets infected with *Paragordius varius*. Source: Adapted from Barquin et al., 2015. License: CC BY-NC-SA 4.0.

manipulations we expected to see based on those transmission modes: Trophic transmission, vector-borne transmission, transmission to a new habitat, and remaining in a host for development. **Learning objective 3**: Be able to provide some classic examples of parasite manipulation of host behaviors. There are 3 primary groups of parasites that always seem to be cited in the literature for behavioral parasitology: Nematomorphs, mermithid nematodes, and acanthocephalans (with a few trematodes and protozoans thrown in). **Learning objective 4:** Understand the evolutionary principles of parasite manipulation of host behaviors. An adaptation is any character that increases the fitness of an individual. In order for parasite-induced behavioral changes to be an adaptation they must increase the fitness of the parasite by increasing its survival so it can reproduce, increase its reproductive/transmission output, or increase its chance to make it to the next host or habitat in order to complete its life cycle. **Learning objective 6:** Think critically about host-parasite relationships yet to be investigated from a behavioral standpoint. Throughout the section, call out boxes urged you to stop and think. These were meant to be a pause in the reading so that you could assess whether what was being conveyed could be applied to a new scenario.

Advanced Questions

Indeed, the questions addressed throughout this section are only a few of the questions one can ask about this interesting relationship between parasites and their hosts. See also the following papers to investigate a few more relevant questions. In Poulin (2010), Moore (2002; 2013), Libersat et al. (2018), Poulin and Maure (2015), Lefèvre et al. (2009), Thomas et al. (2010), Hughes et al. (2012), numerous questions are asked, such as:

- Are some taxonomic groups of parasites more likely manipulate host behavior than others?
- Why do some parasites alter behaviors and others do not?
- How effective is host manipulation?
- What behavioral changes might occur in hosts with more than 1 species of parasite?
- What other parasite-induced behavioral alterations that may benefit the host?
- How do hosts alter their behavior in order to compensate for their eventual sexual demise?
- What role might parasites that manipulate host behavior play on the ecology of the habitat in which they are found?
- What are the evolutionary mechanisms by which parasites evolve behavioral manipulation?
- What research is being conducted to determine the physical mechanism of parasite-induced altered behavior?

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7

Parascript Approaches

Biostatistics for Parasitologists:

A Painless Introduction

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Chapter 7

Biostatistics for Parasitologists: A Painless Introduction

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Introduction

Students of veterinary or human epidemiology, evolutionary biologists, and ecologists alike, are often asked how heavily a particular host species (or population, or herd, etc.) is infected by parasites. Further questions arise in comparisons regarding which one is more infected, or which one is more subjected to more pathogenic pressure than the others. After carefully reading this chapter, you won't be able to answer such questions–simply because such questions make no sense.

The occurrence of parasites within the host population, just like the harm exerted by them, is a complex pattern that cannot be described by a single statistical measure. Different indices capture different aspects of infection. Statistical indices have to be chosen that have clear (easy to understand) and distinct (non-overlapping) biological interpretations, and appropriate statistical tests must be chosen that are not based on assumptions that are not fulfilled in host-parasite systems. Unfortunately, some of the most widespread indices have vague if any biological interpretation, or they merely statistically predict each other, causing a redundancy of information.

Further, when applying appropriate indices to describe infection, it is a common situation that one index is higher in the host population A, the other index of infection is higher in population B, and so on. Even if all indices appear to be higher in one population than the other, we can never exclude the possibility that further meaningful indices can be proposed. A definite answer like "sample A is more infected than B" arises only in some rare and self-evident–and frankly not really interesting scientifically—cases when parasites are totally absent from the latter.

The aim of the present chapter is to advise readers how to choose appropriate statistical indices, and then, to choose the appropriate statistical tests to handle them. Finally, we offer a free statistical toolset to carry out the recommended statistical procedures in a relatively painless manner. The text below is based closely on a review paper by the authors of this chapter (Reiczigel et al., 2019a).

Taking Samples

Constrained by time, and financial and ethical limitations, investigators usually cannot collect and analyze every individual of a **host-parasite system**. Rather they take a random sample from the whole, with the hope that the sample will represent the unknown totality with reasonable accuracy. Of course, the larger the sample, the better accuracy we get. When taking a sample of a host-parasite system, typically, host individuals serve as ordinary units of sampling. First, a sample of host individuals is collected to represent the host population and, second, their bodies are searched for parasites. It is usually presumed that all parasites harbored by a particular host individual are found and identified, which may not be true.

Thus, we collect groups of parasite individuals inhabiting the same host individual, so-called **parasite infrapopulations** (Bush et al., 1997). Statistically speaking, **random sampling** of hosts implies **cluster sampling** of parasites. The size of these infrapopulations is most often expressed as the number of parasite individuals, thus we limit the discussion here to this particular situation.

Frequency Distribution of Host Individuals across Infection Classes

For sake of simplicity, first we focus our interest on the occurrence of a single species of parasite within a sample of

hosts. After collecting a sample, all **conspecific** parasite individuals need to be identified and counted from each host. Then host individuals are characterized by the number of parasites they harbor, then they can be grouped into so-called **infection classes**, such as the group of non-infected hosts, the next group of hosts each harboring 1 parasite, the next group of those harboring 2 parasites, etc. Alternatively, wider categories are often applied, such as 0, 1–10, 11–20, etc. It is a common practice to replace the number of host individuals by the proportion (%) or probability (0–1 scale) that host individuals belong to a particular infection class. Such frequency distributions are visualized as **histograms**, and often used to characterize host-parasite systems.

Host-parasite **frequency distributions** do not approximate a normal distribution (a symmetric bell curve) nor a uniform distribution. Rather the distribution of parasites always exhibits an **aggregated** (also known as **right-skewed**, or **positively-skewed**) **distribution**: The majority of hosts harbor 0, or just a very few, parasites, a few hosts harbor more, and only a very few hosts harbor many more of them (see Figure 1; Crofton, 1971). The experienced frequency distributions, as visualized by histograms, can be approximated by mathematical models. In the case of natural infections by macroparasites, the so-called **negative binomial distribution model** often provides a good approximation.

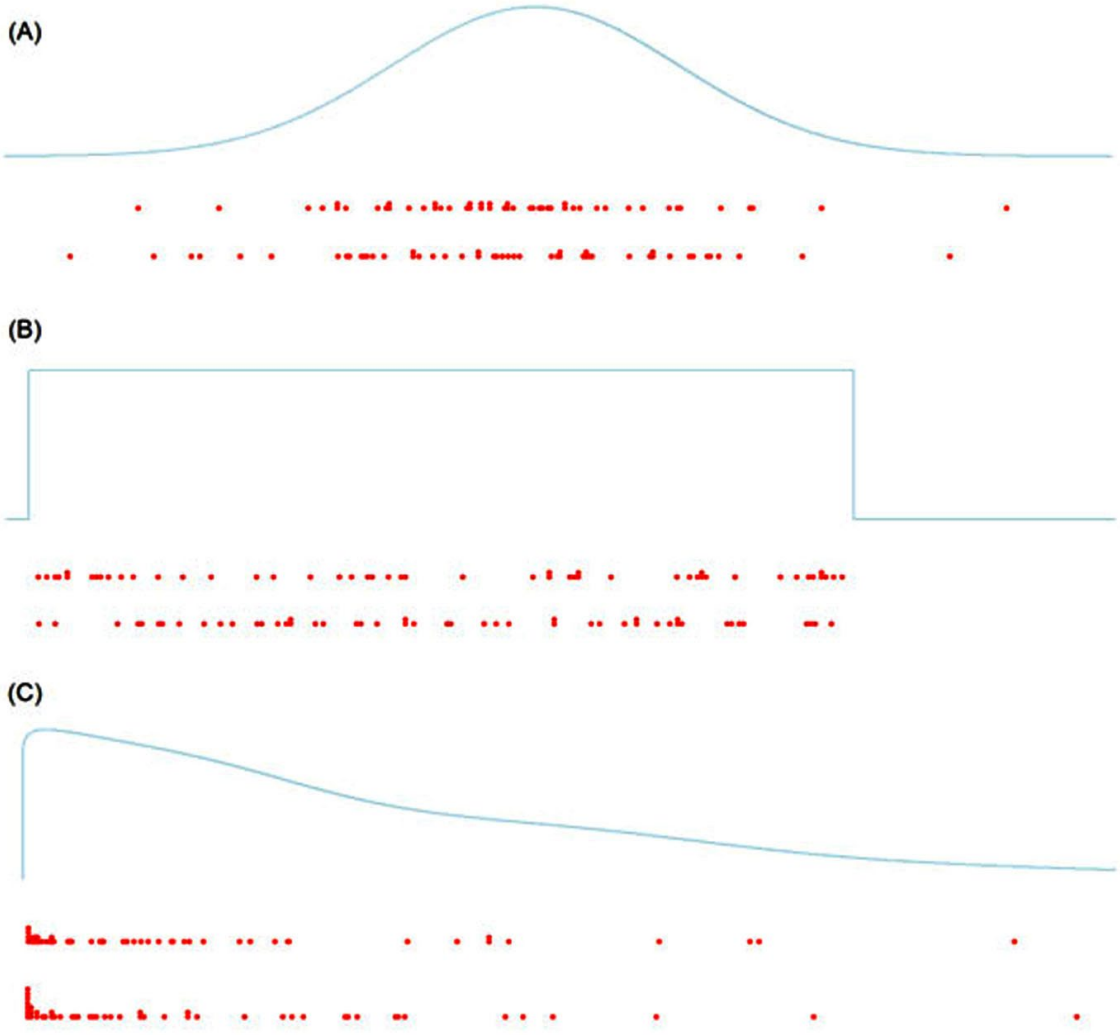

Figure 1. Density function (light blue) and dot plots of samples (n = 50) taken from different distributions. A) **Normal distribution**, where the mean is the most frequent value and the exceedingly smaller or greater values are exceedingly rare. B) **Uniform distribution**, where all values in a certain interval are equally likely. C) **Aggregated (or right-skewed) distribution**, where low values are frequent but high values are rare. Hosts grouped into parasite infection classes typically exhibit this type of distribution. Source: J. Reiczigel, M. Marozzi, F. Ibolya, and L. Rózsa. License: CC BY-NC-SA 4.0.

Sample Size

Providing information on **sample size** is essential partly because it affects the accuracy of the sample estimates, and partly because low sample sizes tend to bias some of the estimated indices of infestation/infection (Reiczigel and Rózsa, 2017). Since hosts usually act as natural sampling units, authors typically express sample size as the number of host individuals. However, in certain cases (see below), the number of parasites collected/examined may remain totally unknown–a shortcoming that should be carefully avoided.

Prevalence

Prevalence (also called extensity in the early literature) is the proportion of infected individuals, traditionally expressed as a percentage (0–100% range) or as a probability (the probability that a randomly chosen individual is infected, 0–1 range). **Sample prevalence** is an estimate of the unknown true population prevalence and, thus, its 95% confidence interval (CI) must be calculated to express its precision or uncertainty: The wider the CI, the lower the precision of the estimate (or the higher the uncertainty).

There are several methods that can be used to calculate a CI for a proportion. It is traditional to apply the Clopper and Pearson's (1934) method. Alternatively, Sterne's (1954) method and Blaker's (2000) method provide narrower, and thus more informative, interval estimates (see Reiczigel, 2003 for a comparison of their efficacy).

In epidemiology, the proportion of host individuals developing new infections within a specified period is called **incidence** or **cumulative incidence**. If calculated for a year (or month, week, etc.) it is called **incidence rate** or **incidence density**. The incidence expresses the risk of developing new infection in a certain time period. From a statistical point of view, incidence is handled similarly to prevalence, often modeled by the Poisson distribution.

Naturally, studies based on methods that can only differentiate the infected versus uninfected status of examined hosts (like serological methods) will report only sample size and prevalence (sample prevalence and its CI) to quantify results.

Mean Intensity

Intensity is the number of parasites found in an infected host. **Sample mean intensity** is the mean number of these values calculated for a sample, with all the 0 values of uninfected hosts excluded. Given the typical aggregated nature of parasite distributions, this value does not characterize a typical (say, characteristic, or usual) level of infection, rather it is highly dependent on the presence or absence of 1 or a very few highly infected host individuals. However, provided that sample size and prevalence are already known, mean intensity exactly defines the total number of parasites found in the sample. It is advisable to provide its 95% CI enabling readers to extrapolate it as an estimation of true **population mean intensity**. This CI is calculated by means of the bias-corrected and accelerated (BCa) bootstrap method of Efron and Tibshirani (1993).

Do not apply the scheme 'mean ± SD,' because it is meaningful only for symmetrical distributions, but not for the aggregated ones so characteristic to parasites. Thus, nonsense expressions like 'mean intensity = 10 ± 20' (erroneously suggesting that mean intensity can have negative values) are also avoided.

Before the era of computer-intensive methods, investigators often log-transformed raw values in order to normalize the data set. Then they calculated the mean of these transformed data, and statistically compared these means by parametric tests (like Student's t test or ANOVA) applied on the log-scale, and finally back-transformed the mean and obtained the 'geometric mean.' However, log-transformed parasite distributions very poorly approximate the normal distribution model, and the resulting index, the 'geometric mean' of intensity is hard to interpret biologically. Given that computer-intensive methods like bootstrap have opened new avenues of statistical analyses, using geometric means should now be abandoned.

Median Intensity

Median intensity, unlike mean intensity, is not strongly affected by the values of the very few highly infected host individuals, thus it is more suitable to provide information about a typical (characteristic, usual) level of infection. Thus, while mean intensity (combining host sample size and prevalence) defines the number of parasites collected, median intensity informs about a characteristic state of infected hosts (of course, with the uninfected hosts excluded).

A 95% CI of median intensity is useful to express the accuracy of estimating population median intensity. For this purpose, the method introduced by Arnold et al. (2008) is followed. Due to the discreteness of data, it is often impossible to construct exact 95% confidence limits, thus, the shortest interval that reaches at least the desired confidence level is reported instead.

The most common method for the comparison of 2 medians is the non-parametric Wilcoxon-Mann-Whitney U-test (WMW). However, it should be noted that, without imposing some rather restrictive assumptions on the population distributions, WMW does not compare medians (there are examples where the sample medians are exactly equal and WMW detects a significant difference between the samples). One such assumption is that the frequency distributions to be

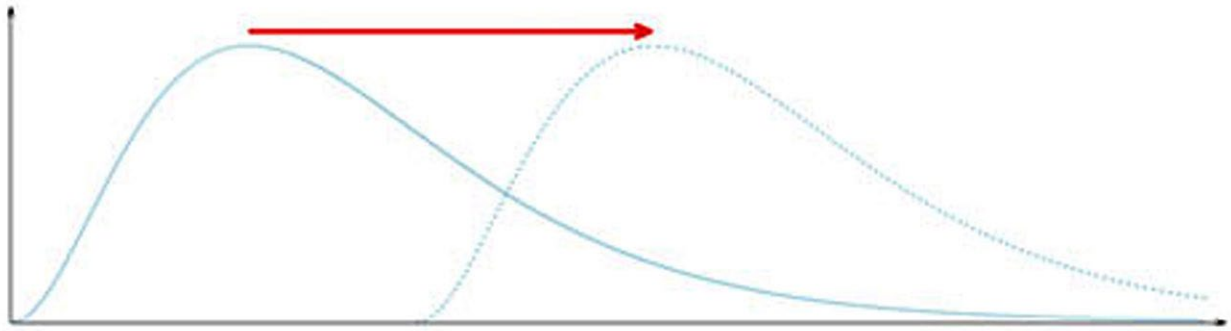

Figure 2. The classical assumption of the Wilcoxon-Mann-Whitney U-test is that the distributions to be compared have same shapes (and therefore same variances) but may be shifted along the horizontal axis (above). Unfortunately, real host-parasite systems do not fulfill this assumption, thus results of the WMW test are difficult to interpret. Source: J. Reiczigel, M. Marozzi, F. Ibolya, and L. Rózsa. License: CC BY-NC-SA 4.0.

compared have the same shape, the only difference between them is a shift along the horizontal axis (see Figure 2). There are other assumptions, but all of them are similarly restrictive, and most parasite distributions do not seem to fulfill them. If none of these assumptions hold, the result of the WMW test can be misleading (Divine et al., 2018). If the test detects a significant difference, the most one can say is that the distributions (rather than the means or medians) differ. Therefore, if differences between medians are of interest, the best choice is Mood's Median Test (Sen, 1998).

Stochastic Equality of Intensities or Abundances

The bootstrap test for **stochastic equality of distributions** (Reiczigel et al., 2005a) is a variant of the WMW test. It compares pairs of values taken from the 2 samples and tests whether the probability of getting higher values from one sample than from the other is same (50%–50%) or different. If using this method, the question regards only *how often* a value taken from one sample is higher than that from the other sample, but not *how much higher*. Therefore, if this test shows that infections in one sample tend to exceed those in the other, it does not necessarily mean that the latter sample hosts fewer parasites.

Abundance

Abundance is defined and treated similarly to intensity, but the 0 values of non-infected host individuals are not excluded. Due to the inclusion of the infection class 0 (non-infected hosts), the frequency distribution of abundance classes is more aggregated and, thus, their analysis is less accurate than that of the intensity classes, resulting in wider CIs and weaker statistical tests (greater *p*-values). Therefore, it is preferable to calculate intensity rather than abundance, and to avoid confusion, it is best to not provide both indices. Presuming that sample size (*N* hosts) and prevalence are provided, readers already have all the information about the non-infected hosts, thus, the further inclusion of these calculations in quantitative descriptions is redundant. The relationship between mean abundance, mean intensity, and prevalence can be described by a simple formula, enabling calculation of any 1 of them when knowing the other 2 of them:

mean abundance = prevalence * mean intensity

Median abundance is a less informative measure, in particular, because, by definition, it equals 0 whenever prevalence is less than 50%, irrespective of the actual prevalence and the intensity values of infected hosts.

Overall, abundance measures (mean and median, their CIs) combine information on prevalence and intensity. Apply them only if such a combined index is definitely needed.

Crowding

Crowding is the size of the infrapopulation to which an individual parasite belongs (Reiczigel et al., 2005b). Although this equals intensity, intensity is defined as a host character, while crowding is a character of the parasite individual. Therefore, mean intensity refers to the intensity values averaged over host individuals, but mean crowding is obtained by averaging the crowding (= intensity) values over the parasite individuals. Say, mean intensity for 3 individuals infected by 1, 2, and 6 parasites is (1 + 2 + 6) / 3 = 3, while mean crowding for the parasites in the same sample is (1 + 2 + 2 + 6 + 6 + 6 + 6 + 6 + 6) / 9 = 4.56. Note that, due to the aggregated shape of distributions, an 'average' individual lives in a host that is more 'crowded' by conspecific parasites than the mean number of parasites per hosts (here: 4.56 > 3). Mean crowding is a rarely used index; however, it is a potentially meaningful measure of infection when speaking about

Box 1. Money Flows Like Parasites

Since counting money is much closer to our everyday experience than counting parasites, here is a surprising parallelism between them.

Most people possess little if any money, while a very few people are extremely rich. Thus, money, just like parasites, exhibits an aggregated distribution across human (analogous to host) individuals. The value of average richness is affected differently by different individual changes. It is very sensitive to the presence or absence of a single very rich person, but much less sensitive to the presence or absence of a single poor person. Similarly, **mean intensity** (or **mean abundance**) of infection is sensitive to the presence or absence of one or few highly infected individuals. Therefore, mean values do not reliably characterize the wealth of "average people;" likewise, neither the infection of a "typical" host individual.

There are similar causes responsible for the rise of aggregated distributions both in monetary and epidemiological systems. First, money (just like parasites) tends to move from one person to another in groups, such as sums of money, similar to multiple infections by more than one propagule at the same time. Second, some people are inherently good at earning and accumulating money, while others consistently spend all the money they happen to have–just like individual differences between susceptible and resistant individual hosts. Finally, money can multiply itself if hosted by a careful person; this is termed interest on capital. Similarly, most parasites can multiply themselves within the body of a susceptible host.

For such reasons, money behaves very much like parasites, at least from a statistical point of view.

density-dependent parasite characters (such as body size, fecundity, or sex ratio) in relation to the putative social environment of parasites.

Due to the usual sampling, that is, sampling the hosts, there are dependencies between the crowding values of parasite individuals: All of the conspecific parasites infecting the same host have identical values and, therefore, all of these values change simultaneously whenever a parasite is added or removed. This makes crowding values notoriously hard to handle statistically. As random sampling from the parasite population is practically infeasible, statistical methods assuming independence of the sample values—practically all classical methods, that is—cannot be validly used for the analysis of crowding.

A CI (confidence interval) for **mean crowding** can be created by the BCa bootstrap method as demonstrated by Efron and Tibshirani (1993). A 95% CI is useful to characterize the accuracy of sample mean crowding as an estimate of the true population value. Statistical comparisons of mean crowding across 2 (or more) different samples are also based on CIs. First, 97.5% CIs are generated for both samples. If these intervals overlap, the difference between the 2 samples is non-significant at the prescribed level of 0.05, that is, $p > 0.05$ (Reiczigel et al., 2005b). Unfortunately, the power of this testing method is rather low. Therefore, Neuhäuser et al. (2010) proposed applying Lepage's (1971) location-scale test as a more suitable alternative.

From a purely mathematical point of view, diversity and crowding are closely related notions; one can be transformed into the other (Lang et al., 2017).

Levels of Aggregation

While all natural, and most experimental parasite infections exhibit an aggregated frequency distribution across host individuals, the level of aggregation may differ considerably from sample to sample. The most frequent indices to quantify these levels are, 1) The variance-to-mean ratio of abundance, 2) the exponent k of the negative binomial model fitted to the data (presuming acceptable fit of the model), and 3) Poulin's (1993) 'index of discrepancy,' which includes a modified version of the so-called Gini-coefficient (a well-known index in the literature of economics).

Although these indices aim to quantify the same feature (level of aggregation) of frequency distributions,

unfortunately, their values do not exactly predict each other, thus, they cannot be transformed into each other and the are not interchangeable.

Just like in the case of mean crowding, these indices can be compared across samples by testing the potential overlap between their 97.5% CIs.

Parasite Sex Ratio

Samples of dioecious parasites can be characterized by their **sex ratios**. Note that the term *sex ratio* is quite misleading. Mathematically speaking, a 'ratio' should be expressed as the frequency of 1 sex divided by the frequency of the other sex. However, the index males/females would be unfavorable to apply; for example, it cannot be calculated for samples without females (since one cannot divide a number by 0). Instead, it is traditional to apply the proportion of males among adult dioecious parasites as a measure of sex ratio. Thus, the index called *sex ratio* actually means ***male-proportion***. As it is a proportion, the recommended statistical tests are identical to those of prevalence.

Parasite Species Richness

Species richness is a simple and frequently used index to quantify diversity. Unfortunately, small samples tend to underestimate the true parasite species richness in populations of animals. General advice about the required sample size cannot be given because it depends on many other factors such as the levels of aggregation, interactions between parasite species, etc. There are several methods that have been designed to extrapolate sample values to the true parasite species richness harbored by the whole host population, so as to correct for this sample size bias. Walther and Morand (1998) compared the reliability of several methods using real parasitological datasets and found that the first-order jackknife (Heltshe and Forrester, 1983) and the Chao2 estimators performed best (Chao, 1987; Chao and Chiu, 2016). This latter method estimates the number of unobserved parasite species from the number of rare species (those occurring only in 1 or 2 hosts in the sample). Thus, the estimation fails in the absence of rare species in the sample, but it performs well if the number of rare species is < 50% of all parasite species in the dataset. It is also advised that a large sample of hosts is needed to obtain a reliable estimate, a sample size of at least a few hundred host individuals is recommended, but of course this depends on the estimated size of the population under study.

Interactions Between Parasite Species

Two parasite species coexisting in the same host population may exhibit a positive or negative interaction, making their co-occurrence in a particular host individual more or less likely than expected by chance. The simplest method to analyze such interactions is to summarize the presence or absence of the 2 species on each host in a 2 × 2 contingency table and apply Fisher's Exact Test to analyze it. The sensitivity of this method, unfortunately, may be rather poor because the difference between hosting 0 or 1 parasite individuals is often negligible. Therefore, computing the Spearman Rank Correlation coefficient to explore potential interactions between abundance values of the 2 parasite species is recommended as it provides a more robust or sensitive estimate.

Quantitative Parasitology on the Web (QPweb)

Misuse of biostatistics and misinterpretation of statistical results are very common in the parasitological literature. Therefore, we have published a brief overview of the suitable biostatistical tools together with some new methods proposed by us (Rózsa et al., 2000) to address these important issues. The Rózsa et al. (2000) paper was accompanied by freely distributed software called Quantitative Parasitology (QP) to make the recommended statistical procedures easily accessible. Subsequent software versions QP1.0, QP2.0, and QP3.0 followed with increasing numbers of new functions. These were made available as downloadable software that ran on Windows PCs. Each was capable of handling only 1 type of parasite per host sample, thus, multispecies infections or sex ratios could not be analyzed. Finally, we introduced Quantitative Parasitology on the Web (QPweb) in 2013, which is an R-based interactive web service capable of communicating with computers via an internet browser, independently of the operating system used. Contrary to former versions, this one is already capable of representing different types of parasites (different species, different sexes, and so on) co-occurring in the same host sample, opening new possibilities for analyzing parasite communities.

Parallel to the introduction of subsequent software versions, we also published new biostatistical procedures potentially useful in characterizing the infection level of a sample or comparing infection indices across samples of hosts (Reiczigel, 2003; Reiczigel et al., 2005a; 2005b; 2008). All these new procedures were incorporated into the newer software versions. The latest version of QPweb (v1.0.15, as of 2020, and still in 2024) is freely available on the web (Reiczigel et al., 2019b; available at https://www2.univet.hu/qpweb/qp10/index.php) to carry out most of the procedures mentioned above, including a simple users' guide to help work through potential technical difficulties (Figure 3).

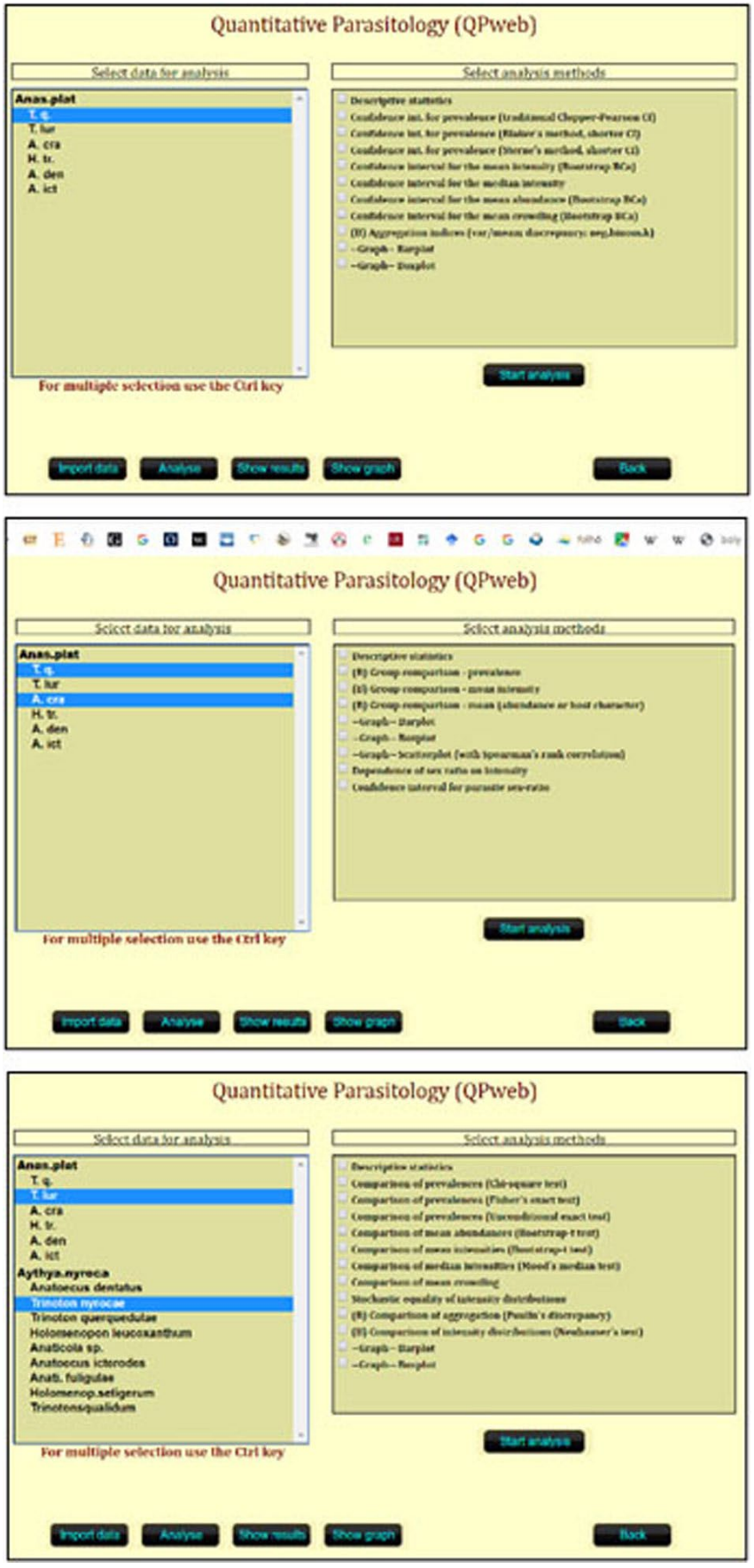

Figure 3. Analysis tools offered by QPweb when choosing different combinations of samples. Top: One species of parasite in 1 sample of host. Middle: Two species of parasites in 1 sample of host. Bottom: Two species of parasites in 2 samples of hosts. Source: J. Reiczigel, M. Marozzi, F. Ibolya, and L. Rózsa. License: CC BY-NC-SA 4.0.

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8

Parascript Approaches

Distributional Ecology of Parasites

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Chapter 8

Distributional Ecology of Parasites

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Introduction

Organisms in general experience an array of factors in shaping their geographic distributions. These factors are studied in the field that is coming to be called **distributional ecology** and range from spatial to environmental and historical to current; as such, the complexity of the situation is quite impressive. The field of distributional ecology is simultaneously pretty old (Grinnell, 1914; 1917a; 1917b) and quite new and novel (Soberón and Nakamura, 2009; Peterson et al., 2011)—distributional ecology centers around the question of why populations of a species are where they are, and why they are not where they are not. These ideas became popular with the development of large-scale and openly accessible data resources (Peterson et al., 2016), and of sophisticated computational algorithms for relating known occurrences of species to raster (that is, grid-based) GIS datasets to discover dimensions ostensibly of the fundamental **ecological niche** (Escobar and Craft, 2016). This old-and-new field has now seen intensive research attention from across the fields of ecology, biogeography, and systematics, and even fields as far afield as public health, invasion biology, and agricultural planning (for example, Mainali et al., 2015; Reddy and Nyári, 2015; Samy et al., 2016; Ramírez-Gil et al., 2019).

Parasites, of course, present several additional levels and dimensions of complexity for distributional ecology. The distributions of many free-living organisms (for example, plants, birds, fish) were hypothesized originally by Grinnell (1917b) to be shaped primarily by **abiotic** factors (for example, temperature, soil pH, precipitation; the important point is that these factors are unaffected by the presence of the species in question). However, parasites often have additional constraints. In particular, Hutchinson (1957) outlined a more complex and comprehensive niche theory that included both abiotic and **biotic** dimensions—these latter biotic dimensions may or may not be important in shaping geographic-scale distributions of species (Anderson, 2017). As a consequence, Peterson et al. (2011) proposed the **Eltonian Noise Hypothesis**, the proposition that biotic interactions do not frequently constrain *geographic-scale* distributions of species (Peterson et al., 2011). This hypothesis—to the extent that it holds true—allows researchers to focus on ecological niches in terms of abiotic factors solely (Peterson et al., 2011). Of course, parasite distributions may be much more complicated in that biotic interactions are at times absolute: Some parasites may be incapable of surviving without specific host species being present. In sum, careful thinking about the distributional ecology of parasites will involve more complexity than is required for free-living organisms (Peterson, 2008; 2014; Escobar and Craft, 2016).

This chapter will provide a review of conceptual bases for distributional ecology. However, distributional ecology is a broad area of inquiry, such that a full and exhaustive review of the field would be too lengthy. As such, in this chapter, the focus is on what is presently perhaps the most popular methodology—that of **correlative ecological niche modeling**—in the parasitology literature over the past couple of decades. Still, without a doubt, other approaches and ideas should also be brought to bear on these questions, as insights based on multiple, complementary sets of analyses from distinct perspectives will generally be more robust and more likely to prove true in the long run.

Conceptual Framework

Early thinking about parasite distributional ecology was laid out by Pavlovsky (1966), who posited that foci ('nidi') of pathogen **transmission** are driven by interactions among various components of ecosystems. However, a genuinely synthetic understanding is still lacking (it is also lacking more generally for free-living, non-parasitic organisms, by the way!). That is to say that, yes, several concepts are well-known: The fundamental ecological niche, which represents an upscaling of organismal environmental physiology, and relates the persistence or fitness of a population or set of populations to a particular set of environmental conditions (Peterson et al., 2011). The fundamental ecological niche can be modified by **biotic interactions** to yield the realized ecological niche (Hutchinson, 1957); most treatments have assumed that these interactions are *negative* (for example, competition, parasitism, predation), but *positive* interactions can also exist. These various niches translate into the **geographic distribution** of the population or species, but in non-specific and non-linear ways, thanks to the complexities of the relationships between geographic and environmental spaces, which has been termed the **Hutchinsonian Duality** (Colwell and Rangel, 2009).

Early contributions in distributional ecology included the concept of an ecological niche that is defined in terms of physical characteristics of the environment (Grinnell, 1917a; 1917b), which has been termed the **Grinnellian niche**, and is roughly equivalent to a fundamental ecological niche defined only in abiotic (non-interactive) dimensions. Later came the idea of the niche being defined in multidimensional spaces and the contrasting ideas of fundamental and realized niches (Hutchinson, 1957). Perhaps least famous but most important is the idea of the existing niche as the subset of the fundamental niche that is manifested on regions that have been accessible to the species (known in previous literature as potential niche; Pulliam, 2000). Although different terminologies do exist (Sillero, 2011), the focus here is on what is probably the most comprehensive theoretical framework in distributional ecology as regards ecological niches of species (Soberón and Peterson, 2005; Soberón and Nakamura, 2009; Peterson et al., 2011).

Grinnell (1917b) developed his niche ideas in terms of tolerances with respect to physical characteristics of the environment, so these environmental dimensions are now called **Grinnellian environmental variables** (Tingley et al., 2009). In modern terminology, those physical characteristics are termed **non-interactive variables**, as they are independent of the presence of the species in question: The presence or absence or high or low abundance of the species in question does not affect Grinnellian variables, such as annual mean temperature (Peterson et al., 2011). Hutchinson (1957) introduced the idea of biotic interactions as a modifying factor in distributional ecology—these biotic factors (for example, presence of prey or a host, absence of a predator, absence of a pathogen) are now known as **interactive variables** (Peterson et al., 2011), and are those that are affected by the presence of the species in question, as direct feedbacks exist between abundance of the species of interest and these variables—for example, prey density.

The environments manifested across the suite of geographic sites that are within the species' fundamental ecological niche are referred to as the **existing niche**, which is the set of conditions that the species has explored and tested, and where the species could potentially establish populations. Given the challenges of understanding where a species could potentially maintain populations, compared to where it actually is present, Soberón and Peterson (2005) emphasized the idea that geographic distributions are limited not just by niche considerations, but also by dispersal ability and access, such that they proposed the so-called **BAM framework**. According to the **BAM** framework, the *occupied geographic distribution* of a species represents the 3-way intersection of the areas suitable with respect to interactive variables (**B** for **biotic**), areas suitable with respect to non-interactive variables (**A** for **abiotic**), and areas accessible to the species over relevant periods of time (**M** for **mobility**).

Species, however, are distributed simultaneously in 2 linked spaces: The **BAM** diagram is cast in *geographic dimensions*, whereas niches are manifested in *environmental dimensions*. This dual-space nature of distributions of species is referred to as the **Hutchinsonian Duality** (Colwell and Rangel, 2009), which is the complex and non-linear set of connections between geographic and environmental spaces, and the idea that the species must maintain a non-null distribution in both spaces continuously and simultaneously. This concept leads to the discussion of distributions of species in environmental dimensions as different sorts of niches and distributions of those same species in geographic dimensions as geographic distributional areas. The **fundamental niche** represents that set of environmental conditions (in non-interactive dimensions) within which the species can maintain populations without immigrational subsidy. The intersection of the fundamental niche with the set of environments represented across **M** (the area accessible to the species over relevant time periods) is termed the **existing niche** (equivalent to the putative potential niche of Pulliam, 2000), and the reduction of the existing niche by the set of environments that are suitable for the species in interactive (biotic) dimensions is the **realized niche** (Peterson et al., 2011). These ideas are presented diagrammatically in Figure 1, as is the idea that the biotic influences themselves reflect **BAM**-type interactions of each interacting species.

In sum, the above is a brief, text-based summary of major concepts in distributional ecology. In effect, in hand, is a **taxonomy** of distributional areas and ecological niches, such that one can be explicit and clear in discussing and describing distributional phenomena. It is not enough to say, "I am developing a niche model" or "I am developing a distribution model" (see title of Godsoe, 2010: "I can't define the niche but I know it when I see it ..."), because the question then has to be asked as to *which niche* or *which distribution* is the object of modeling. Rather, if distributional ecology is to be a rigorous area of inquiry, explicit terminology becomes crucial; the above description is an attempt to provide such a framework for such a terminology (see Table 1 for detailed definitions of each of these concepts).

Relevant Questions in Distributional Ecology

Hutchinson's Duality indicates that the field of distributional ecology can (and indeed must) explore both geographic and environmental dimensions of distributions of species. That is, on one side, questions are feasibly addressed that have to do with geographic distributions. For example,

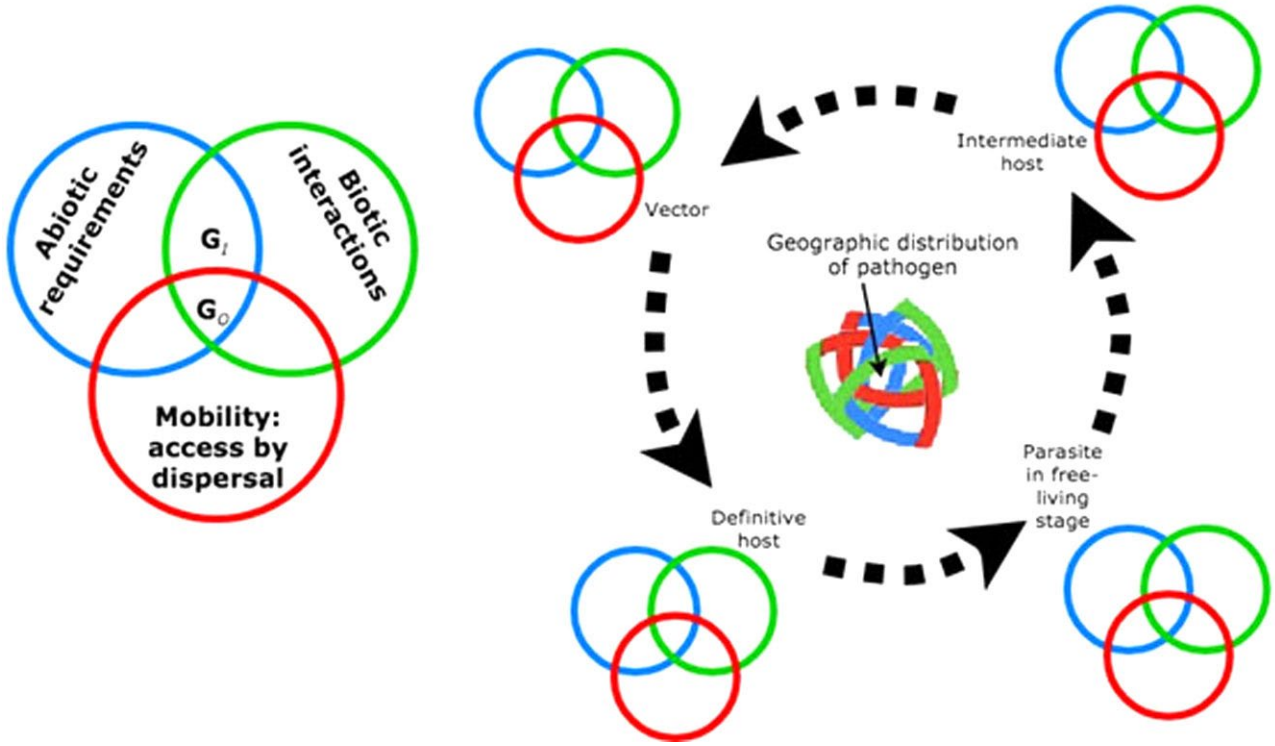

Figure 1. Summary of basic principles of distributional ecology, adapted to parasite biology. Specifically, at the left is the **BAM** diagram, a heuristic useful for conceiving of a species' geographic distribution as the geographic area that (1) fits the species' abiotic requirements (**blue** circle), (2) includes all necessary biotic conditions (**green** circle), and (3) is accessible to the species via dispersal (**red** circle). At the right is a hypothetical parasite life cycle, in which a parasite passes through a free-living stage, and subsequently infects an intermediate host, and is passed by a vector to a definitive host. Each of these steps in the cycle involves a set of interactions with abiotic and biotic environments, and access to a restricted set of areas (that is, a **BAM** intersection for each species in the parasite cycle), such that the 4-way interaction shown in the center of the life cycle would be a hypothesis of the possible geographic distribution of the parasite. Source: A. T. Peterson, 2019. License: CC BY-NC-SA 4.0.

Table 1. Summary of concepts and ideas relevant to species' geographic and environmental distributions. Note that the operator η(**X**) indicates the set of environments associated with some area **X** in geographic space.

Concept	Notation	Relationship	Concept	Notation
Fundamental niche	$\mathbf{N}_F$	$\eta(\mathbf{A}) \subseteq \mathbf{N}_F$	Abiotically (non-interactive) suitable area	**A**
Existing niche	$\mathbf{N}_F$*	$\mathbf{N}_F \cap \eta(\mathbf{M})$		
Realized niche	$\mathbf{N}_R$	$\mathbf{N}_F$*$\cap \eta(\mathbf{B})$		
		$\mathbf{A} \cap \mathbf{B} \cap \mathbf{M}$	Occupied distributional area	$\mathbf{G}_O$
		$\mathbf{A} \cap \mathbf{B}$	Potential distributional area	$\mathbf{G}_P$
		$\mathbf{G}_P$ - $\mathbf{G}_O$	Invadable distributional area	$\mathbf{G}_I$
			Biotically (interactive) suitable area	**B**
			Accessible area	**M**
			Presence sites for the species	$\mathbf{G}_+$

what is the full geographic distribution of a parasite and what host species likely remain to be discovered and documented? If closely related species tend to share the same fundamental ecological niche (Peterson et al., 1999; Peterson, 2011), then these techniques can also be used to make predictions regarding the location of undescribed species (Raxworthy et al., 2003; Peterson and Navarro-Sigüenza, 2009). Similarly, if fundamental ecological niches remain stable across time and if one has raster data layers that describe environmental conditions both at present and in the future or past, one can assess or anticipate future or past potential distributional patterns of the species.

On the environmental side, one can feasibly explore the suites of conditions associated with the distribution of a species, interpreting those conditions as manifestations of the species' realized ecological niche. For parasites in particular, questions of realized versus existing niches emerge, as the degree to which a parasite's range is a function of its own requirements versus those of its host(s) is a critical question in distributional ecology (Maher et al., 2010). Ideally, a deep and detailed understanding of the various niches of a species (that is, realized, existing, fundamental) should permit a predictive understanding of its distribution in time and space, and in relation to other species, including parasites, vectors, hosts, and other competitor parasites. Of particular interest is the opportunity to estimate the fundamental niche, as a fundamental niche represents an evolved characteristic of a species and should be able to be transferred to diverse sets of environmental conditions to hypothesize distributional potential.

Methodology and Study Design

Ecological niche modeling requires 2 major data inputs, and a number of decisions regarding strategy and parameter values (see Figure 2 for a diagrammatic summary, and book-length methodological summaries: Franklin, 2010; Peterson et al., 2011; Peterson, 2014; Guisan et al., 2017). The first data input is that of **species occurrence data**—that is, geographic coordinate pairs that correspond to locations where the species is known to have occurred. Of course, these data need to be explored, and erroneous or inconsistent records need to be detected and removed (Chapman, 2005; Cobos et al., 2018); frequently, geographic coordinates and associated uncertainty measures and documentary metadata need to be added to the data records (Chapman and Wieczorek, 2006). Finally, the occurrence data must be inspected for areas of overly intense sampling, duplicate records, or imprecise records, to avoid introducing biases.

The other major data input is that of **environmental data**, in the form of raster GIS data layers. Most niche-modeling algorithms require that these data layers have the same grid system (that is, spatial resolution, origin, and orientation),

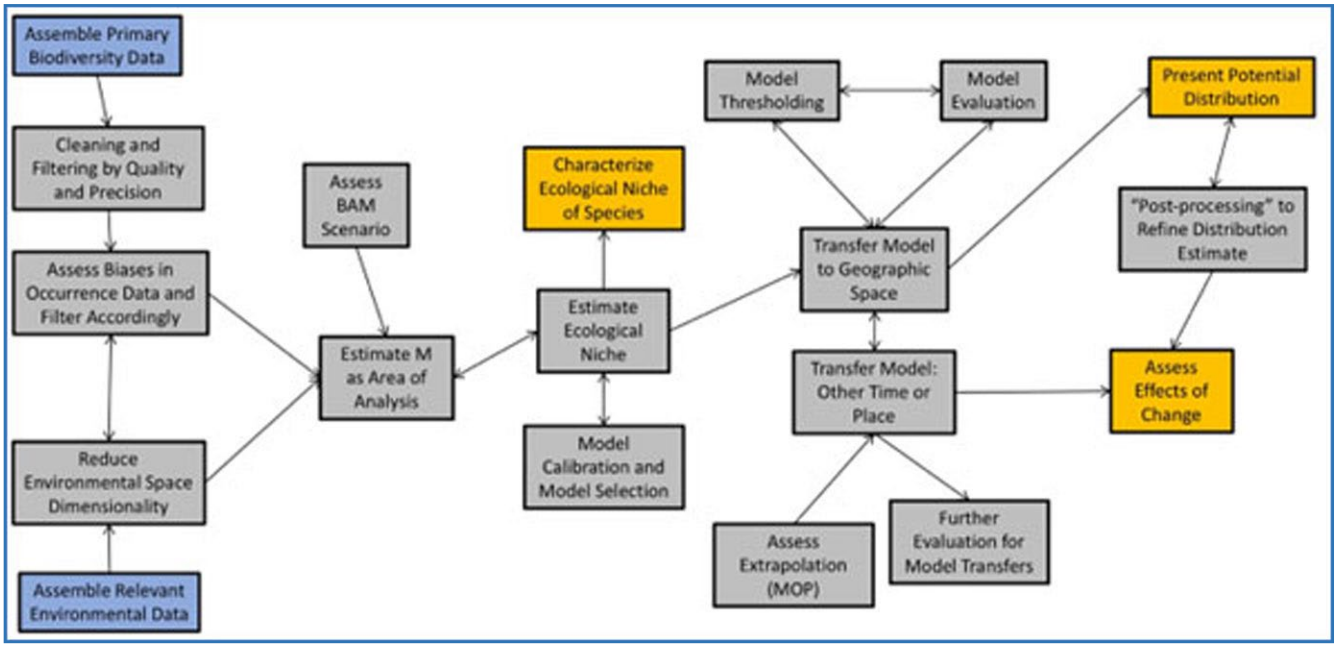

Figure 2. General summary of flow of work, inputs, and products, in ecological niche modeling. Blue boxes indicate data inputs, gray boxes are steps in the process, and gold boxes are outputs. Arrows direction denotes the flow of information. Source: A. T. Peterson, 2019. License: CC BY-NC-SA 4.0.

and indeed most studies have centered on a single climate summary (Hijmans et al., 2005), but one must think more deeply than just that. Rather, in ecological niche modeling, the modeler does not have much freedom to explore massive numbers of environmental dimensions because of problems with model overfitting in too-highly-dimensional environments (Peterson, 2007), so modelers must choose carefully the most interesting or relevant dimensions associated with the persistence of populations of a species. Of course, one approach is simply to "let the data choose," and use the niche modeling algorithm as a sort of data-mining algorithm, but generally a better approach is to assess what is known of the species' natural history, and to pick environmental data layers accordingly.

Once the data streams are identified and prepared, then the niche modeler must begin to **integrate** them. A first step is that of estimating the accessible area **M**, which ends up being the key area over which models should appropriately be calibrated (Barve et al., 2011). A further step is that of assessing or approximating the relative configuration of the **BAM** diagram for that particular species in that particular situation, because certain **BAM** configurations invariably lead to bad models that have little or no predictive power (Saupe et al., 2012; Qiao et al., 2015). A few adjustments can be made, though some situations simply are not appropriate for modeling.

Actual **niche model calibration** is accomplished by means of various algorithms (see illustrations in Figure 3). The algorithms range from the simplest, BIOCLIM, which is an approach to delineating niche estimates as orthogonal tolerance limits in different dimensions based on observed ranges of values, to complex multivariate statistical and machine-learning approaches. Each of this diversity of approaches to estimating niches has its own complexities about how it can and should be calibrated and executed (Muscarella et al., 2014; Sánchez-Tapia et al., 2017). At the end of the model calibration process, the model is generally **evaluated** via some sort of test of its ability to predict independent data sets, usually in geographic space. These tests can be threshold-dependent or threshold-independent, but all devolve into testing how well the model anticipates the independent occurrence data sets in the smallest area possible (Fielding and Bell, 1997). Once models are calibrated and evaluated, they can be **interpreted**, **or transferred** to other times or other regions.

A Worked Example

Here, as an example of the concepts described above, and a bit of an illustration of the inferences that can and cannot be derived from ecological niche modeling of parasites. The wasp *Vespula austriaca* is analyzed as an obligate parasite of its congener *V. rufa* (Taylor, 1939). Occurrence data were gathered for the 2 species from the Global Biodiversity Information Facility (February 28, 2019; queries are available at doi: 10.15468/dl.blijyg and doi: 10.15468/dl.w6spai), and reduced their coverage to western Europe, where point densities were greatest, as a proxy of areas where the species have established successful populations. Figure 3 presents visualizations of the distribution of the 2 species in geographic and environmental spaces.

A first consideration is that of how to characterize the fundamental niches of the species, and many methodological options are available. Focusing for the moment on *Vespula rufa*, the host species, one of the classic approaches to ecological niche modeling is the so-called BIOCLIM approach (Nix, 1986), which basically consists of defining tolerance limits independently in each environmental dimension, creating a multidimensional parallelepiped (Figure 4). This area nicely incorporates all (or nearly all) of the records of the species, but it also tends to include too much environmental space. More modern methods, however, such as Maxent, boosted regression trees, random forests, and general additive models, tend to be more complex in the response types that they reconstruct, which has been seen as an advantage (shown diagrammatically in Figure 4; Elith et al., 2006). However, an emerging realization is that such highly complex reconstructions of response types may not be particularly biologically realistic, as theory and experimental results from physiological studies suggest that fundamental niches should be relatively simple, and effectively convex in environmental space (Maguire, 1973). As such, a more appropriate model of a fundamental niche might enforce the simple and convex nature of these niches (see Figure 4, ellipsoid model).

A final point regards the parasite and its distribution. Several studies in the literature indicate that *Vespula austriaca* is an obligate parasite that focuses on *V. rufa* across its European distributional area. This idea is borne out by the co-distribution of the 2 species, such that no sites are apparent where *V. austriaca* exists in areas where *V. rufa* is not at least close by (Figure 3). As such, one can take the environmental distribution of the host as defining the biotically suitable area **B** for the parasite; the final panel of Figure 4 shows the environmental distributions of the 2 species together and points out some possible niche limitation of *V. austriaca* even within the bounds set by the ecological niche of *V. rufa*. Note that the niche of the parasite remains undefined on 2 sides simply because sites presenting environments in those directions are either 1) not accessible to the parasite or 2) not within the niche of the parasite's obligate host.

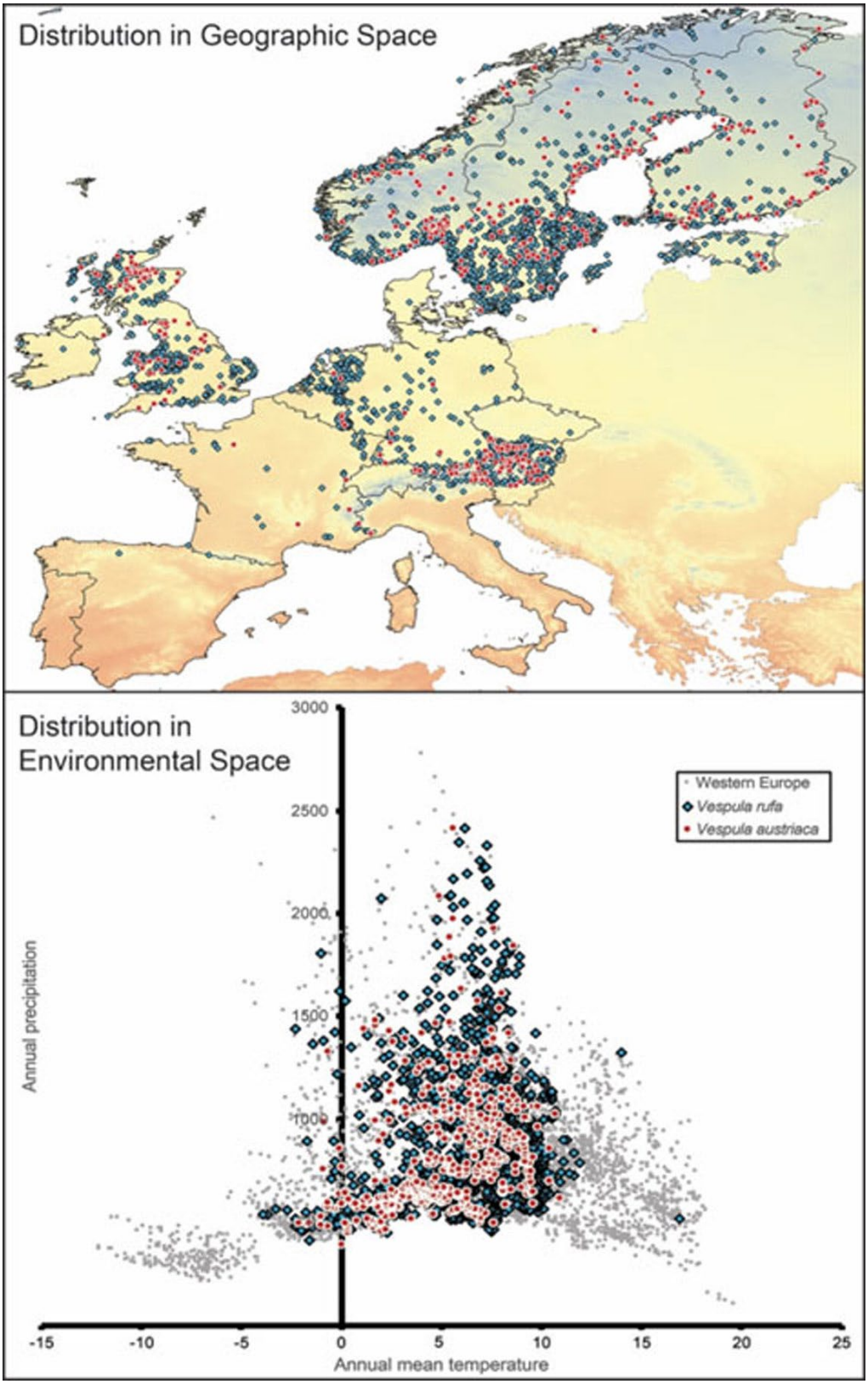

Figure 3. Summary of the distribution of one host-parasite system (*Vespula rufa* and *V. austriaca*, respectively, across western Europe, shown on top of the annual mean temperature data set (**red** = high, **blue** = low) (Hijmans et al., 2005). In the lower panel, the 2 species are shown in relation to the environments available across the region (in medium gray). Source: Adapted from Hijmans et al. (2005). License: CC BY-NC-SA 4.0.

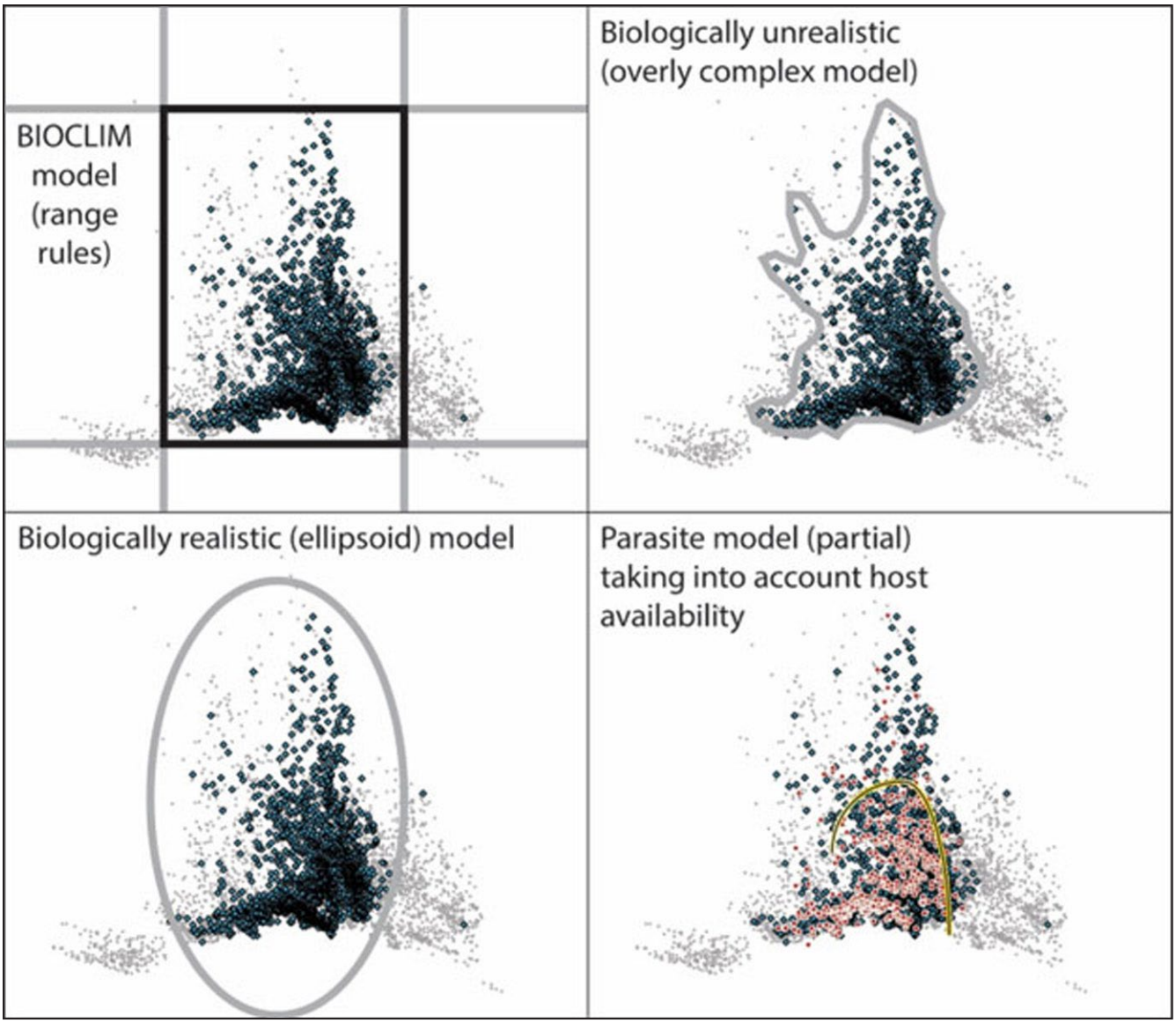

Figure 4. An illustration of methods and some key ideas in ecological niche modeling. Top panels and bottom-left panel are focused on *Vespula rufa* (the host species): Gray dots show the set of environments that is accessible to the species across western Europe, whereas the blue diamonds are the occurrences of the species. The gray and black lines show the set of environments that might be "chosen" as within the species niche under different approaches. Finally, the bottom-right panel shows the parasite (*V. austriaca*) distribution on top of that of the host and the available environments. The **yellow**-and-black line separates the distribution of the parasite (**red** points) from areas in which the host is available (blue diamonds), yet few parasite records are available (note that the great bulk of the parasite records comes from below the **yellow**-and-black curve), suggesting niche limits for the parasite, independent of the host's niche. Source: A. T. Peterson, 2019. License: CC BY-NC-SA 4.0.

Published Examples

Parasitology has a rich history of interest in distributions and environmental constraints on distributions, yet it has not seen an abundance of distributional ecology studies, in the modern, quantitative sense. Where parasites have been analyzed in greatest detail is certainly as regards pathogenic organisms, including viruses (for example, Kearney et al., 2009; Oliveira et al., 2013; Campbell et al., 2015; Escobar et al., 2015a), bacteria (for example, Eisen et al., 2006; Giles et al., 2010; Escobar et al., 2015b), simple eukaryotes (for example, Foley et al., 2008; Kulkarni et al., 2010; Gurgel-Gonçalves et al., 2012; Escobar et al., 2014; Ramsey et al., 2015), and a cutting edge papers on macroparasites (for example, Botero-Cañola et al., 2019; Botero-Cañola and Gardner,

2023; Haverkost et al., 2010; Gentry et al., 2016). However, some of pathogen-related studies mentioned above generally assess the occurrence of the disease per se, and often neglect the independent distributional potentials of the parasite and host. That is, they treat the disease transmission system as a black box that results in human, other (non-human) animal, or plant disease (Peterson, 2014). Black box models have the advantage of integrating over the entire transmission cycle of a parasite or pathogen, but have the failing of not focusing on the ecological niche of any species in particular, and of being easily biased by regional differences in sampling intensity, diagnostic capacities, or reporting frequency (Waller et al., 2007).

Distributions

Most parasite-oriented studies in distributional ecology have focused on distributional questions. That is, most studies have taken known occurrences and have attempted to predict the full geographic distribution of the disease (for example, Sehgal et al., 2010; Machado-Machado, 2012). Rarer are studies that include careful testing with independent data (for example, Escobar et al., 2015a; Botero-Cañola et al., 2019; Botero-Cañola and Gardner, 2023). Other studies include model transfers to future conditions, where distributional shifts are anticipated that will likely manifest eventually as changing disease occurrence patterns (Rödder et al., 2010; Rose and Wall, 2011; Suwannatrai et al., 2017; Alkishe et al., 2018).

Perhaps most interesting is the potential for developing fine-resolution distributional summaries for species, even across complex and poorly sampled landscapes. Here, when fine-resolution occurrence data, such as those that are derived from GPS georeferencing for recent field records, are available, they can be integrated with equally fine-resolution environmental data deriving from remote sensing. The result is a highly precise and detailed mapping of the distributional potential of the species across broad landscapes, thanks to the pairing of fine-resolution data on both occurrence and environment. Examples include applications to understanding the spatial distribution of likely avian influenza risk across Southeast and East Asia (Gilbert et al., 2007; Xiao et al., 2007; Gilbert et al., 2008; Dhingra et al., 2016) and other regions (Bodbyl-Roels et al., 2011), fine-scale predictions of triatomine distributions in Mexico (López-Cárdenas et al., 2005), and others, although exploration of the full diversity of remote-sensing data products is likely still in its infancy in distributional ecological studies.

Finally, it is worth mentioning that studies of this general sort that are specifically interpreted in the context of infection risk—that is, including additional processing beyond just modeling the niche and estimating **A** in the **BAM** diagram—are relatively rare (Ostfeld et al., 2006; Estrada-Peña et al., 2014; Ostfeld et al., 2018). The ideas central to this step (that is, risk mapping) are treated in detail in a book-length contribution (Peterson, 2014).

Niches

On the niche and environment side, this suite of techniques has perhaps seen much less application to those questions. An early contribution (Costa et al., 2014) explored ecological niche variation within a key complex of vector insects that transmit Chagas disease, but failed to distinguish between fundamental and existing niches, which wasn't well appreciated at that time. A later contribution, also focused on Chagas vectors, documented niche differentiation within the *Triatoma dimidiata* complex more rigorously (Gómez-Palacio et al., 2015), including detailed background similarity testing (Warren et al., 2008), to avoid misinterpreting existing niche differentiation as fundamental niche differentiation.

Niches and Distributions

On a more synthetic level, one suite of analyses has gone deep into the interaction between sampling and reporting of pathogen occurrences and their likely geographic distributions (Del Valle et al., 2018), with deep integration of dispersal opportunity and ecological niche, to get at transmission risk more or less rigorously (Escobar et al., 2016). Another study, focused on the plague transmission system, assembled information on human cases, animal detections of the pathogen, and the broader distributions of the host mammal species, to test whether the distribution of plague is a function of the distributions of its hosts, or rather on its own distributional potential (Maher et al., 2010). This work was echoed later in an assessment of a plant-parasite system (Lira-Noriega and Peterson, 2014). Finally, one early analysis focused on using distributional estimates from ecological niche models to predict the mammal hosts of triatomine bugs in the Protracta group of species within the genus *Triatoma*, and the predictions turned out to be quite predictive of host-parasite associations (Peterson et al., 2002). This sort of deeper, and more synthetic, application of distributional ecology tools to parasite distributions is rare, but is quite promising as regards making concrete contributions to understanding parasite distributions.

For macroparasites, early explorations managed to outline the potential of these methods and demonstrate some of the interest in their potential (Haverkost et al., 2010), and regionally focussed studies have recently been published, focusing on a *Echinococcus multilocularis,* a pathogenic cestode by Botero-Cañola et al. (2019) and a general test of latitudinal

variation in parasitism using museum collections based data (Botero-Cañola and Gardner, 2023). Meanwhile global geographic summaries of key groups have also been published (Feidas et al., 2014). Chaiyos et al. (2018) developed detailed niche models for a number of macroparasites in humans in Thailand and explored their results in both geographic and environmental spaces. Lira-Noriega et al. (2013) developed detailed analyses to assess whether biotic drivers (that is, host associations) versus Grinnellian niches drove distributions of parasitic mistletoe distributions.

Future Perspectives

Distributional ecology has progressed from a descriptive effort (for example, making a map by hand) to a quantitative effort, and the quantitative approaches have moved from shots in the dark ("look, this works!") to steps that are firmly based in ecological theory, in just a few decades. As such, the field is exciting and vibrant, and is seeing intensive research attention across many taxa and across many fields. Still, applications in parasitology have lagged somewhat, leaving many opportunities for exciting steps forward in understanding geographic and environmental distributions of many types of parasites.

Parasite applications in distributional ecology may be more complicated than most such studies, because of the frequent negation of the Eltonian Noise Hypotheses—that is, interactions with other species often *do* matter to parasites, at least in many cases. Indeed, one of the most useful testing frameworks has almost never been applied in parasitology: If one has a hypothesis about a biotic interaction, one can build ecological niche models that include and exclude that interacting species (for example, a host). One can then assess quantitatively whether the models with the interactor are better (for example, in predictive challenges, or in terms of maximum likelihood) than the models without the interactor (Atauchi et al., 2018). Such simple assessments have the potential eventually to understand some of the most fundamental elements of distributions of parasites—are their distributions governed by the niches of their hosts, or do they have meaningful niche constraints on their own?

More fundamentally, though, applications of ideas from distributional ecology to questions in parasitology must weigh very carefully the conceptual framework of the question, in order to proceed to deeper and more interesting questions. That is, a world of exciting questions abounds, such as the environmental dimensions of and constraints on the process of host-parasite co-speciation, or micro-scale versus macro-scale niche dimensions that may constrain parasite distributions at multiple scales, and how different types of niches (for example, realized or fundamental) may be broader or narrower at different spatial scales. The challenge, however, is to assemble a methodology that responds first to the conceptual foundations, and then is adapted and applied to the specific case of the parasite in question. Once such conceptual rigor is in hand, exciting distributional ecology results will emerge for parasitology.

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and
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University of Nebraska Lincoln

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Part V: ECTOPARASITES

Preface

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IMPETUS FOR PREPARING THIS BOOK

The United Nations (UN) has declared education as a basic human right. One of the UN's sustainable development goals is a call to ensure "inclusive and equitable quality education and promotion of lifelong learning opportunities for all" (United Nations, 2023; see also WOERC, 2012). Depending on the specifics of their implementation, financing, and dissemination models, open educational resources (OERs) have the potential to help in the effort to achieve equitable learning across the globe (Orr et al., 2015; Lee and Lee, 2021; see also Bali et al., 2020).

Open educational resources are "teaching, learning, and research materials in any medium that reside in the public domain or have been released under an open license that permits their free use and re-purposing by others" (Creative Commons, 2014). Wiley (2020) cites the Creative Commons' framing of OERs as providing explicit permission to "retain, re-use, revise, remix, and redistribute" openly-accessible educational material.

Aside from the obvious benefit of saving students money, OERs have been shown to promote equity among students. Their use has been shown to contribute to maintenance or improvement of student success, especially with respect to retention in school, course completion, grade point average, and subsequent educational attainment (Colvard et al., 2018; Griffiths et al., 2022; Fischer et al., 2015).

HOW TO USE THIS BOOK

Scope

This is a textbook covering concepts in animal parasitology. It is meant to be used by students, teachers, professors, researchers, and members of the public who are interested in learning about animal parasite biology, systematics, taxonomy, zoogeography, and ecology. The primary intended audience is upper-level undergraduate or graduate university students who have knowledge of basic biology and, particularly, basic animal biology.

Organization of the Book

This textbook was conceived to fill a gap in educational materials about parasitology. One of the main goals in both teaching and learning about parasites and parasitology is to understand the diversity of parasites and of parasitism as a way of life on Earth. With this in mind, the editors made a decision to treat the organization of the book as though led by the organisms themselves—a sort of bottom-up approach—and present the parasitic organisms as a parasitologist will first find them in nature, as in: Where they tend to exist in relation to their host, and more specifically, whether inside or outside the host animal. Therefore, the book includes sections covering a few taxonomic groups representing just some of the millions of extant endoparasite (Greek: **endo** = inside; **para** = beside; **sitos** = food) and ectoparasite (Greek: **ektos** = outside) species.

Examples of endoparasites are parasitic trematodes or nematodes that live inside the respiratory systems or gastrointestinal tracts of their hosts. Ectoparasites include lice and ticks, almost all fleas, many mites, a few platyhelminths that live on echinoderms, and even some chordates like the lamprey and vampire bat. Some groups of animals, such as monogeneans and mites, are not neatly categorized and may live part of their lives as endoparasites and part of their lives as ectoparasites or as free-living animals. Despite these myriad variations, the editors believe that the basic division between endo- and ecto- serves well enough to organize the chapters.

In approaching the organization in this way, the focus of the book is primarily at the level of species and other lower level taxonomy as opposed to higher-level groupings which are notoriously constantly in flux. The classification of parasites based on phylogenies is useful and necessary to understand the diversity, diversification, and evolution of parasites, but classification does not dictate the book's primary organization. Instead, the concept of biodiversity of parasites and their animal hosts is the main factor that motivates the research and teaching in the Harold W. Manter Laboratory of Parasitology (University of Nebraska State Museum, Lincoln, Nebraska, United States) where editor Scott L. Gardner conducts his work. It is this push toward understanding biological diversity of parasites that overarchingly informs the organization of this book.

Note about Bibliographical References

The citations in the book are formatted to promote finding usable copies, they are not meant to serve as an archival resource. As such, and to save space, only the first four authors are listed for each resource. A digital object identifier (doi) is included whenever one could be found; but the dois are not

hot linked since these links would often take readers to paywalled versions. Readers are encouraged instead to attempt to locate free, legal versions of the resources included in the references whenever possible. For example, free-to-read versions (and sometimes also open access versions) of the papers may be available in institutional repositories, on authors' personal websites, or from academic social media sites.

Note about Images

When selecting images, the editors relied on the guidelines included in Egloff et al. (2017) regarding copyrightability of images that serve as biodiversity data. Beyond this broad framework to guide selection, the images in the book were chosen ultimately based on the following criteria: Conceptual applicability, quality, allowable copyright and permissions, and (for human subject images) an acceptable declaration of informed consent (see Roguljić and Wager, 2020). Due to the constraints of these criteria, there are several sections in the book that are lightly illustrated. Where images are sparse or lacking, instructors are encouraged to insert their own images or select images from other sources, including those used under applicable fair use/fair dealing or educational use guidelines.

Accompanying Glossary

A supplemental glossary is in the process of preparation. Until the glossary is completed, a work that may be used in its stead for many of the terms found in the book is the Dictionary of Invertebrate Zoology (Maggenti et al., 2017) available online for free: https://digitalcommons.unl.edu/zeabook/61/

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Disclaimers

Although students of pre-medical studies, medical studies, or veterinary studies may use this text to learn foundational concepts in animal parasitology, it is not a medical or veterinary text. Further, it is not meant for any medical- or veterinary-related purposes whatsoever. When medical or veterinary topics are touched upon in the text, this is for educational purposes for those studying or interested in the biological sciences generally. *No medical or veterinary advice of any kind is offered or implied anywhere in this textbook*. *No* medical or veterinary diagnoses, treatments, or conclusions of any kind may be construed using the knowledge offered herein.

For studies specifically related to medical parasitology, readers may consult any of a number of qualified texts in the subject, including Medical Parasitology: A Textbook (Mahmud et al., 2017), Medical Parasitology (Satoskar, 2009), and Modern Parasitology: A Textbook of Parasitology, 2nd edition, (Cox et al., 2009), among others. Numerous medical periodicals are also appropriate sources of knowledge about medical parasitology. For medical diagnoses, qualified practitioners of medicine may be consulted directly.

For studies specifically related to veterinary parasitology, readers may consult any of a number of qualified texts in the subject, including Veterinary Parasitology, 4th edition, (Taylor et al., 2015) and Georgis' Parasitology for Veterinarians, 11th edition, (Bowman, 2020), among others. Numerous veterinary parasitology periodicals are also appropriate sources of knowledge about veterinary parasitology. For veterinary diagnoses, qualified practitioners of veterinary medicine may be consulted directly.

Use of material from United States federal agencies *does not constitute its endorsement or recommendation* by the US Government, Department of Health and Human Services, or Centers for Disease Control and Prevention (CDC). The material from the CDC is otherwise available on the agency website for no charge.

Invitation to Review and Give Feedback

If any qualified readers would like to serve as a reviewer for any of the sections, you are invited to please contact one of the editors to discuss the possibility of being assigned the task of reviewing. You will be credited in revisions if you ultimately serve as a selected reviewer. In addition, if readers discover factual or typographical errors in the content, please contact one of the editors.

HOW THE BOOK WAS DEVELOPED

Origin of the Book

The concept for this book arose in 2018 around the time there was a concerted push to create open educational resources in universities (Austin, 2018; Sennott et al., 2015). This push seemed well-timed to the editors. In fact, the rising costs of textbooks has become a major problem for students to the point where it is basically untenable to expect students to pay for them anymore. The editors reasoned that it would be a good time to call on their esteemed and accomplished colleagues in academia to help create a new textbook in a massively collaborative endeavor, if they were willing to participate.

Also driving the idea of a new textbook, the seminal English-language parasitology textbook of our time, Gerald R. Schmidt and Larry S. Roberts' Foundations of Parasitology, 9th edition (Roberts et al., 2012), has recently gone out of print and there are no plans to update it. John J. Janovy, Jr., the lead author of the last several editions of the Schmidt and Roberts book, agreed that the creation of a new textbook was a good and timely idea.

Contributing to the decision to attempt the creation of a large-scale textbook project was the public access/open access platform available to the editors, namely, the Zea Books imprint of the University of Nebraska–Lincoln Libraries. In line with the OER ethos driving the creation of the content, this publishing imprint operates under a diamond open access model, such that neither the authors nor the readers have to pay to publish nor to read any work published as a Zea Book.

Development of the Book

At the time of the conception of the book idea, the edi-tors capitalized on the availability of visiting scholars in the Harold W. Manter Laboratory of Parasitology (Lincoln, Ne-braska, United States)—Griselda Pulido-Flores, Scott Monks, and Donald Gettinger, as well as local colleagues John J. Janovy, Jr. and Gabor Rácz, and student-colleagues Auggie Tsogtsaikhan Dursahinhan and Guin Drabik—and called to-gether a couple of meetings to discuss their idea with the group. They asked them to envision what they would like to see in a new textbook, one that would be available online for anyone with a computer connection to access for free. Among many other good ideas they shared, they suggested that the book could possibly include numerous links to other sources and interactive modules, and pointed out that the information may be kept more current than was possible with a printed volume. Colleagues Paul Royster, Linnea Fredrickson, Catherine Fraser Riehle, and Mary Bolin in the University of Nebraska–Lincoln Libraries (Lincoln, Nebraska, United States) also provided encouragement and expertise that helped the project on its way.

When preparing to solicit manuscripts for this project, based on the preliminary conversations with colleagues, the editors first prepared an outline of the concepts desired to have covered and then created streamlined style requirements (the instructions for authors and references style guide are available online here: https://digitalcommons.unl.edu/parasittext/). They then asked numerous colleagues—all experts in their subareas of parasitology—to contribute one or more sections based on the outline. So many of them agreed to write sections that it seemed that it really might be possible to create a high-quality work with the input of so many fine experts. Every one of them submitted manuscripts quickly.

The editors gave the authors quite a bit of latitude regarding how to approach their assignment to write sections. They provided an optional template to work from (available here), but use of this format was optional. They wanted the authors to be able to express themselves in the way they each felt was best to demonstrate knowledge of their respective areas of interest within the larger subject of animal parasitology. This liberal approach naturally resulted in some variation in presentation styles, which is perhaps a plus for the reader. It breaks up the tone and emphases from section to section, and the reader gets a sense of each author's different voice and approach. The editors have worked to retain much of each author's preferred style of presentation, but with normalizing of typography and other style elements to help the manuscript finally cohere as a unified whole.

Some of the sections were sent out for review. This review process was open, so the authors knew who was reviewing their work and the reviewers were aware that the authors knew they were reviewing. Reviewed sections are marked as such with the reviewer's name and affiliation. Whether reviewed or not, all of the sections were editor-reviewed by both editors: Sue Ann Gardner edited primarily for bibliographic details and style elements, and Scott L. Gardner edited primarily for content.

Delayed Publication

With best-laid plans, the editors started to review and edit the sections as soon as they were submitted. Then a great number of both quite-dire and less-dire issues arose that interfered with the ability to complete the editing and production in as timely a manner as intended (selected challenges include: The SARS-CoV-2 pandemic requiring remote teaching, a computer crash, a death in the family that then required weeks away from work and home, radical changes in administrations at the university, and other issues). With those issues finally

receding in impact, five years after the project began, the book will be published at long last.

Demographic Data About the Authors

With editor Scott L. Gardner's large network of expert parasitologist colleagues, it was possible to seek out scholars who are experts in their field. While the first consideration when deciding who to invite to participate was expertise, the editors further worked toward the desired goal of equity and inclusion in the selection of authors. One result was a 1:2 ratio of women to men. While this does not represent parity, it is an improvement over days past when the majority of authors would likely have been men. Another result of efforts at equity and inclusion was the participation of many au-thors from outside the United States. Approximately 40% of authors are US-American and the remaining 60% are from one of 14 other countries (Argentina, Brazil, Australia, Japan, Mongolia, Bulgaria, Czechia, Germany, Hungary, Norway, Russia, Spain, Mexico, or Canada). Almost half of the authors (44%) do not have English as their first language.

Spanish-Language Version

In late 2018, the Office of the President at the University of Nebraska–Lincoln (Lincoln, Nebraska, United States) issued a call for proposals for Inclusive Excellence Development at the university. The editors were awarded funds to go toward translation of the textbook. With this, the editors partnered with a local professor of Spanish-language translation, Yoanna Esquivel Greenwood, who has created Spanish-language versions for numerous chapters in the book. Thanks to her work, and perhaps with the added input of some of the Spanish speakers among the authors, a comprehensive Spanish-language translation is forthcoming.

Acknowledgement of Authors' Contributions

From the Editors, Scott L. Gardner and Sue Ann Gardner

We sincerely thank all of the authors of this collaborative work. Your excellent contributions and dedication to the ad-vancement of knowledge of animal parasitology have the po-tential to positively change the lives of countless students and teachers worldwide.

While we were grappling with challenges and distractions that delayed the editing of the manuscript of this book, we lost a few of our esteemed author colleagues. We wish to posthumously acknowledge Bernie Fried, Akira Ito, and Robin M. Overstreet for what turned out to be some of their truly late-career contributions. We miss them, and we feel so fortunate to have benefitted from their long-acquired knowledge and their willingness to join in on this project.

Dedication

From the Editors, Scott L. Gardner and Sue Ann Gardner

This book is dedicated to **all** of our academic forebears and mentors who made this effort possible—some of whom are authors* of sections of the book! We can't list everyone, but we can provide a truncated list to commemorate some people especially.

Sydney Anderson
Odile Bain
Mary Bolin
Alain Chabaud
Patricia Coty
Lee Couch
Donald W. Duszynski*
William F. Font, Jr.
Bernard Fried*
Donald Heyneman
Akira Ito*
John J. Janovy, Jr.*
Armand Maggenti
Harold W. Manter
Brent B. Nickol
Robert M. Overstreet*
Mary Lou Pritchard
Robert L. Rausch
Virginia R. Rausch
Peter Raven
Constance Rinaldo
Larry S. Roberts*
Klaus Rohde*
Gerald R. Schmidt
Franklin Sogandares-Bernal
Robert M. Storm
Annegret Stubbe
Michael Stubbe
Sam Telford
Terry L. Yates

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