
Parasitology
(Study of Parasites)
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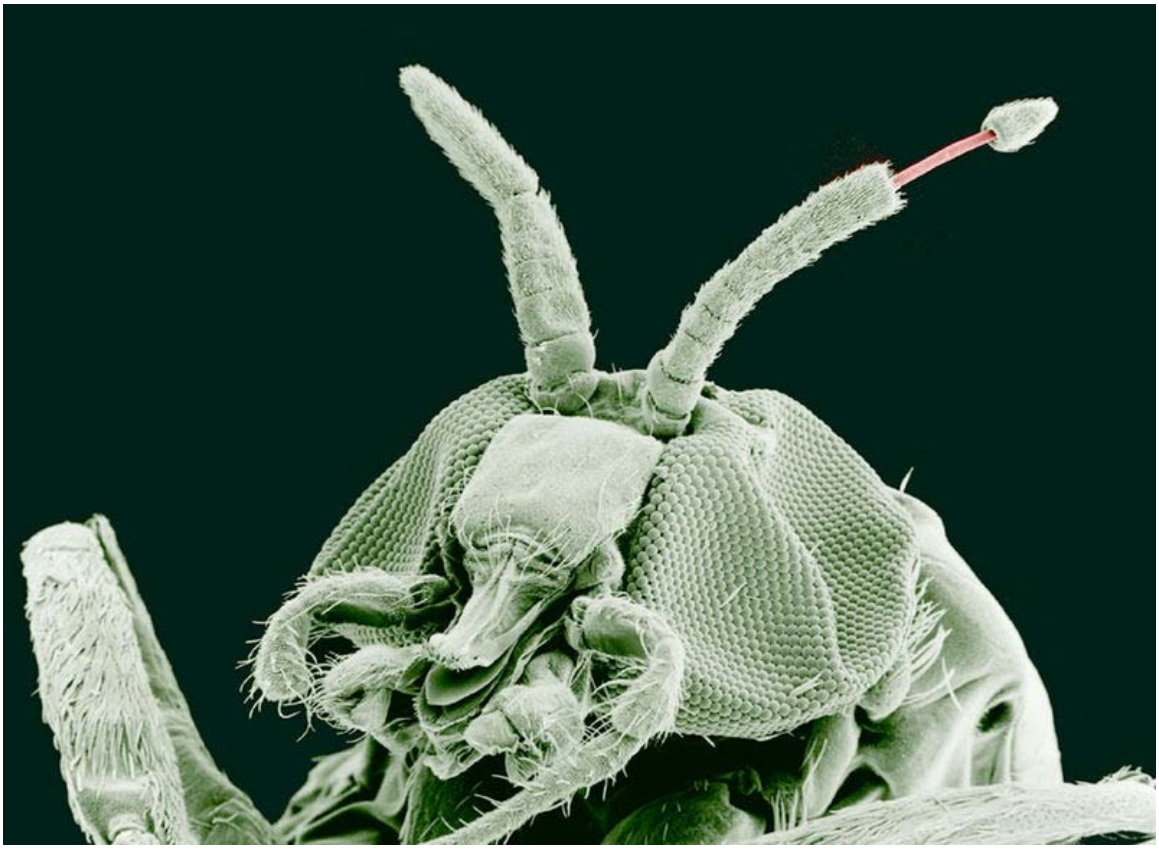
Parasitology



Adult black fly (*Simulium yahense*) with (*Onchocerca volvulus*) emerging from the insect's antenna. The parasite is responsible for the disease known as river blindness in Africa. Sample was chemically fixed and critical point dried, then observed using conventional scanning electron microscopy.

Parasitology is the study of parasites, their hosts, and the relationship between them. As a biological discipline, the scope of parasitology is not determined by the organism or environment in question, but by their way of life. This means it forms a synthesis of other

disciplines, and draws on techniques from fields such as cell biology, bioinformatics, biochemistry, molecular biology, immunology, genetics, evolution and ecology.

Fields

The study of these diverse organisms means that the subject is often broken up into simpler, more focused units, which use common techniques, even if they are not studying the same organisms or diseases. Much research in parasitology falls somewhere between two or more of these definitions. In general, the study of prokaryotes fall under the field of bacteriology rather than parasitology.

Medical parasitology

One of the largest fields in parasitology, medical parasitology is the subject which deals with the parasites that infect man, the diseases caused by them, clinical piture and the response generated by man against them. It's also concerned with the various methods of their diagnosis, treatment and finally their prevention & control. A parasite is an organism that live on or within another organism called the host. These include organisms such as:

- *Plasmodium* spp., the protozoan parasite which causes malaria. The four species of malaria parasites infective to humans are *Plasmodium falciparum*,*Plasmodium malariae*, *Plasmodium vivax* & *Plasmodium ovale*.
- *Leishmania donovani*, the unicellular organism which causes leishmaniasis
- multicellular organisms such as *Schistosoma* spp., *Wuchereria bancrofti* and *Necator americanus*

Medical parasitology can involve drug development, epidemiological studies and study of zoonoses.

Veterinary parasitology

The study of parasites that cause economic losses in agriculture or aquaculture operations, or which infect companion animals. Examples of species studied are:

- *Lucilia sericata*, a blowfly, which lays eggs on the skins of farm animals. The maggots hatch and burrow into the flesh, distressing the animal and causing economic loss to the farmer
- *Otodectes cynotis*, the cat ear mite, responsible for Canker.
- *Gyrodactylus salaris*, a monogenean parasite of salmon, which can wipe out populations which are not resistant.

Structural parasitology

This is the study of structures of proteins from parasites. Determination of parasitic protein structures may help to better understand how these proteins function differently

from homologous proteins in humans. In addition, protein structures may inform the process of drug discovery.

Quantitative parasitology

Parasites exhibit an aggregated distribution among host individuals, thus the majority of parasites live in the minority of hosts. This feature forces parasitologists to use advanced biostatistical methodologies.

Parasite ecology

Parasites can provide information about host population ecology. In fisheries biology, for example, parasite communities can be used to distinguish distinct populations of the same fish species co-inhabiting a region. Additionally, parasites possess a variety of specialized traits and life-history strategies that enable them to colonize hosts. Understanding these aspects of parasite ecology, of interest in their own right, can illuminate parasite-avoidance strategies employed by hosts

Conservation biology of parasites

Conservation biology is concerned with the protection and preservation of vulnerable species, including parasites. A large proportion of parasite species are threatened by extinction, partly due to efforts to eradicate parasites which infect humans or domestic animals, or damage human economy, but also caused by the decline or fragmentation of host populations and the extinction of host species.

Taxonomy and phylogenetics

The huge diversity between parasitic organisms creates a challenge for biologists who wish to describe and catalogue them. Recent developments in using DNA to identify separate species and to investigate the relationship between groups at various taxonomic scales has been enormously useful to parasitologists, as many parasites are highly degenerate, disguising relationships between species.

Chapter- 2

Parasitism

Brood parasitism is a common form of parasitism

Parasitism is a type of symbiotic relationship between organisms of different species where one organism, the **parasite**, benefits at the expense of the other, the host. Traditionally **parasite** referred to organisms with lifestages that went beyond one host (e.g. Taenia solium), which are now called macroparasites (typically protozoa and helminths). Parasites can now also refer to microparasites, which are typically smaller, such as viruses and bacteria and can be directly transmitted between hosts of one species.

Unlike predators, parasites are generally much smaller than their host, although both are special cases of consumer-resource interactions. Parasite show a high degree of specialization for their mode of life, and reproduce at a faster rate than their hosts. Classic examples of parasitism include interactions between vertebrate hosts and diverse animals such as tapeworms, flukes, the *Plasmodium* species, and fleas. Parasitism is differentiated from parasitoidism, a relationship in which the host is normally killed by the parasite and which occurs in some species of moth, butterfly, ant, fly and other insects.

The harm and benefit in parasitic interactions concern the biological fitness of the organisms involved. Parasites reduce host fitness in many ways, ranging from general or specialized pathology (such as castration), impairment of secondary sex characteristics, to the modification of host behaviour. Parasites increase their fitness by exploiting hosts for resources necessary for the parasite's survival: (i.e. food, water, heat, habitat, and dispersal).

Although the concept of parasitism applies unambiguously to many cases in nature, it is best considered part of a continuum of types of interactions between species, rather than an exclusive category. Particular interactions between species may satisfy some but not all parts of the definition. In many cases, it is difficult to demonstrate that the host is harmed. In others, there may be no apparent specialization on the part of the parasite, or the interaction between the organisms may be short-lived. In medicine, only eukaryotic organisms are considered parasites, with the exclusion of bacteria and viruses. Some branches of biology, however, regard members of these groups as parasitic.

Etymology

First attested in English 1539, the word *parasite* comes from the Medieval French *parasite*, from the Latin *parasitus*, the latinisation of the Greek παράσιτος (*parasitos*), "one who eats at the table of another" and that from παρά (*para*), "beside, by" + σῖτος (*sitos*), "wheat". Coined in English 1611, the word *parasitism* comes from the Greek παρά (*para*) + σιτισμός (*sitismos*) "feeding, fattening".

Types of parasitism

Parasites are classified based on their interactions with their hosts and on their life cycles.

Parasites that live on the surface of the host are called **ectoparasites** (e.g. some mites) and those that live inside the host are called **endoparasites** (including all parasitic worms). Endoparasites can exist in one of two forms: intercellular (inhabiting spaces in the host’s body) or intracellular (inhabiting cells in the host’s body). Intracellular parasites, such as bacteria or viruses, tend to rely on a third organism which is generally known as the carrier or vector. The vector does the job of transmitting them to the host. An example of this interaction is the transmission of malaria, caused by a protozoan of the genus *Plasmodium*, to humans by the bite of an anopheline mosquito.

An *epiparasite* is one that feeds on another parasite. This relationship is also sometimes referred to as *hyperparasitism* which may be exemplified by a protozoan (the hyperparasite) living in the digestive tract of a flea living on a dog.

Social parasites take advantage of interactions between members of social organisms such as ants or termites. In *kleptoparasitism*, parasites appropriate food gathered by the host. An example is the brood parasitism practiced by many species of cuckoo and cowbird, which do not build nests of their own but rather deposit their eggs in nests of other species and abandon them there. The host behaves as a "babysitter" as they raise the young as their own. If the host removes the cuckoo's eggs, some cuckoos will return and attack the nest to compel host birds to remain subject to this parasitism. The cowbird's parasitism does not necessarily harm its host's brood; however, the cuckoo may remove one or more host eggs to avoid detection, and furthermore the young cuckoo may heave the host's eggs and nestlings from the nest.

Parasitism can take the form of isolated *cheating* or *exploitation* among more generalized mutualistic interactions. For example, broad classes of plants and fungi exchange carbon and nutrients in common mutualistic mycorrhizal relationships; however, some plant species known as myco-heterotrophs "cheat" by taking carbon from a fungus rather than donating it.

Parasitoids are organisms whose larval development occurs inside or on the surface of another organism, resulting in the death of the host. This means that the interaction between the parasitoid and the host is fundamentally different from that of a true parasite and shares some of the characteristics of predation.

An adelpho-parasite is a parasite in which the host species is closely related to the parasite, often being a member of the same family or genus. An example of this is the citrus blackfly parasitoid, *Encarsia perplexa*, unmated females of which may lay haploid eggs in the fully developed larvae of their own species. These result in the production of male offspring. The marine worm *Bonellia viridis* has a similar reproductive strategy, although the larvae are planktonic.

Evolutionary aspects

Biotrophic parasitism is a common mode of life that has arisen independently many times in the course of evolution. Depending on the definition used, as many as half of all animals have at least one parasitic phase in their life cycles, and it is also frequent in plants and fungi. Moreover, almost all free-living animals are host to one or more parasite taxa.

Restoration of a *Tyrannosaurus* with parasite infections. A 2009 study showed that holes in the skulls of several specimens might have been caused by *Trichomonas*-like parasites

Parasites evolve in response to defense mechanisms of their hosts. Examples of host defenses include the toxins produced by plants to deter parasitic fungi and bacteria, the complex vertebrate immune system, which can target parasites through contact with bodily fluids, and behavioral defenses. An example of the latter is the avoidance by sheep of open pastures during spring, when roundworm eggs accumulated over the previous year hatch en masse. As a result of these and other host defenses, some parasites evolve adaptations that are specific to a particular host taxon and specialize to the point where they infect only a single species. Such narrow host specificity can be costly over evolutionary time, however, if the host species becomes extinct. Thus, many parasites are capable of infecting a variety of host species that are more or less closely related, with varying success.

Host defenses also evolve in response to attacks by parasites. Theoretically, parasites may have an advantage in this evolutionary arms race because of their more rapid generation time. Hosts reproduce less quickly than parasites, and therefore have fewer chances to adapt than their parasites do over a given span of time.

In some cases, a parasite species may coevolve with its host taxa. In theory, long-term coevolution should lead to a relatively stable relationship tending to commensalism or

mutualism, in that it is in the evolutionary interest of the parasite that its host thrives. A parasite may evolve to become less harmful for its host or a host may evolve to cope with the unavoidable presence of a parasite to the point that the parasite's absence causes the host harm. For example, although animals infected with parasitic worms are often clearly harmed, and therefore parasitized, such infections may also reduce the prevalence and effects of autoimmune disorders in animal hosts, including humans.

The presumption of a shared evolutionary history between parasites and hosts can sometimes elucidate how host taxa are related. For instance, there has been dispute about whether flamingos are more closely related to the storks and their allies, or to ducks, geese and their relatives. The fact that flamingos share parasites with ducks and geese is evidence these groups may be more closely related to each other than either is to storks.

Parasitism is part of one explanation for the evolution of secondary sex characteristics seen in breeding males throughout the animal world, such as the plumage of male peacocks and manes of male lions. According to this theory, female hosts select males for breeding based on such characteristics because they indicate resistance to parasites and other disease.

Co-speciation

In rare cases, a parasite may even undergo co-speciation with its host. One particularly remarkable example of co-speciation exists between the simian foamy virus (SFV) and its primate hosts. In one study, the phylogenies of SFV polymerase and the mitochondrial cytochrome oxidase subunit II from African and Asian primates were compared. Surprisingly, the phylogenetic trees were very congruent in branching order and divergence times. Thus, the simian foamy viruses may have co-speciated with Old World primates for at least 30 million years.

Ecology

Quantitative ecology

When considering the distribution of a single parasite species, one finds that they exhibit an aggregated distribution among host individuals, which means that most hosts harbour few parasites, while a few hosts carry the vast majority of parasite individuals. This poses considerable problems for students of parasite ecology: the use of parametric statistics should be avoided. Log-transformation of data before the application of parametric test, or the use of non-parametric statistics is recommended by several authors. However, these give rise to further problems. Therefore, modern day quantitative parasitology is based on more advanced biostatistical methods.

Diversity ecology

Hosts represent discrete habitat patches that can be occupied by parasites. A hierarchical set of terminology has come into use to describe parasite assemblages at different host scales.

Infrapopulation
: All the parasites of one species in a single individual host.

Metapopulation
: All the parasites of one species in a host population.

Infracommunity
: All the parasites of all species in a single individual host.

Component community
: All the parasites of all species in a host population.

Compound community
: All the parasites of all species in all host species in an ecosystem.

The diversity ecology of parasites differs markedly from that of free-living organisms. For free-living organisms, diversity ecology features many strong conceptual frameworks including Robert MacArthur and E. O. Wilson's theory of island biogeography, Jared Diamond's assembly rules and, more recently, null models such as Stephen Hubbell's unified neutral theory of biodiversity and biogeography. Frameworks are not so well-developed for parasites and in many ways they do not fit the free-living models. For example, island biogeography is predicated on fixed spatial relationships between habitat patches ("sinks"), usually with reference to a mainland ("source"). Parasites inhabit hosts, which represent mobile habitat patches with dynamic spatial relationships. There is no true "mainland" other than the sum of hosts (host population), so parasite component communities in host populations are metacommunities.

Nonetheless, different types of parasite assemblages have been recognised in host individuals and populations, and many of the patterns observed for free-living organisms are also pervasive among parasite assemblages. The most prominent of these is the interactive-isolationist continuum. This proposes that parasite assemblages occur along a cline from interactive communities, where niches are saturated and interspecific competition is high, to isolationist communities, where there are many vacant niches and interspecific interaction is not as important as stochastic factors in providing structure to the community. Whether this is so, or whether community patterns simply reflect the sum of underlying species distributions (no real "structure" to the community), has not yet been established.

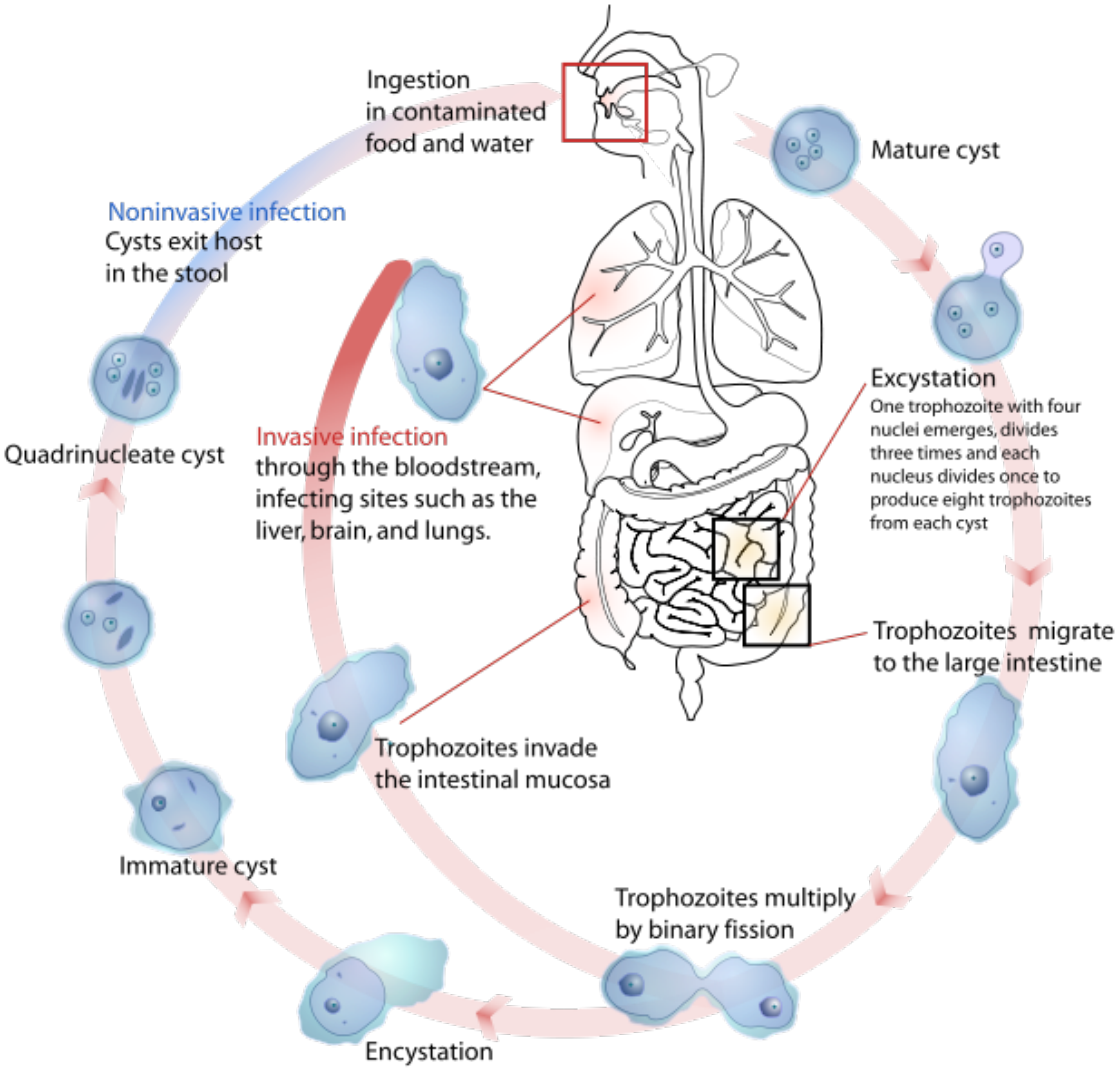
Adaptation

Parasites infect hosts that exist within their same geographical area (sympatric) more effectively. This phenomenon supports the "Red Queen hypothesis - which states that interactions between species (such as host and parasites) lead to constant natural selection for adaptation and counter adaptation." The parasites track the locally common host

phenotypes, therefore the parasites are less infective to allopatric (from different geographical region) hosts.

Experiments published in 2000 discuss the analysis of two different snail populations from two different sources- Lake Ianthe and Lake Poerua in New Zealand. The populations were exposed to two pure parasites (digenetic trematode) taken from the same lakes. In the experiment, the snails were infected by their sympatric parasites, allopatric parasites and mixed sources of parasites. The results suggest that the parasites were more highly effective in infecting their sympatric snails than their allopatric snails. Though the allopatric snails were still infected by the parasites, the infectivity was much less when compared to the sympatric snails. Hence, the parasites were found to have adapted to infecting local populations of snails.

Transmission



Life cycle of *Entamoeba histolytica*, an anaerobic parasitic protozoan

Parasites inhabit living organisms and therefore face problems that free-living organisms do not. Hosts, the only habitats in which parasites can survive, actively try to avoid, repel, and destroy parasites. Parasites employ numerous strategies for getting from one host to another, a process sometimes referred to as parasite *transmission* or *colonization*.

Some endoparasites infect their host by penetrating its external surface, while others must be ingested. Once inside the host, adult endoparasites need to shed offspring into the external environment in order to infect other hosts. Many adult endoparasites reside in the host's gastrointestinal tract, where offspring can be shed along with host excreta. Adult stages of tapeworms, thorny-headed worms and most flukes use this method.

Among protozoan endoparasites, such as the malarial parasites and trypanosomes, infective stages in the host's blood are transported to new hosts by biting-insects, or vectors.

Larval stages of endoparasites often infect sites in the host other than the blood or gastrointestinal tract. In many such cases, larval endoparasites require their host to be consumed by the next host in the parasite's life cycle in order to survive and reproduce. Alternatively, larval endoparasites may shed free-living transmission stages that migrate through the host's tissue into the external environment, where they actively search for or await ingestion by other hosts. The foregoing strategies are used, variously, by larval stages of tapeworms, thorny-headed worms, flukes and parasitic roundworms.

Some ectoparasites, such as monogenean worms, rely on direct contact between hosts. Ectoparasitic arthropods may rely on host-host contact (e.g. many lice), shed eggs that survive off the host (e.g. fleas), or wait in the external environment for an encounter with a host (e.g. ticks). Some aquatic leeches locate hosts by sensing movement and only attach when certain temperature and chemical cues are present.

Some parasites modify host behaviour to make transmission to other hosts more likely. For example, in California salt marshes the fluke *Euhaplorchis californiensis* reduces the ability of its killifish host to avoid predators. This parasite matures in egrets, which are more likely to feed on infected killifish than on uninfected fish. Another example is the protozoan *Toxoplasma gondii*, a parasite that matures in cats but can be carried by many other mammals. Uninfected rats avoid cat odours, but rats infected with *T. gondii* are drawn to this scent, a change which may increase transmission to feline hosts.

Roles in ecosystems

Modifying the behaviour of infected hosts to make transmission to other hosts more likely is one way parasites can affect the structure of ecosystems. For example, in the case of *Euhaplorchis californiensis* (discussed above) it is plausible that the abundance of local predator and prey species would be different if this parasite were absent from the system.

Although parasites are often omitted in depictions of food webs, they usually occupy the top position. Parasites can function like keystone species, reducing the dominance of superior competitors and allowing competing species to co-exist.

Many parasites require multiple hosts of different species to complete their life cycles and rely on predator-prey or other stable ecological interactions to get from one host to another. In this sense, the parasites in an ecosystem reflect the "health" of that system.

Chapter- 3

Veterinary Parasitology

Veterinary parasitology is the study of animal parasites, especially relationships between parasites and animal hosts, and their interactions. Parasites of domestic animals (livestock and pet animals) as well as wildlife animals are considered. Veterinary parasitology studies genesis and development of parasitoses in animal host. Veterinary parasitology also studies taxonomy and systematics of parasites, morphology, life cycles, and living needs of parasites in environment and in animal host. Diagnosis, treatment, and prevention of animal parasitoses are designed using procured observations. Data obtained from parasitological research in animals helps in veterinary practice and improve animal breeding. Major goal of veterinary parasitology is to protect animals and improve their health status. Moreover, a number of animal parasites are transmitted to humans. Therefore, veterinary parasitology is also important for public health.

Diagnostic methods

A number various methods are used to identify and to diagnose parasites in animals:

- **Coprological examination**
  - Flotation
  - Sedimentation
  - McMaster method
  - Baermann method
- **Haematology**
  - Bloodsmear and Giemsa-stain
  - Diff-Quick
  - QBC
- **Skin scraping**
- **Immunological methods**
  - Indirect immunofluorescence
  - ELISA
  - Immunoblotting (Western blot)
  - Complement fixation test
- **Molecular biological methods**

- PCR
- RFLP-PCR

Divisions of Veterinary Parasitology

Veterinary Protozoology

- focused on veterinary important protozoans

Examples of protozoan parasites:

- *Trypanosoma brucei*
- *Trypanosoma equiperdum*
- *Leishmania donovani*
- *Leishmania infantum*
- *Giardia duodenalis*
- *Trichomonas gallinae*
- *Tritrichomonas foetus*
- *Histomonas meleagridis*
- *Cryptosporidium parvum*
- *Balantidium coli*
- *Eimeria acervulina*
- *Eimeria tenella*
- *Isospora canis*
- *Toxoplasma gondii*
- *Neospora caninum*
- *Hammondia hammondi*
- *Besnoitia besnoiti*
- *Babesia divergens*

Veterinary Helminthology

- focused on veterinary important helminth parasites

Examples of helminth parasites:

- *Fasciola hepatica*
- *Fascioloides magna*
- *Dicrocoelium dendriticum*
- *Schistosoma bovis*
- *Trichobilharzia regenti*
- *Paragonimus westermani*
- *Dipylidium caninum*
- *Taenia pisiformis*
- *Taenia saginata*
- *Taenia solium*

- *Echinococcus granulosus*
- *Dictyocaulus bovis*
- *Muellerius capillaris*
- *Haemonchus contortus*
- *Ostertagia ostertagi*
- *Strongyloides canis*
- *Ancylostoma duodenale*
- *Trichuris suis*
- *Syngamus trachea*
- *Ascaris suum*
- *Toxocara canis*
- *Strongylus vulgaris*
- *Metastrongylus*
- *Trichinella spiralis*

Veterinary Entomology (Arachnoentomology)

- focused on veterinary important Arachnids, Insects, and Crustaceans

Examples of arachnid, insect, and crustacean parasites:

- *Sarcoptes equi*
- *Psoroptes ovis*
- *Ixodes ricinus*
- *Dermacentor marginatus*
- *Caligus clemensi*
- *Caligus cuneifer*
- *Caligus elongatus*
- *Caligus rogercresseyi*
- *Cimex colombarius.*
- *Cimex lectularius*
- *Culex pipiens*
- *Culicoides imicola*
- *Demodex bovis*
- *Gasterophilus intestinalis*
- *Haematobia irritans*
- *Hypoderma bovis*
- *Knemidocoptes mutans*
- *Lepeophtheirus salmonis (sea louse)*
- *Lucilia sericata*
- *Musca domestica*
- *Nosema apis*
- *Notoedres cati*
- *Oestrus ovis*
- *Otodectes cynotis*
- *Phlebotomus*

- *Pulex irritans*
- *Rhipicephalus sanguineus*
- *Sarcophaga carnaria*
- *Tabanus atratus*
- *Triatoma*
- *Ctenocephalides canis*
- *Ctenocephalides felis*

Chapter- 4

Structural Parasitology and Quantitative Parasitology

Structural parasitology

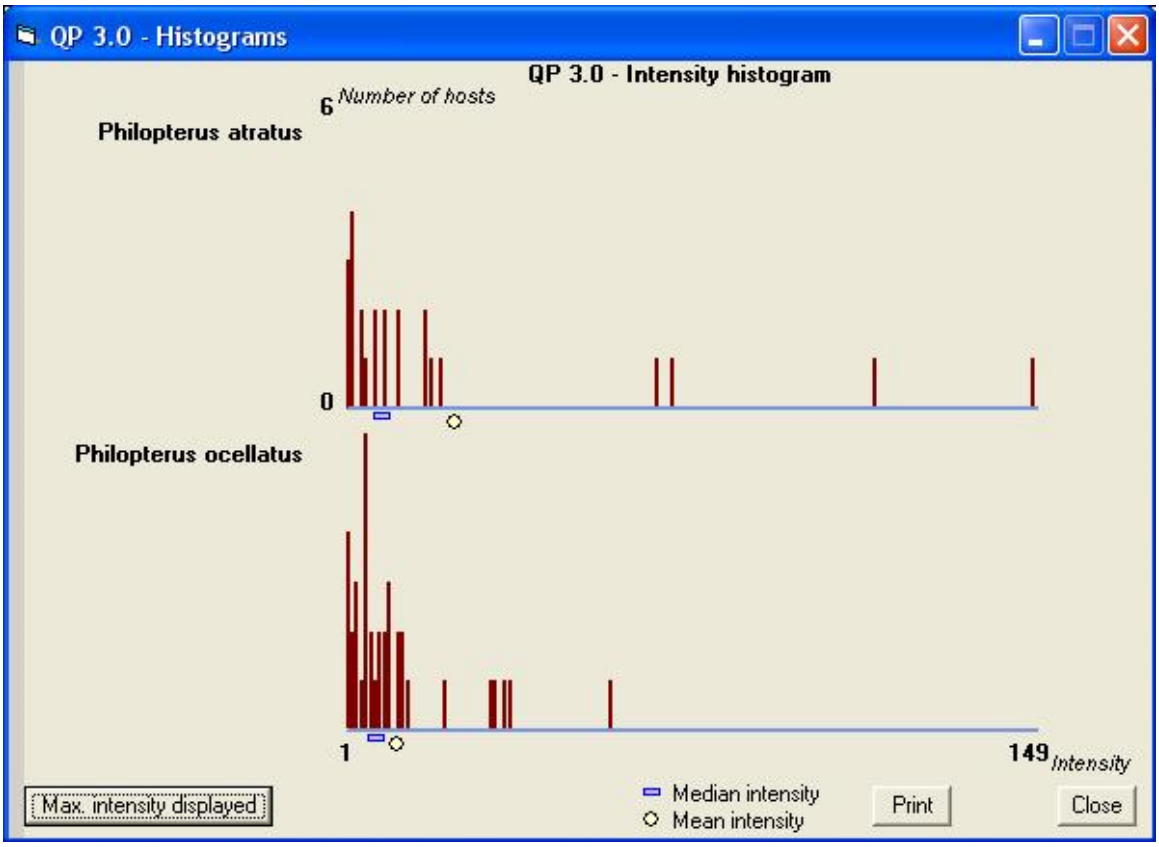
Structural parasitology is the study of the structures of proteins for interesting parasites. It applies the techniques of structural biology (such as X-ray crystallography or NMR) to determine the 3-D structures of protein molecules involved in a parasitic relationship. One goal is to distinguish the workings of functional pathways in these organisms in comparison to humans. More importantly, it is hoped that structures of parasite proteins will lead to faster discovery of drugs for diseases neglected by pharmaceutical companies.

This is a challenging field because parasite proteins are often more difficult to express using a heterologous system. The challenge is particularly great for proteins from eukaryotic parasites. Once expressed, many parasitic proteins are also resistant to crystallization because they contain inserts which are not commonly found in human or prokaryotic proteins.

Parasites of interest include Plasmodium, Trypanosoma, Leishmania, Giardia, Entamoeba, Cryptosporidium, Helminth and Toxoplasma, most of which are agents for Neglected Diseases.

Many academic labs around the world study structural parasitology. Two groups in particular have contributed many parasite structures: the SGPP (Structural Genomnics of Pathogenic Protozoa) and the SGC (Structural Genomics Consortium).

Quantitative parasitology



Intensity histograms are helpful to get a first impression about the differences of infection between 2 or more samples. Horizontal axis: infection classes, vertical axis: the number of host individuals belonging to each class.

Counting parasites

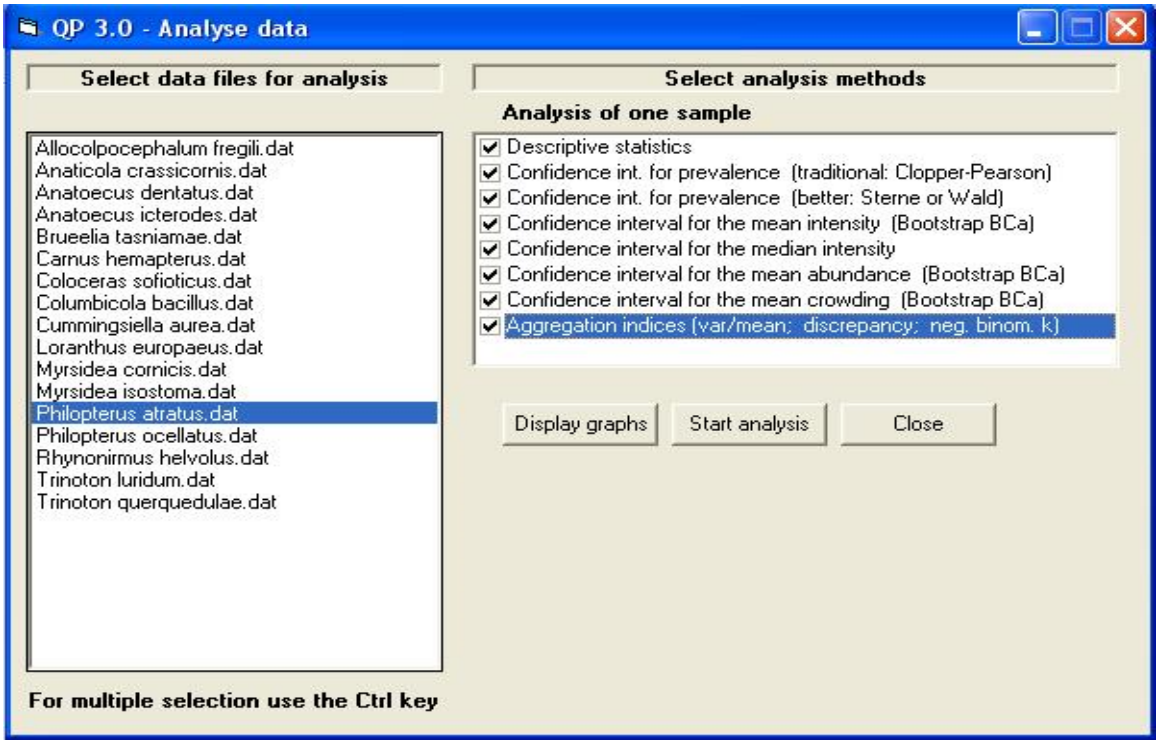
Quantifying parasites in a sample of hosts or comparing measures of infection across two or more samples can be challenging.

The parasitic infection of a sample of hosts inherently exhibits a complex pattern that cannot be adequately quantified by a single statistical measure. As the use of two or more separate indices is advisable, only two or more separate statistical tests can reliably compare infections different samples of hosts.

A few of the available statistical measures have markedly different biological interpretations, while others have more-or-less overlapping interpretations or no interpretations at all. Therefore, one should apply measures that have clear and separate biological interpretations thus do not predict each other.

Parasite individuals typically exhibit an aggregated (right-skewed) distribution among host individuals; most hosts harbour few if any parasites and a few hosts harbour many of them. This quantitative feature of parasitism renders many of traditional statistical methods obsolete and requires the use of advanced computer-intensive statistical methods.

How to describe the parasitic infection of a sample of hosts



Statistical procedures to characterize the infection/infestation of a sample of hosts

Always give the host **sample size**. In most cases, this is expressed as the number of hosts individuals examined. (Exceptionally, other units may also be used for special cases.)

Describe **prevalence**. This is the proportion of infected hosts among all the hosts examined. Give the confidence interval (CI) of prevalence (either as a Clopper-Pearson interval or as adjusted Wald/Sterne's interval) to indicate the accuracy of the estimation (use of the confidence intervals belonging to the 95% probability is advisable).

Describe **mean intensity**. This is the mean number of parasites found in the infected hosts (the zeros of uninfected hosts are excluded). Since sample size and prevalence are known, mean intensity defines the quantity of parasites found in the sample of hosts. Given the typical aggregated (right-skewed) distribution of parasites, its actual value is highly dependent on a few extremely infected hosts. Also give CI to indicate the accuracy of the estimation. Use bias-corrected and accelerated bootstrap (BCa Bootsrap) to get this confidence interval.

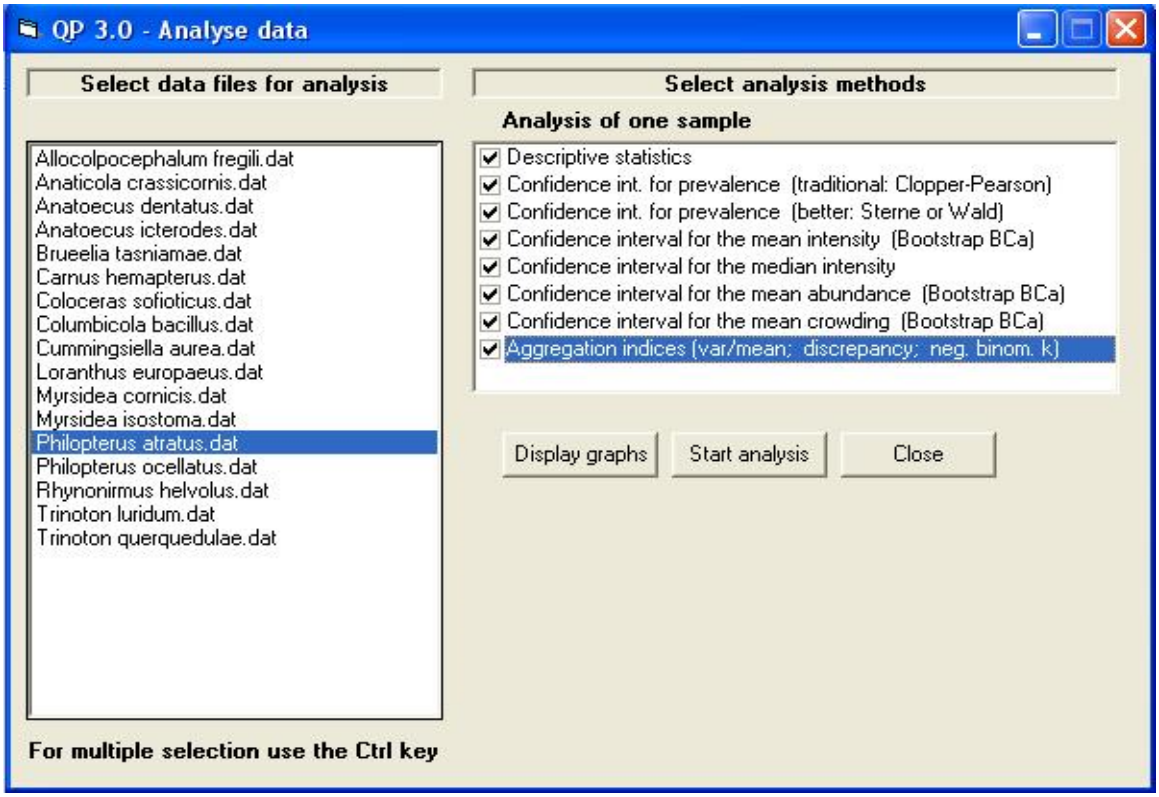
Describe **median intensity**. This is the median number of parasites found in infected hosts (the zeros of uninfected hosts are excluded). Median intensity shows a typical level of infection among the infected hosts. Use exact CI to indicate the accuracy of the estimation.

In certain cases one may prefer to use **mean abundance** instead of mean intensity. This is the mean number of parasites found in all hosts (involves the zero values of uninfected hosts). Give BCa Bootsrap confidence interval to indicate the accuracy of this estimation. This measure unifies two of the former ones: prevalence and mean intensity. Do not use it, unless you have a clearly specified a reason why to prefer it.

Describing **mean crowding** (intensity values averaged across parasite individuals) and its confidence interval is essential only for those who study density-dependent characters of parasites. BCa Bootsrap CI can be used to indicate the accuracy of the estimation.

Finally, quantify **levels of skewness** of the parasites' distribution among hosts. There are 3 indices widely used for this purpose, but their interpretation is quite similar. They predict each other rather well, thus it is not necessary to use all the 3 of them.

How to compare the parasite burdens across two or more samples



Statistical procedures to compare levels of infection/infestation across two or more samples of hosts

Compare **prevalences by Fisher's exact test**. This will show whether the proportion of infected individuals differs significantly between the two (or more) samples. The time need of this test may increase dramatically when several samples are involved. The use Chi-square test for the same purpose may be advisable in such cases.

Compare **mean intensities by a Bootstrap t-test**. This will show whether parasite quantities differ significantly between the infected proportions of the two samples.

Compare **median intensities by Mood's median test**. This will show whether the typical level of infection differs significantly between the infected proportions of the two samples.

One can also compare the **frequency distributions of intensities by a Stochastic equality test**. It compares several random pairs of individual values taken from the two samples to test whether or not there is a significant tendency to get higher values from one sample than from the other.

In certain cases, one may also decide to compare **mean abundances by a Bootstrap t-test**. This will show whether parasite quantities differ significantly between two samples. This comparison unifies two of the former ones: the comparison of prevalences and the comparison of mean intensities.

Finally, **mean crowding can be compared** across samples by a simple method: provided that the two 97.5% confidence intervals do not overlap, we conclude that the two values are different at a 95% level of significance.

Avoid typical mistakes

Do not use geometric mean because this measure is hard to interpret biologically.

Do not apply the usual form of *arithmetic mean ± standard deviation (mean ± SD)* to describe levels of infection because this is useful only for normal distributions, and not for the aggregated (right-skewed) distributions that characterize parasites. Use confidence intervals to quantify the accuracy of estimations.

Chapter- 5

Conservation Biology of Parasites and Archaeoparasitology

Conservation biology of parasites

A large proportion of living species on Earth live a parasitic way of life. Parasites have traditionally been seen as targets of eradication efforts, and they have often been overlooked in conservation efforts. In the case of parasites living in the wild – and thus harmless to humans and domesticated animals – this view is changing.

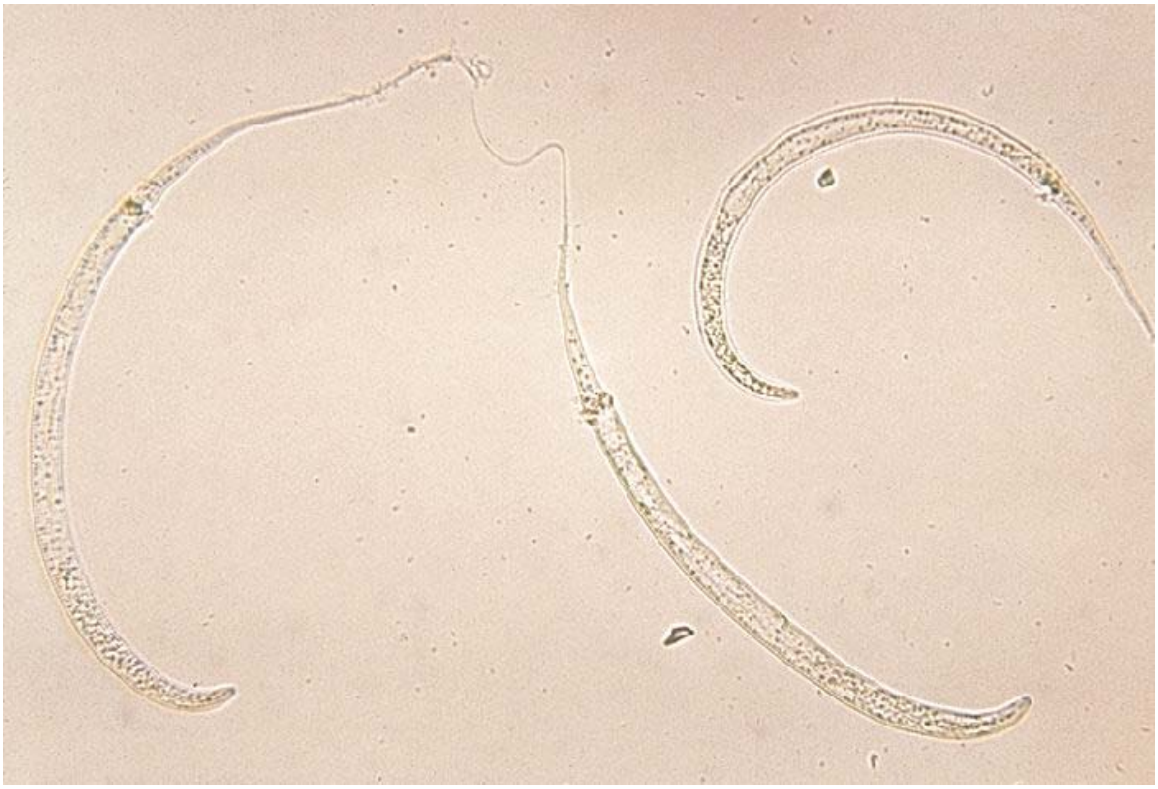
Endangered parasite species

A note published in 1990 pointed out that the captive breeding and reintroduction program to save the black-footed ferret would cause the loss of its specific parasites and demanded *"equal rights for parasites!"*. Then a paper in 1992 has warned that not only the loss of certain host species from the wild, but even host population bottlenecks or the fragmentation of host populations would predictably lead to the extinction of several host specific parasite species. It also noted that parasites are not only components of biodiversity by definition, but they also exert selective pressures upon their host populations that increase host genetic diversity. Firstly, this view met with open scepticism. Soon after, it became clear that the co-extinction of hosts and their specific parasites is likely to increase the current estimates of extinction rates significantly. A decade later, a study focusing on some highly host-specific groups (such as fig wasps, parasites, butterflies, and myrmecophil butterflies) estimated the number of co-endagered species (i.e. endagered by the endagered status of the host) at about 6300. Other authors argued that host specific parasite faunae have an unexpected advantage for conservation scientists. Their genealogies and population genetic patterns may help to illuminate their hosts' evolutionary and demographic history. Recently, scientists suggested that rich parasite faunae are inevitably needed for healthy ecosystem functioning and also that parasites and mutualists are the most endangered species on Earth. Even veterinarians have started to argue about the conservational values of parasite species.

Example: extinct avian lice

The list below follows that of Mey (2005)

- *Acutifrons caracarensis* parasite of the extinct Guadalupe Caracara *(Caracara lutosa)*, Guadelupe Island, Mexico;
- *Longimenopon dominicanum* parasite of the extinct Guadalupe Storm-petrel, *Oceanodroma macrodactyla*, Guadelupe Island, Mexico;
- *Campanulotes defectus* parasite of the extinct Passenger Pigeon *(Ectopistes migratorius)*, North-America;
- (*Columbicola extinctus* another parasite of the extinct Passenger Pigeon (*Ectopistes migratorius*). Interestingly, recent taxonomic studies show that it was conspecific with the lice living on Band-tailed Pigeon *(Columba fasciata)*, thus it is not extinct as a species) ;
- *Rallicola piageti* parasite of the extinct New Caledonian Rail *(Gallirallus lafresnayanus)*, New-Caledonia;
- *Halipeurus raphanus* parasite of the extinct Guadalupe Storm-petrel *(Oceanodroma macrodactyla)*, Guadelupe Island, Mexico;
- *Puffinoecus jamaicensis* parasite of the extinct Jamaica Petrel *(Pterodroma caribbaea)*, Jamaica;
- *Nitzschiella hemiphagae* parasite of the extinct Norfolk Island Pigeon (*Hemiphaga novaeseelandiae spadicea*), Norfolk Island, New-Zealand;
- *Patellinirmus restinctus* parasite of the extinct Norfolk Island Pigeon (*Hemiphaga novaeseelandiae spadicea*)), Norfolk Island, New-Zealand;
- *Rallicola extinctus* parasite of the extinct Huia *(Heteralocha acutirostris)*, New-Zealand;
- *Philopteroides xenicus* parasite of the extinct Bushwren *(Xenicus longipes)*, New-Zealand;
- *Psittacobrosus bechsteini* parasite of the extinct Cuban Red Macaw *(Ara tricolor)*, Cuba;
- *Colpocephalum californici*, parasite of the California Condor *(Gymnogyps californianus)*. The host have been saved by captive breeding and repatriation programs, however, the parasite have been lost, either spontaneously or perhaps exterminated by wildlife vets.



Larvae of the Guinea Worm: probably the next species to exterminate.

Extermination by purpose

Naturally, medical (and veterinary) science and practice aim to exterminate parasites and pathogens living in humans (and in domesticated animals). In case of the few highly host-specific pathogens, this equals the extinction of the pathogen species. Throughout human history, however, only a single one species, i.e. smallpox virus, was eradicated from the Globe. The last cases of smallpox occurred 1978. However, secured stocks still exist in the United States and Russia for defensive purposes such as developing new vaccines, antiviral drugs, and diagnostic tests. It is not known whether or not these superpowers have shared their stocks with some of their allies during the Cold War.

A second candidate for purposeful extermination is the Guinea Worm *(Dracunculus medinensis)*. Once widespread across some 20 nations of Africa and Asia, the parasite nowadays is much withdrown occurring only in a few countries of Sub-Saharan Africa. Prevalent civil wars in the region, such as the War in Darfur have ensured the survival of this species up to the present.

Archaeoparasitology

Archaeoparasitology, a multi-disciplinary field within paleopathology, is the study of parasites in archaeological contexts. It includes studies of the protozoan and metazoan parasites of humans in the past, as well as parasites which may have affected past human societies, such as those infesting domesticated animals.

Reinhard suggested that the term "archaeoparasitology" be applied to "... all parasitological remains excavated from archaeological contexts ... derived from human activity" and that "the term 'paleoparasitology' be applied to studies of nonhuman, paleontological material." (p. 233) Paleoparasitology includes all studies of ancient parasites outside of archaeological contexts, such as those found in amber, and even dinosaur parasites.

The first archaeoparasitology report described calcified eggs of *Bilharzia haematobia* (now *Schistosoma haematobium*) from the kidneys of an ancient Egyptian mummy. Since then, many fundamental archaeological questions have been answered by integrating our knowledge of the hosts, life cycles and basic biology of parasites, with the archaeological, anthropological and historical contexts in which they are found.

Parasitology basics

Parasites are organisms which live in close association with another organism, called the host, in which the parasite benefits from the association, to the detriment of the host. Many other kinds of associations may exist between two closely allied organisms, such as commensalism or mutualism.

Endoparasites (such as protozoans and helminths), tend to be found inside the host, while ectoparasites (such as ticks, lice and fleas) live on the oustide of the host body. Parasite life cycles often require that different developmental stages pass sequentially through multiple host species in order to successfully mature and reproduce. Some parasites are very host-specific, meaning that only one or a few species of hosts are capable of perpetuating their life cycle. Others are not host-specific, since many different hosts appear to harbor and pass on the infective stages of the parasite.

Most archaeoparasitology reports involve species which are considered to be true parasites of humans today. However, incidental parasitism (referred to by some authors as "pseudoparasitism", "false parasitism" or "accidental parasitism") occurs when a parasite which does not normally utilize a host for the perpetuation of its lifecycle is found in that host incidentally. One example is finding the eggs of *Cryptocotyle lingua* (a fish parasite) in the stomach contents of an Eskimo mummy. It is estimated that 70% of the "parasite" species reported from present-day humans are actually only incidental parasites. Some incidental parasites do cause harm to the infested pseudohosts.

Sources of material

In archaeological contexts, endoparasites (or their eggs or cysts) are usually found in (i) fossilized human or animal dung (coprolites), (ii) the tissues and digestive contents of mummified corpses, or (iii) soil samples from latrines, cesspits, or middens (dumps for domestic waste). A cyst of *Echinococcus granulosus* was even retrieved from cemetery soil in Poland. Ectoparasites may be found on the skin or scalp, as well as wigs, clothing, or personal grooming accessories found in archaeological sites. Ectoparasite eggs may be found attached to individual hairs. The International Ancient Egyptian Mummy Tissue Bank in Manchester, England, provides tissue samples for a variety of uses, including parasitological studies.

Since 1910, parasite remains have been found in archaeological samples from Africa, the Americas, Asia, Europe, the Middle East, and New Zealand. The age of archaeological sites yielding human parasite remains ranges from approx. 25,000-30,000 years ago to late 19th-early 20th century. Parasite remains have also been found in domestic animal remains at archaeological sites.

Human skeletal remains may exhibit indirect evidence of parasitism. For example, hookworm (*Ancyslostoma duodenale*) parasitism may lead to anemia, and anemia is one factor associated with the skeletal changes of cribra orbitalia and porotic hyperostosis. Thus, hookworm parasitism *may* be a causal factor in observed cribra orbitalia and porotic hyperostosis, though dietary factors may also lead to anemia.

Information on the presence of intermediate hosts, required for life cycle completion by many parasites, is also useful in determing the likelihood that a parasite may have infected a particular ancient society. One example is the identification of molluscan intermediate hosts of schistosomiasis in an Islamic archaeological context.

Artifacts depicting the appearance of individuals may also indicate cases of parasitism. Examples include the characteristic facial deformities of leishmaniasis found on pre-Columbian Mochica pottery, and morphological features of certain ancient Egyptian figurative art. Literary sources also provide valuable information regarding not only the parasites present in historic societies, but also the knowledge and attitudes that the people had towards their parasitic infestations. However, specific parasitological diagnoses reported in ancient and medieval texts must always be read with some degree of skepticism.

Techniques and methods

Parasite remains in archaeological samples are identified by a variety of techniques. Very durable remains, such as eggs and cysts, may remain intact for many thousands of years. In some cases, relatively intact soft-bodied adult helminths and ectoparasitic arthropods have been found. All of these forms can be identified to the family, genus or species level by compound or electron microscopy.

In cases where the intact bodies of parasites are not found, protein or DNA from the parasite may still be present. Antigenic and immunological assays (including enzyme-linked immunoassay - ELISA), and DNA sequencing are used to identify the source of these chemical remains, often to the species level.

Fundamental questions

Archaeoparasitological studies have provided information on many fundamental archaeological, historical, and biogeographical questions. These questions may be grouped into the following broad categories: past dietary and farming practices, animal domestication, migration patterns, climate change, sanitary practices, cultural contacts, ethnomedicine, and the overall health of various human societies. Archaeoparasitology data, combined with our knowledge of present host-parasite associations, also contributes to our understanding of the co-evolution of human host-parasite interactions. Our understanding of the geographic origins, evolution and biogeography of the parasites themselves and human diseases associated with them has also benefitted tremendously from archaeoparasitological studies.

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Paleoparasitology

Paleoparasitology (or "palaeoparasitology") is the study of parasites from the past, and their interactions with hosts and vectors; it is a subfield of Paleontology, the study of living organisms from the past. Some authors define this term more narrowly, as "Paleoparasitology is the study of parasites in archaeological material." (p. 103) K.J. Reinhard suggests that the term "archaeoparasitology" be applied to "... all parasitological remains excavated from archaeological contexts ... derived from human activity" and that "the term 'paleoparasitology' be applied to studies of nonhuman, paleontological material."

Sources of material

The primary sources of paleoparasitological material include mummified tissues, coprolites (fossilised dung) from mammals or dinosaurs, fossils, and amber inclusions. Hair, skins, and feathers also yield ectoparasite remains. Some archaeological artifacts document the presence of animal parasites. One example is the depiction of what appear to be mites in the ear of a "hyaena-like" animal in a tomb painting from ancient Thebes.

Some parasites leave marks or traces (ichnofossils) on host remains, which persist in the fossil record in the absence of structural remains of the parasite. Parasitic ichnofossils include plant remains which exhibit characteristic signs of parasitic insect infestation, such as galls or leaf mines and certain anomalies seen in invertebrate endoskeletal remains.

Plant and animal parasites have been found in samples from a broad spectrum of geological periods, including the Holocene (samples over 10,000 years old), Pleistocene (over 550,000 years old), Eocene (over 44 million years old), Cretaceous (over 100 million years) and even Lower Cambrian (over 500 million years).

Evidence of parasitism

One of the most daunting tasks involved in studying parasitic relationships from the past is supporting the assertion that the relationship between two organisms is indeed

parasitic. Organisms living in "close association" with each other may exhibit one of several different types of trophic relationships, such as parasitism, mutualism, and commensalism. Demonstration of true parasitism between existing species typically involves observing the harmful effects of parasites on a presumed host. Experimental infection of the presumed host, followed by recovery of viable parasites from that host also supports any claim of true parasitism. Obviously such experiments are not possible with specimens of extinct organisms found in paleontological contexts.

Assumptions of true parasitism in paleontological settings which are based on analogy to known present-day parasitic relationships may not be valid, due to host-specificity. For example, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are both devastating human parasites, but the related subspecies *Trypanosoma brucei brucei* will infect a number of animal hosts, but cannot even survive in the human blood stream, much less reproduce and infect a human host. So a related (or unidentifiable) species of *Trypanosoma* found in a paleontological or archaeological context may not be a true human parasite, even though it appears identical (or very similar) to the modern parasitic forms.

The most convincing evidence of paleoparasitism is obtained when a presumed parasite is found in direct association with its presumed host, in a context that is consistent with known host-parasite associations. Some examples include helminths caught in amber in the process of escaping from the body of an insect, lice found in the fur of guinea pig mummies, protozoans in the alimentary canal of flies in amber, nematode larvae found embedded in animal coprolites, and a mite caught in amber in the process of apparently feeding on a spider.

Fossil organisms which are related to present-day parasites often possess the morphological features associated with a parasitic lifestyle, such as blood-feeding mouthparts. So fossil ticks and hematophagous insects are generally assumed to be ectoparasites, even when their remains are found in the absence of a host.

The presence of structures resembling leaf miner trails in leaf fossils provide indirect evidence of parasitism, even if remains of the parasite are not recovered. The dramatic tissue aberrations seen in present-day plant galls and gall-like structures in some invertebrates are direct physiological reactions to the presence of either metazoan parasites or microbial pathogens. Similar structures seen in fossil plant and invertebrate remains are often interpreted as evidence of paleoparasitism.

Host-parasite interactions today are often exploited by other species, and similar examples have been found in the fossil record of plant galls and leaf mines. For example, there are species of wasps, called inquilines, which are unable to induce their own plant galls, so they simply take up residence in the galls that are made by other wasps. Another example is the predation of plant galls or leaf mines, to eat the trapped insect larva inside the gall or mine.

Knowledge gained from ancient animal and plant parasites

Studies of parasite remains and traces from the past have yielded a vast catalog of ancient host-parasite associations. Genetic sequence data obtained directly from ancient animal parasites, and inferences of past relationships based on genetic sequences of existing parasite groups are also being applied to paleoparasitological questions. Data obtained by all of these methods are constantly improving our understanding of the origin and evolution of the parasites themselves and their vectors, and of the host-parasite and vector-parasite associations.

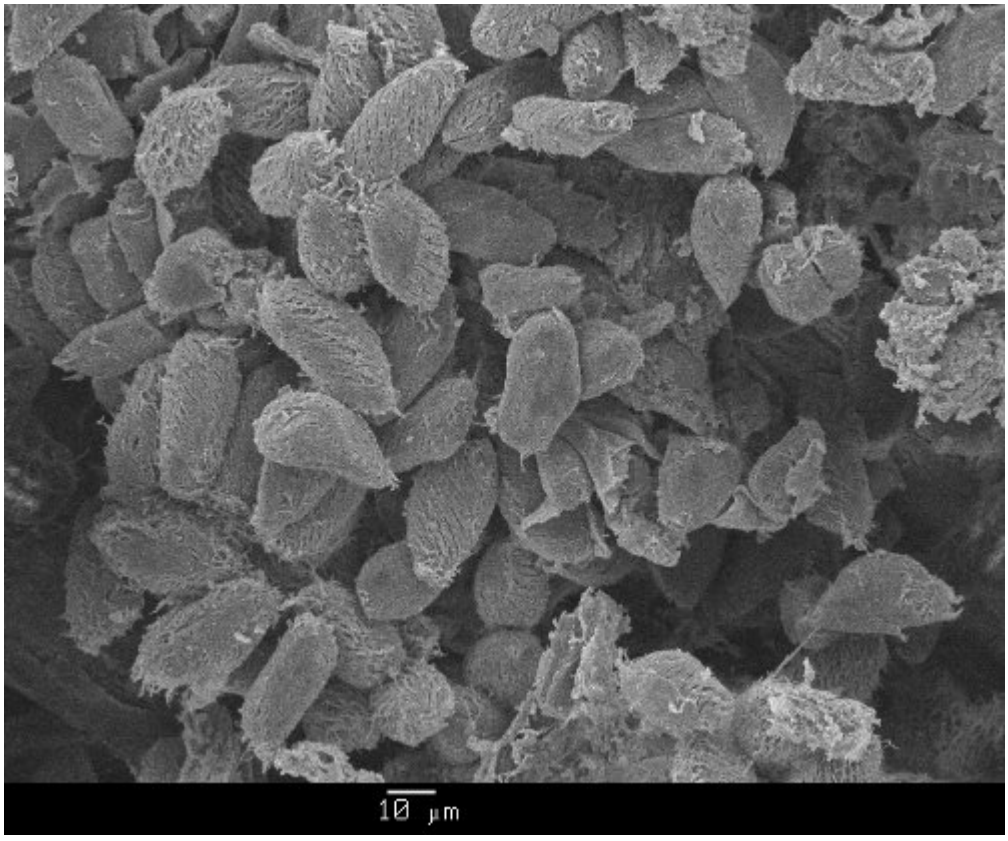
In some cases, presumed host-parasite relationships of the past seem quite different from those known in the present, such as a fly which appears to be a parasite of a mite

Paleoparasitological studies have also provided insight into questions outside the realm of parasitology. Examples include the migration and phylogeography of marine mammal hosts, the identity of domestic animal bones based on the known hosts of parasite remains found at the site, and the possible role of climatic changes on animal host genetic diversity.

Chapter- 7

Parasitoid and Parasitoid Wasp

Parasitoid



SEM image of endoparasitoid ciliates of the genus *Collinia*, which can cause mass mortality in affected krill populations

A **parasitoid** is an organism that spends a significant portion of its life history attached to or within a single host organism, which it ultimately kills (and often consumes) in the process. Thus they are similar to typical parasites except in the certain fate of the host. In

a typical parasitic relationship, the parasite and host live side by side without lethal damage to the host. Typically, the parasite takes enough nutrients to thrive without preventing the host from reproducing. In a parasitoid relationship, the host is killed, normally before it can produce offspring. When treated as a form of parasitism, the term **necrotroph** is sometimes (though rarely) used.

This type of relationship seems to occur only in organisms that have fast reproduction rates, such as insects or (rarely) mites. Parasitoids are also often closely coevolved with their hosts. Most biologists use the term *parasitoid* to refer only to insects with this type of life history, but some argue the term should be used more embracively to include parasitic nematodes, seed weevils, and certain bacteria and viruses (e.g., bacteriophages), all of which obligately destroy their host.

The term *parasitoid* was coined in 1913 by the German writer O. M. Reuter (and adopted in English by his reviewer, W. M. Wheeler) to describe the strategy in which, during its development, the parasite lives in or on the body of a single host individual, eventually killing that host, the adult parasitoid being free-living.

Types of parasitoids

Idiobiont parasitoids are those that prevent further development of the host after initial parasitization; this typically involves a host life stage that is immobile (e.g., an egg or pupa), and almost without exception, they live outside the host. **Koinobiont** parasitoids allow the host to continue its development and often do not kill or consume the host until the host is about to either pupate or become an adult; this therefore typically involves living within an active, mobile host. Koinobionts can be further subdivided into **endoparasitoids**, which develop inside of the prey, and **ectoparasitoids**, which develop outside the host body, though they are frequently attached or embedded in the host's tissues.

It is not uncommon for a parasitoid itself to serve as the host for another parasitoid's offspring. The latter is commonly termed a **hyperparasite**, but this term is slightly misleading, as both the host *and* the primary parasitoid are killed. A better term is **secondary parasitoid**, or **hyperparasitoid**; most such species known are in the insect order Hymenoptera.

Insects

About 10% of described insect species are parasitoids. There are four insect orders that are particularly renowned for this type of life history. By far, the majority are in the order Hymenoptera. The largest and best-known group comprises the so-called "Parasitica" within the Hymenopteran suborder Apocrita: the largest subgroups of these are the chalcidoid wasps (superfamily Chalcidoidea) and the ichneumon wasps (superfamily Ichneumonoidea), followed by the Proctotrupoidea and Platygastroidea. Outside of the Parasitica, many other Hymenopteran lineages that include parasitoids, such as most of the Chrysidoidea and Vespoidea, and the rare Symphytan family Orussidae. The flies

(order Diptera) include several families of parasitoids, the largest of which is the family Tachinidae, and also smaller families such as Pipunculidae, Conopidae, and others. The other two orders are the "twisted-wing parasites" (order Strepsiptera), which is a small group consisting entirely of parasitoids, and the beetles (order Coleoptera), which includes at least two families, Ripiphoridae and Rhipiceridae, that are largely parasitoids, and rove beetles (family Staphylinidae) of the genus *Aleochara*. Occasional members of other orders can be parasitoids; one of the more remarkable is the moth family Epipyropidae, which are ectoparasitoids of planthoppers.

Hymenopteran parasitoids often have unique life cycles. In one family, the Trigonalidae, the female wasps deposit eggs into small pockets they cut into the edge of leaves with their ovipositor. A caterpillar chewing these leaves may unknowingly swallow some of the eggs, and when they get into the caterpillar's gut, they hatch and burrow through the gut wall and into the body cavity. Later they search the caterpillar's body cavity for other parasitoid larvae, and it is these they attack and feed on. Some trigonalids, once in a caterpillar or sawfly larva, need their vehicle to fall prey to a social wasp. The wasp carries the caterpillar back to its nest, and there it is butchered and fed to the wasp's young; they will serve as the host for the trigonalid, the eggs of which are in the butchered caterpillar.

Parasitoid wasp

Parasitoid wasp

Quarter-inch-long (6 mm) parasitoid wasp (*Peristenus digoneutis*, Ichneumonoidea: Braconidae) prepares to lay an egg in a tarnished plant bug nymph.
Scientific classification

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Hymenoptera
Suborder:	Apocrita

The term **parasitoid wasp** refers to a large evolutionary grade of hymenopteran superfamilies, mainly in the Apocrita. They are primarily parasitoids of other animals, mostly other arthropods. Many of them, such as the family Braconidae, are considered beneficial to humans because they control populations of agricultural pests. Some of these wasps help pest control in a rather sophisticated manner. Certain types of plants have compounds that work in part with the saliva of caterpillars. When the saliva of the caterpillar and the juices of the plant mix, a fragrance is emitted that certain parasitoid wasps are very attracted to. The parasitoid wasps then kill the caterpillars and often use the carcasses to lay eggs within. This is a form of mutualism between the plant and the wasp and works only when the fragrance from the plant and the saliva combine.

Taxonomy and systematics

Historically, the classification system of Hymenoptera included two divisions or infraorders within the suborder Apocrita: one of these, the **"Parasitica"** containing the parasitoid wasp. However, the use of the name Parasitica (or its alternative, **"Terebrantia"**) has been phased out in recent years, as it is a paraphyletic grouping, and most modern classifications explicitly reject the use of any groups that are not monophyletic.

Presently, it is not clear what the eventual taxonomic fate of these groups will be. A number of clades seem to stand out in newer studies, and these may be treated as unranked taxa or at the ranks of infraorder and division.

Superfamilies

The traditional superfamilies united in the "Parasitica" are:

- Superfamily Ceraphronoidea
- Superfamily Chalcidoidea
- Superfamily Cynipoidea
- Superfamily Evanioidea
- Superfamily Ichneumonoidea
- Superfamily Megalyroidea
- Superfamily Mymarommatoidea (sometimes included in Serphitoidea)
- Superfamily Platygastroidea
- Superfamily Proctotrupoidea
- Superfamily Serphitoidea (fossil)
- Superfamily Stephanoidea

- Superfamily Trigonaloidea

Other than the "Parasitica", there are a few Apocrita that are also sometimes called "parasitic wasps": most of the members of the superfamily Chrysidoidea, as well as most of the families superfamily Vespoidea such as Bradynobaenidae, Mutillidae, Rhopalosomatidae, Sapygidae, Scoliidae, Sierolomorphidae, Tiphiidae, and a few species of Pompilidae. Most of these groups are ectoparasitoids. Among the sawflies, which are not Apocrita, there is only one small parasitic family, Orussidae. This is the Apocrita's closest living relative. It thus appears that the ancestors of bees were parasitic too.

Systematics

According to recent cladistic studies, there are a number of basal lineages among the Apocrita, as well as a diverse group of parasitic wasps that seems to form a major clade. If the Apocrita are divided into infraorders, about 6 of these must be recognized, and several of these warrant further subdivision, with a number of families being moved out of the Proctotrupoidea:

Ichneumonidae (this is possibly *Netelia testaceus* or *Ophion luteus*) are close relatives of the Aculeata.

- Superfamily Ichneumonoidea seems closer to the Aculeata than to other parasitic wasps. If the Aculeata are treated as a division, the Ichneumonoidea would form a basal superfamily in a new infraorder.
- Superfamily Stephanoidea forms a clade or infraorder of its own.
- Another clade or infraorder contains the following groups:
    - Superfamily Megalyroidea
    - Superfamily Trigonaloidea
    - a clade or division containing
        - Superfamily Ceraphronoidea
        - Superfamily Evanioidea.
- A clade or infraorder containing families formerly in the Proctotrupoidea:
    - Family Maamingidae
    - a clade, division or superfamily containing
        - Family Austroniidae
        - Family Diapriidae
        - Family Monomachidae.
- Another clade or infraorder of families formerly in the Proctotrupoidea:
    - Family Proctorenyxidae
    - Family Roproniidae

- The bulk of the parasitic wasps, a clade or infraorder containing two very distinct groups:
    - One clade or division containing:
        - Superfamily Platygastroidea
        - Superfamily Chalcidoidea
        - Superfamily Mymarommatoidea (sometimes included in Serphitoidea)
        - Superfamily Serphitoidea (fossil, tentatively placed here).
    - Another clade or division containing:
        - Superfamily Cynipoidea
        - Superfamily Proctotrupoidea *sensu stricto*

Chapter- 8

Aspidogastrea and Cestoda

Aspidogastrea

Aspidogastrea
Scientific classification
Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Subclass: **Aspidogastrea**
Families
Aspidogastridae
Multicalycidae
Rugogastridae
Stichocotylidae

The **Aspidogastrea** (Ancient Greek: ἀσπίς *aspis* "shield", γαστήρ *gaster* "stomach/pouch") is a small group of flukes comprising about 80 species. It is a subclass of the trematoda, and sister group to the Digenea. Species range in length from approximately one millimeter to several centimeters. They are parasites of freshwater and marine molluscs and vertebrates (cartilaginous and bony fishes and turtles). Maturation may occur in the mollusc or vertebrate host. None of the species has any economic importance, but the group is of very great interest to biologists because it has several characters which appear to be archaic.

Morphology

Shared characteristics

Shared characteristics of the group are a large ventral disc with a large number of small alveoli ("suckerlets") or a row of suckers and a tegument with short protrusions, so-called "microtubercles".

Larval physiology

Larvae of some species have ciliated patches. Those of *Multicotyle purvisi* have four patches on the anterior side of the posterior sucker and six at the posterior side, those of *Cotylogaster occidentalis* have an anterior ring of eight and a posterior ring of six, while larvae of *Aspidogaster conchicola*, *Lobatostoma manteri*, *Rugogaster hydrolagi* lack cilia altogether. Larvae of some species hatch from eggs, others do not.

Excretory system

Like most platyhelminthes, aspidogastreans use flame cells as an excretory mechanism. The two excretory bladders are located dorsally, on the anterior side of the posterior sucker, connected to ducts, and three flame cell "bulbs" on each side of the body; the ducts contain cilia to aid the flow of excreta.

Nervous system

Aspidogastreans have a nervous system of extraordinary complexity, greater than that of related free-living forms, and a great number of sensory receptors of many different types. The nervous system is of great complexity, consisting of a great number of longitudinal nerves (connectives) connected by circular commissures. The brain (cerebral commissure) is located dorsally, in the anterior part of the body, the eyes dorsally attached to it. A nerve from the main connective enters the pharynx and also supplies the intestine. Posteriorly, the main connective enters the sucker.

Sensory receptors are scattered over the ventral and dorsal surface, the largest numbers occurring on the ventral surface, at the anterior end and on the posterior sucker. Electron-microscopic studies revealed 13 types of receptors.

Life cycles

Their life cycle is much simpler than that of digenean trematodes, including a mollusc and a facultative or compulsory vertebrate host. There are no multiplicative larval stages in the mollusc host, as known from all digeneans.

Host specificity of most aspidogastreans is very low, i.e., a single species of aspidogastrean can infect a wide range of host species, whereas a typical digenean trematode is restricted to few species (at least of molluscs). For example, *Aspidogaster*

conchicola infects many species of freshwater bivalves belonging to several families, as well as snails, many species of freshwater fishes of several families, and freshwater tortoises.

Life cycles have been elucidated for a number of species. *Lobatostoma manteri* is an example of a species which has obligate vertebrate hosts. Adult worms live in the small intestine of the snubnosed dart, *Trachinotus blochi* (Teleostei, Carangidae), on the Great Barrier Reef. They produce large numbers of eggs which are shed in the faeces. If eaten by various prosobranch snails, larvae hatch in the stomach and—depending on the species of snail—stay there or migrate to the digestive gland where they grow up to the preadult stage which has all the characteristics of the adult including a testis and ovary.

Evolutionary relationships

Digenean trematodes have been cultured in various, complex, media. However, their parasitic stages die soon in water. Aspidogastreans may survive for many days or even weeks outside a host in simple physiological saline solution). For example, adult *A. conchicola* survived in water for a fortnight, and in a mixture of water and saline solution for up to five weeks. *L. manteri* extracted from fish could be kept alive for up to 13 days in dilute sea water in which they laid eggs containing larvae infective to snails. This has led to the suggestions that aspidogastreans are archaic trematodes, not yet well adapted to specific hosts, which have given rise to the more "advanced" digenean trematodes, and that the complex life cycles of digenean trematodes have evolved from the simple ones of aspidogastreans.

Synapomorphies of the trematodes are presence of a *Laurer's Canal*, a posterior sucker (transformed to an adhesive disc in the Aspidogastrea), and life cycles involving molluscs and vertebrates. DNA studies have consistently supported this sister group relationship. The question of whether vertebrates or molluscs are the original hosts of the trematodes, has not been resolved.

This view is supported by the evolutionary relationships of the hosts which these two subclasses utilise. The hosts of aspidogastreans include chondrichthyan fishes (sharks, rays and chimaeras), a group that is 450 million years old, whereas the digeneans, are known from teleost fishes (210 million years old) as well as from various "higher" vertebrates; very few species have invaded chondrichthyans secondarily.

Families within the Aspidogastrea

Rohde (2001) distinguish four families of Aspidogastrea:

- The **Rugogastridae** include a single genus, *Rugogaster*, with two species from the rectal glands of holocephalan fishes. It is characterised by a single row of rugae (transverse thickenings of the body surface), numerous testes, and two caeca. Species of all other families have a single caecum and either one or two testes.

- The **Stichocotylidae** include the single species *Stichocotyle nephropis* from the intestine of elasmobranchs. It has a single ventral row of well separated suckers.
- The **Multicalycidae** include the single genus *Multicalyx* from the intestine of holocephalans and elasmobranchs. It is characterised by a single ventral row of alveoli.
- The **Aspidogastridae** includes species infecting molluscs, teleosts and turtles. The ventral adhesive disc bears either three or four rows of alveoli. Rohde distinguishes three subfamilies of Aspidogastridae, the *Rohdellinae*, *Cotylaspidinae* and *Aspidogastrinae*.

Gibson further recognized two orders, the **Aspidogastrida** with the single family Aspidogastridae, and the **Stichocotylida** including the Stichocotylidae, Multicalycidae and Rugogastridae. However, similarities between species of these two orders are so great that distinction at the level of orders does not seem justified.

Cestoda

Cestoda	
	
Scolex of *Taenia solium*	
Scientific classification	
Kingdom:	Animalia
Phylum:	Platyhelminthes
Class:	**Cestoda**
Subclasses and orders	
Cestodaria Amphilinidea Gyrocotylidea	

Eucestoda
Aporidea
Caryophyllidea
Cyclophyllidea
Diphyllidea
Lecanicephalidea
Litobothridea
Nippotaeniidea
Proteocephalidea
Pseudophyllidea
Spathebothriidea
Tetraphyllidea
Trypanorhyncha

Cestoda (**Cestoidea**) is the name given to a class of parasitic flatworms, commonly called **tapeworms**, of the phylum Platyhelminthes. Its members live in the digestive tract of vertebrates as adults, and often in the bodies of various animals as juveniles. Over a thousand species have been described, and all vertebrate species can be parasitised by at least one species of tapeworm. Several species parasitise humans after being consumed in underprepared meat such as pork (*T. solium*), beef (*T. saginata*), fish (*Diphyllobothrium* spp.), or in food prepared in conditions of poor hygiene (*Hymenolepis* spp. or *Echinococcus* spp.).

T. saginata, the beef tapeworm, can grow up to 12 m (40 ft); other species may grow to over 30 m (100 ft).

Anatomy

Scolex

The worm's *scolex* ("head") attaches to the intestine of the definitive host. In some species, the scolex is dominated by bothria (tentacles), which are sometimes called "sucking grooves", and function like suction cups. Other species have hooks and suckers that aid in attachment. Cyclophyllid cestodes can be identified by the presence of four suckers on their scolex.

While the scolex is often the most distinctive part of an adult tapeworm, it is often unnoticed in a clinical setting as it is inside the patient. Thus, identifying eggs and proglottids in feces is important.

Body systems

The main nerve centre of a cestode is a cerebral ganglion in its scolex. Motor and sensory innervation depends on the number and complexity of the scolex. Smaller nerves emanate from the commissures to supply the general body muscular and sensory ending. The cirrus and vagina are innervated, and sensory endings around the genital pore are more

plentiful than other areas. Sensory function includes both tactoreception and chemoreception. Some nerves are only temporary. These are in the proglottids, and stop working with a detach.

Proglottids

The body is composed of successive segments (*proglottids*). The sum of the proglottids is called a strobila, which is thin, and resembles a strip of tape. From this is derived the common name "tapeworm". Like some other flatworms, cestodes use flame cells (protonephridia), located in the proglottids, for excretion. Mature proglottids are released from the tapeworm's posterior end and leave the host in feces.

Because each proglottid contains the male and female reproductive structures, they can reproduce independently. Some biologists have suggested that each should be considered a single organism, and that the tapeworm is actually a colony of proglottids.

The layout of proglottids comes in two forms, craspedote, meaning proglottids are overlapped by the previous proglottid, and acraspedote which indicates a non-overlapping conjoined proglottid.

Once anchored to the host's intestinal wall, the tapeworm absorbs nutrients through its skin as the food being digested by the host flows past it and it begins to grow a long tail, with each segment containing an independent digestive system and reproductive tract. Older segments are pushed toward the tip of the tail as new segments are produced by the neckpiece. By the time a segment has reached the end of the tail, only the reproductive tract is left. It then drops off, carrying the tapeworm eggs to the next host.

Reproduction and life cycle

True tapeworms are exclusively hermaphrodites; they have both male and female reproductive systems in their bodies. The reproductive system includes one or many testes, cirrus, vas deferens and seminal vesicle as male organs, and a single lobed or unlobed ovary with the connecting oviduct and uterus as female organs. There is a common external opening for both male and female reproductive systems, known as genital pore, which is situated at the surface opening of the cup-shaped atrium. Even though they are sexually hermaphroditic, self-fertilization is a rare phenomenon. In order to permit hybridization, cross-fertilization between two individuals is often practiced for reproduction. During copulation, the cirrus one individual connects with that of the other through the genital pore, and then exchange their spermatozoa.

The life cycle of tapeworms is simple in the sense that there are no asexual phases as in other flatworms, but complicated in that at least one intermediate host is required as well as the definitive host. This life cycle pattern has been a crucial criterion for assessing evolution among Platyhelminthes. Many tapeworms have a two-phase life cycle with two types of host. The adult *Taenia saginata* lives in the gut of a primate such as a human. Proglottids leave the body through the anus and fall onto the ground, where they may be

eaten with grass by animals such as cows. In the cow's body, the juvenile form migrates and establishes as a cyst in body tissues such as muscles, rather than the gut; they cause more damage to this host than the intestinal form to its host. The parasite completes its life cycle when the grass-eater is eaten by a compatible carnivore—possibly a human with a preference for raw meat—in whose gut the adult *Taenia* establishes itself.

Chapter- 9

Fasciola Hepatica and Heteroecious

Fasciola hepatica

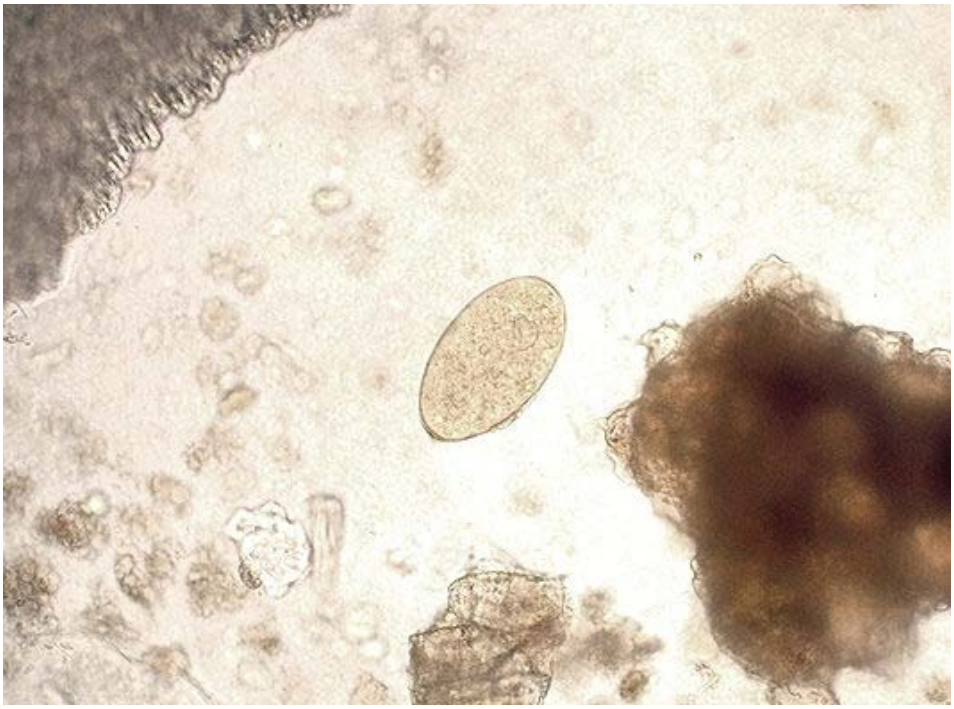
Fasciola hepatica	
	
Fasciola hepatica - adult worm	
Scientific classification	
Kingdom:	Animalia
Phylum:	Platyhelminthes
Class:	Trematoda
Order:	Echinostomida
Family:	Fasciolidae
Genus:	*Fasciola*
Species:	***F. hepatica***
Binomial name	
Fasciola hepatica Linnaeus, 1758	

Fasciola hepatica, also known as the **common liver fluke** or **sheep liver fluke**, is a parasitic flatworm of the class Trematoda, phylum Platyhelminthes that infects liver of various mammals, including humans. The disease caused by the fluke is called fascioliasis (also known as fasciolosis). *F. hepatica* is distributed worldwide and causes great economic losses in sheep and cattle.

Life cycle

In order to complete its life cycle, *F. hepatica* requires an aquatic snail as an intermediate host such as *Galba truncatula*, in which the parasite can reproduce asexually. From the snail, minute cercariae emerge and swim through pools of water in pasture, and encyst as metacercariae on near-by vegetation. From here, the metacercariae are ingested by the ruminant, or in some cases, by humans eating un-cooked foods such as water-cress. Contact with low pH in the stomach causes the early immature juvenile to begin the process of excystment. In the duodenum, the parasite breaks free of the metacercariae and burrows through the intestinal lining into the peritoneal cavity. The newly excysted juvenile does not feed at this stage, but once it finds the liver parenchyma after a period of days, feeding will start. This immature stage in the liver tissue is the pathogenic stage, causing anaemia and clinical signs sometimes observed in infected animals. The parasite browses on liver tissue for a period of up to 5–6 weeks and eventually finds its way to the bile duct where it matures into an adult and begins to produce eggs. Up to 25,000 eggs per day per fluke can be produced, and in a light infection, up to 500,000 eggs per day can be deposited onto pasture by a single sheep.

Disease biology

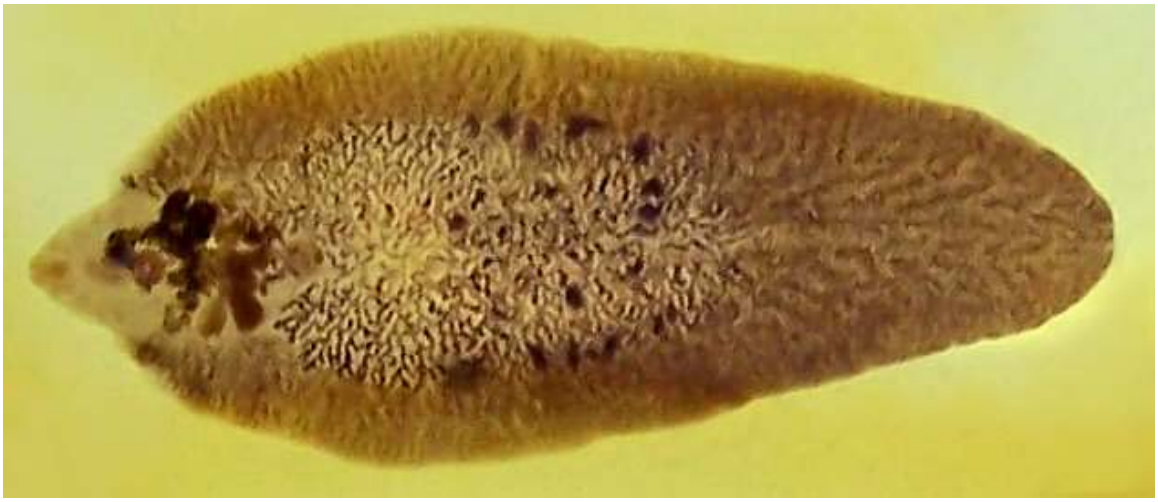


Egg of *F. hepatica*

In the United Kingdom, *Fasciola hepatica* is a frequent cause of disease in ruminants - this is most common between March and December. Cattle and sheep are infected when they consume the infectious stage of the parasite from low-lying, marshy pasture. The effects of liver fluke are referred to as fascioliasis, and include anaemia, weight loss and sub-mandibular oedema. Diarrhea is only an occasional consequence of liver fluke. Liver fluke is diagnosed by yellow-brown eggs in the faeces. They are not distinguishable from the eggs of *Fascioloides magna*, although the eggs of *F. magna* are very rarely passed in sheep, goats or cattle.

A serious consequence of the liver damage caused by fascioliasis is that latent *Clostridium novyi* spores can be activated by the low oxygen conditions in the damaged tracts the parasite forms in the liver - this can lead to "black disease", caused by *Clostridium novyi* type B or immune-mediated haemolytic anaemia (IMHA) leading to haemoglobinuria caused by *Clostridium novyi* type D.

Treatment



Slide showing its internal organs

The drug of choice in the treatment of fasciolosis is triclabendazole, a member of the benzimidazole family of anthelmintics. The drug works by preventing the polymerization of the molecule tubulin into the cytoskeletal structures, microtubules. However, resistance of *F. hepatica* to triclabendazole has already been recorded in Australia and Ireland. Artemether has been shown to be effective in a rat model of fascioliasis.

Heteroecious

A **heteroecious** parasite is one that requires at least two hosts. The *primary host* is the host in which the parasite spends its adult life; the other is the *secondary host*. Both the primary host and an unrelated alternate host are required for the parasite to complete its life cycle. This can be contrasted with an **autoecious** parasite which can complete its life cylce on a single host species. Many rust fungi are prime examples of a heteroecious life cylce.

Parasitic heteroecious fungi include

- *Gymnosporangium* (Cedar-apple rust); the juniper is the primary (telial) host and the apple, pear or hawthorn is the secondary (aecial) host
- *Cronartium ribicola* (White pine blister rust); the primary host are white pines, and currants the secondary.
- *Hemileia vastatrix* (Coffee rust); Primary host is coffee plant; Unknown alternate host
- *Puccinia graminis* (Stem rust of wheat and Kentucky bluegrass); Primary hosts include: Kentucky bluegrass, barley, and wheat; Common barberry is the alternate host.
- *Puccinia coronata* (Crown Rust of Oats and Ryegrass); Oats are the primary host; *Rhamnus spp.* (Buckthorn) is alternate host.
- *Phakopsora meibomiae* and *P. pachyrhizi* (Soybean Rust); Primary host is soybean and various legumes. Unknown alternate host.

History

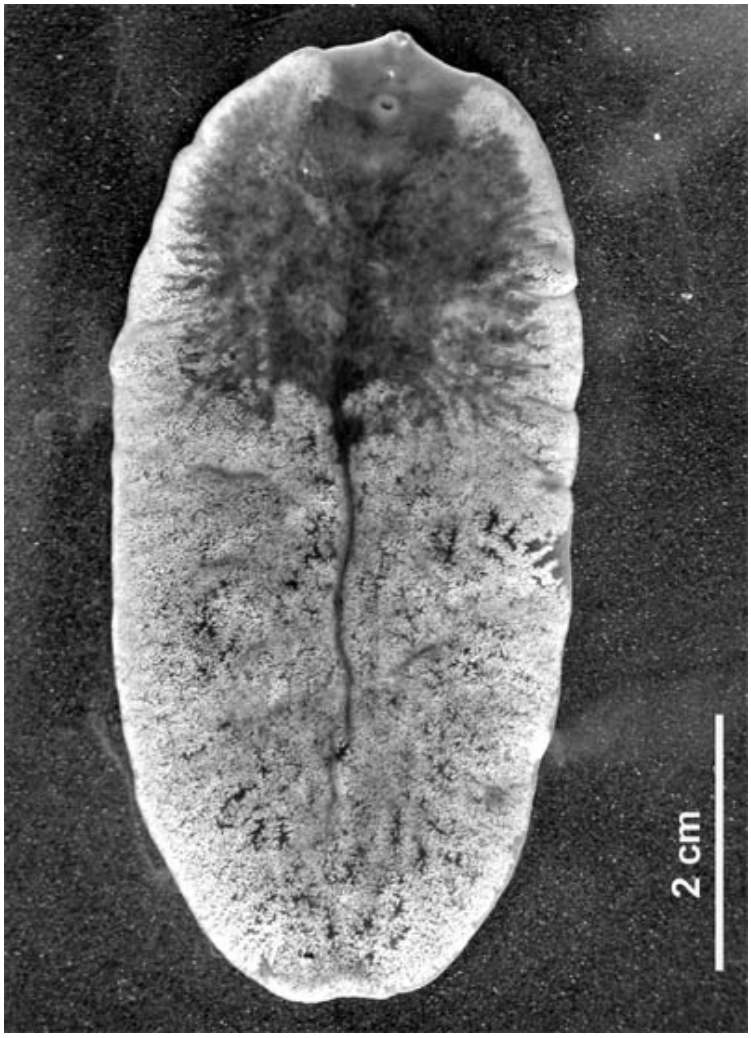
The phenomenon of **heteroecy** was first discovered by A.S. Ørsted in 1863.

- Ørsted, A.S. (1963) Om Sygdomme hos Planterne, som foraarsages af Snyltesvampe, navnlig om Rust og Brand og om Midlerne til deres Forebyggelse. Kjøbenhavn.

Chapter- 10

Fascioloides Magna

Giant liver fluke



Scientific classification

Kingdom: Animalia

Phylum:	Platyhelminthes
Class:	Trematoda
Subclass:	Digenea
Order:	Echinostomida
Family:	Fasciolidae
Genus:	*Fascioloides*
Species:	***F. magna***

Binomial name

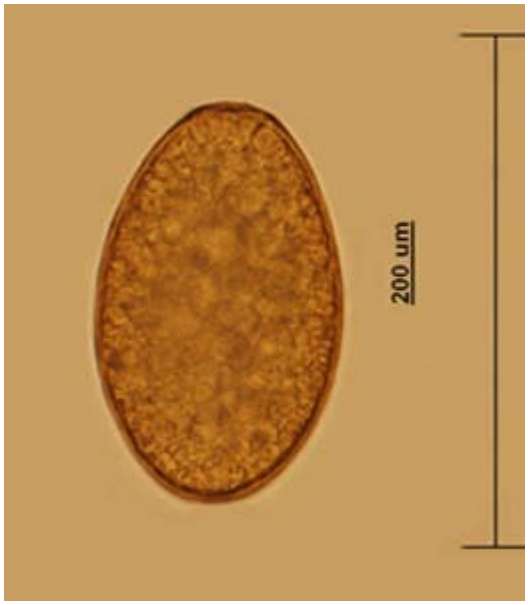
Fascioloides magna
Bassi, 1875

Fascioloides magna, also known as **giant liver fluke**, **large American liver fluke** or **deer fluke**, is an important parasite of a variety of wild and domestic ruminants in North America and Europe. Adult flukes occur in the liver of the definitive host and feed on blood. Mature flukes measure 4 to 10 cm in length × 2 to 3.5 cm in width, and have an oval dorso-ventrally flattened body with oral and ventral sucker. The flukes are reddish-brown in colour and are covered by tegument. Similarly to that in other digenean trematodes, the life cycle includes intramolluscan phase in snails.

History

Fascioloides magna is essentially of North American origin but the parasite was introduced into Europe with imported game animals at the second half of the 19th century. In spite of being native to North America the fluke was first described in Italy. In 1875, Bassi observed massive deaths of red deer in the Royal Park near Torino, Italy. The signs were similar to well known fasciolosis in sheep. He named it *Distomum magnum*. The author believed that the parasite was introduced into the park in wapiti imported from USA in 1865. Most workers did not accept Bassi's species because of his poor description. From 1882 to 1892, the fluke was recorded from different areas of the United States and described separately by many authors. Later, Stiles (1894) pointed out that the American findings are identical with species described previously by Bassi. Stiles made a complete morphological description of the adult fluke and named it *Fasciola magna* (Bassi 1875) Stiles 1894. In 1917, Ward showed that owing to the lack of the distinct anterior cone and the fact that vitellaria are confided to the region ventral to the intestinal branches, he established a new genus *Fascioloides* and rename it to *Fascioloides magna* (Bassi 1875) Ward 1917. In 1895, Stiles suggested that the life cycle of the fluke is very similar to *Fasciola hepatica*, i.e. it includes an aquatic snail as an intermediate host. He gave a comparative description of the egg and miracidium of the fluke. However, first reported intermediate hosts of *F. magna* were not published until 1930's. The complete life cycle of *F. magna*, including a description of all the larval stages, was described by Swales (1935) in Canada.

Life cycle



The egg of *F. magna*

The life cycle of *F. magna* is relatively complex and is similar to the development of the related fluke, *F. hepatica*. A detailed account of the *F. magna* life cycle was given by Swales (1935), Erhardová-Kotrlá (1971), and reviewed by Pybus (2001).

The cercariae of *F. magna* shedded from the snail

Adult flukes occur in pairs or groups within a fibrous capsule in the liver parenchyma of the definitive host. Mature flukes release eggs which are collected in the cavity of the capsule. The capsule contains a great mass of eggs and has duct connections to bile ducts.

The eggs are passed together with bile into the bile collecting system, enter the small intestine, and leave the definitive host along with the faeces. The eggs which are passed out in the faeces into the environment are undeveloped and undergo embryonation outside the host. Several physical-chemical factors, especially temperature, humidity and oxygen tension, are known to influence embryonation. During the embryonation of the egg, a larva called a miracidium develops from germinal cells. Fully developed miracidium releases the operculum of the egg using several proteases. The embryonation period varies from 27 to 44 days in natural conditions. Ciliated miracidia hatch in water and actively seek suitable intermediate hosts that are freshwater snails from family Lymnaeidae. After attaching to a suitable snail host, the miracidium penetrates into the snail body. After shedding its ciliated cell layer it is called a sporocyst. The sporocysts are found in the foot, the snail body, digestive glands, reproductive organs, and in the pulmonary sac of the snail. The sporocysts contain germinal cells that give rise to 1-6 mother rediae. Developed mother rediae are released from the sporocyst and migrate into digestive glands, renal organ, reproductive organs, and pulmonary sac of the snail body. Each mother redia can produce up to 10 daughter rediae. However, only 3 to 6 daughter rediae complete their development and leave the mother rediae. In turn, each daughter redia may produce 1-6 cercariae in experimentally infected snails and 16-22 cercariae under natural conditions. Cercariae emerge from the rediae and mature usually in digestive glands of the snail. Mature cercarie spontaneously emerge from the snail host and swim actively in water for up to two hours before encysting on vegetation. After encystment the flukes are called metacercariae. Development within the snail takes 40 to 69 days depending upon the temperature and the species of snail. The definitive host ingests vegetation containing the metacercariae. In the stomach and the intestine, the metacercariae are stimulated to emerge from the cyst (excystation). Newly excysted juvenile flukes penetrate the wall of the intestine and migrate in the abdominal cavity. Juvenile flukes penetrate the Glisson's capsule of the liver and continue migrating in the liver tissue. Rarely juvenile flukes penetrate other organs, such as lungs or kidneys. In these organs, however, flukes do not survive and not attain maturity. In the liver, flukes migrate within the parenchyma to search another fluke. If the fluke meet another one, they stop moving, and the fibrous capsule is formed around them. In the capsule, the parasite completes its development and starts egg-laying. Prepatent period varies 3–7 months and is dependent on host species. Adult *F. magna* can survive in the liver of the host up to 7 years.

Distribution

Distribution of *F. magna* in North America

Currently, *F. magna* occurs only in North America and Europe where suitable habitat exists and susceptible intermediate hosts are found. However, sporadic works reported unique appearance of the fluke in other continents. *F. magna* was found in imported animals in South Africa, Australia and Cuba. In all cases, infected animals (brahman heifer, ox, and elk, respectively) were imported from USA or Canada.

North America

During the 20th century, *F. magna* was reported in these American states: Arkansas, California, Colorado, Illinois, Iowa, Kansas, Louisiana, Michigan, Minnesota, Montana, New York, Oklahoma, Oregon, South Carolina, Texas, Washington, and Wisconsin. In Canada, the fluke was reported in Alberta, British Columbia, Ontario, and Quebec. Currently, *F. magna* is enzootic in five major areas: (1) the Great Lakes region; (2) the Gulf coast, lower Mississippi, and southern Atlantic seaboard; (3) northern Pacific coast; (4) the Rocky Mountain trench; and (5) northern Quebec and Labrador. However, within

these broad ranges, actual presence of giant liver flukes varies from locally abundant to locally absent.

Europe

Fascioloides magna was first reported by Bassi in Torino, Italy. In spite of Bassi's work, no other data concerning the occurrence of F. magna in Europe were reported until 1930's. In the Czech territory, Ullrich reported the first appearance of *F. magna* in fallow deer as late as 1930. At the same time, Salomon (1932) diagnosed the fluke in one hunted red deer near Görlitz (Saxony) in Germany. Other isolated findings of the fluke were recorded in Italy and Poland. From 1948 till 1961, sporadic occurrence of the parasite in red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) were reported by several authors in former Czechoslovakia. However, all reports were published on the basis of incident discoveries in hunted deer and no massive infections were documented.

In 1960's, a number of *F. magna* outbreaks in cervids were reported in some areas of former Czechoslovakia. The prevalence of infection varied from 70 to 80 % in red deer and maximum parasite burden was 144 worms. In addition, sudden deaths were documented in free or game ranging deer. The highest mortality was reported in free ranging roe deer in Písek County in the South Bohemia of former Czechoslovakia. In the same region, moreover, the parasite was found in livers of slaughtered cattle.

Distribution of *F. magna* in Europe

Erhardová-Kotrlá (1971) confirmed red deer, fallow deer and roe deer as main definitive hosts of *F. magna* in Europe. In 1960's, *F. magna* was enzootic in former Czechoslovakia in following four major areas: (1) České Budějovice and Třeboň county, including Nové Hrady Mountains; (2) the area along the Vltava River on the Vltava-Týn hills near Hluboká and Bechyně; (3) Písek and Milevsko county; (4) the Brdy mountains and the Hřebeny mountains. In following years, *F. magna* was only reported from these areas. Recently, geographical distribution of *F. magna* in cervids was determined in the Czech Republic. The giant liver fluke was confirmed in the same areas as reported in 1960's. However, seven new endemic areas of *F. magna* were discovered suggesting that the parasite is spreading in the Czech Republic. Moreover, the appearance of *F. magna* in the Šumava Mountains has epizootiological importance due to possibility of spread of the parasite into the German territory (Bavaria). During the last few years, a new European enzootic area has established in the Danube watershed in Central Europe. In 1988, *F. magna* was isolated from a 3-year old red deer female found dead near the Gabčíkovo water plant at the Danube River in Slovakia. The parasite has spread through whole Slovakian Danube watershed.

Soon after the Slovakian first report, *F. magna* was found in red deer in Hungarian parts of Danubian floodplain forests. The prevalence reported by the same authors was up to 90 %. *F. magna* infection of cervids is a considerable problem in northern part of Hungary (Szigetköz) and the southern Danubian territory in the Gemenc area. Since the autumn of 2000, *F. magna* has been found in Austrian territory, east of Vienna. In years 2000-2001, the prevalence of the giant liver fluke in red deer in Austrian parts of Danube (east of Vienna) was 66.7 %. Appearance of American liver fluke was reported in Croatia in January 2000. The prevalence of fascioloidosis among red deer in Hungary was 21,1-60,7 % between 1998-2005. During the necropsy of 459 deer livers (using Egri's method) the number of flukes per host ranged from 1 to 138 in the same period. Regarding the origin of *F. magna* enzootic area in the Danube River watershed, it is essential to point out that cervids were not introduced into these localities, neither recently nor in the past. Origin of the *F. magna* population in Danubian floodplain forests in Central Europe remains therefore unclear.

Definitive hosts

White-tailed deer - a typical definitive host of the giant liver fluke

Natural infections of *F. magna* occur primarily in cervids and bovids. Although many species are susceptible to infection, only a few cervid species contribute significantly to maintaining populations of the fluke. In North America, the common definitive hosts of the giant liver fluke are wapiti (*Cervus elaphus canadensis*), white-tailed deer (*Odocoileus virginianus*) and caribou (*Rangifer tarandus*). In Europe, *F. magna* occurs commonly in red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*). Domestic ruminants are also susceptible to natural infection with *F. magna*. However, the infection is not patent, and domestic ruminants do not contribute to the propagation of the parasite in the environment. In North America, the giant liver fluke is commonly found in cattle, sheep and goats in areas where *F. magna* is enzootic in deer. In contrast, *F. magna* occurs rarely in domestic ruminants in Europe. The list of all natural definitive hosts of *F. magna* is presented in **Table**.

The only indigenous primary definitive host of *F. magna* is white-tailed deer. This species has been parasitized by the fluke for the longest time in historical context. Wapiti and caribou are of Eurasian origin and entered North America during the Pleistocene epoch, and overlapped with white-tailed deer in some parts of North America. They might have encountered *F. magna* in these shared biotopes.

Common name of species	Latin name of species
NORTH AMERICA	
Bison	*Bison bison*
Black-tailed deer	*Odocoileus hemionus columbianus*
Caribou	*Rangifer tarandus*
Cattalo	*Bos taurus* × *Bison bison*
Cattle	*Bos taurus*
Collared peccary	*Dicotyles tajacu*
Goat	*Capra hircus*
Horse	*Equus caballus*
Llama	*Lama glama*
Moose	*Alces alces*
Mule deer	*Odocoileus hemionus hemionus*
Pig	*Sus scrofa* var. *domesticus*
Sheep	*Ovis aries*
Wapiti	*Cervus elaphus canadensis*
White-tailed deer	*Odocoileus virginianus*
Wild boar	*Sus scrofa*
Yak	*Bos grunniensis*
EUROPE	
Blue bull	*Bosephalus tragocamelus*
Cattle	*Bos taurus*
Fallow deer	*Dama dama*
Goat	*Capra hircus*
Horse	*Equus caballus*
Red deer	*Cervus elaphus*
Roe deer	*Capreolus capreolus*
Sambar	*Cervus unicolor*
Sheep	*Ovis aries*
Sika deer	*Sika nippon*
White-tailed deer	*Odocoileus virginianus*
Wild boar	*Sus scrofa*

Clinical signs, pathology and pathophysiology

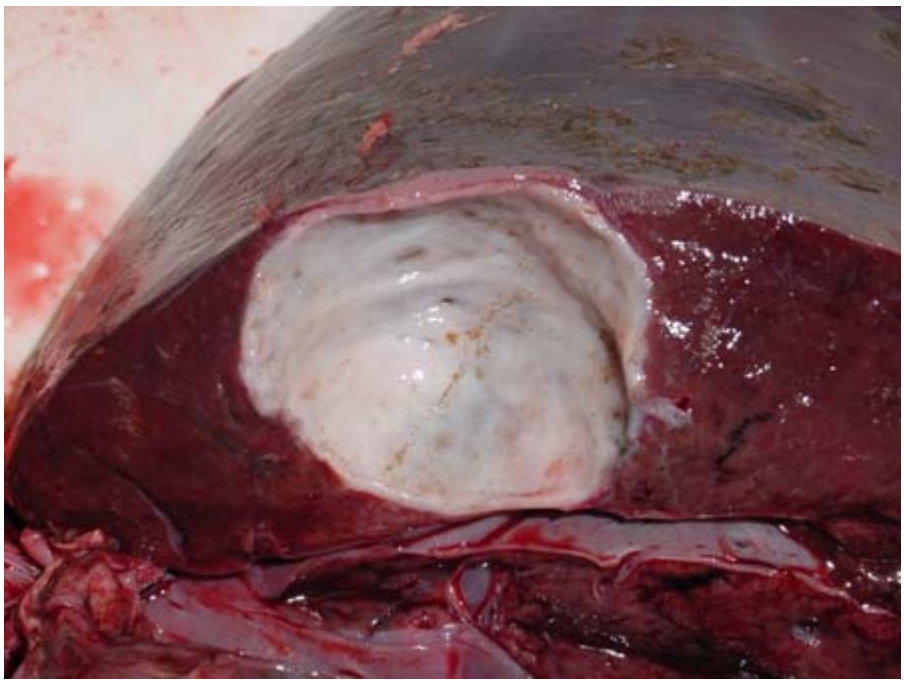
According to several American authors, three types of definitive host exist:

- (1) **definitive hosts**
- (2) **dead-end hosts**

- (3) **aberrant hosts**

Pathology of *F. magna* infection varies according to host type but some features are shared by all three types. Primary lesions usually occur in the liver and are associated with mechanical damage due to migrating juvenile flukes or fibrous encapsulation of sedentary adult flukes. The most common feature of *F. magna* infection is black pigmentation in abdominal or thoracic organs, especially in the liver. The hematin pigment is produced by flukes as a byproduct of feeding on blood. Pigment within tissues is a result of migrating of juvenile flukes and it accumulates within hepatic cells without resorption.

(1) Definitive hosts



The fibrous capsule in the liver parenchyma of red deer infected with *F. magna*

Definitive hosts are primarily New World and some Old World cervids. In definitive hosts, flukes are encapsulated in thin-walled fibrous capsules communicating to the bile system. The eggs are passed through the bile system, enter the small intestine, and leave the host with faeces. Therefore, the infection is patent. The capsules are a result of the defence response of the host to the parasite and are pathognomonic for *F. magna* infection. They contain two to five flukes, greyish-black fluid with eggs and cell detritus.

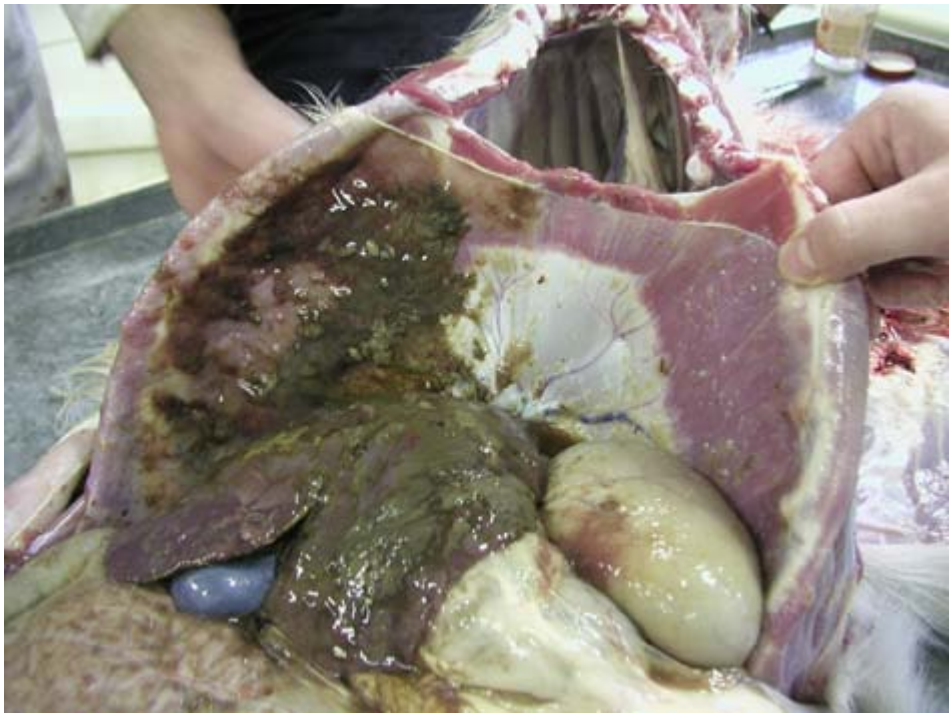
F. magna infections in definitive hosts are usually subclinical. However, massive deaths caused by the fluke in red-, fallow- and roe deer were reported. Lethargy, depression, weight loss and decreased quality of antlers can occur sporadically. In addition, nervous symptoms were observed very rarely. In the first case, urging motion followed by apathy was reported in one experimentally infected fallow deer. Authors suggested that these symptoms were associated with hepatocerebral syndrome. Other author has observed

partial paralysis in naturally infected wapiti caused by migrating juvenile flukes in the spinal cord. Biochemical and haematological profiles are little investigated in definitive hosts. A decrease of haemoglobin, elevation of γ-globulins, and increase of eosinophils in serum was observed in experimentally infected white-tailed deer.

(2) Dead-end hosts

Dead-end hosts are represented by large bovids, suids, llamas, horses and some Old World cervids. Infections in dead-end hosts are characterized by excessive fibrosis, thick-walled encapsulation of flukes within hepatic parenchyma, and black pigmentation of various tissues. Both afferent and efferent bile ducts are totally occluded and are marked by tracts of fibrous tissue. The eggs can not be passed into the bile system, and, therefore, the infection is not patent. In addition, flukes rarely mature in dead-end hosts probably due to strong immune response. Nevertheless, appearance of *F. magna* eggs in the faeces of single experimentally infected calf has been documented. Pathophysiology or clinical symptoms in dead-end hosts have been rarely studied. In cattle, significant elevations of eosinophil counts in periphery blood but only slight increases of AST and GGT have been observed. While American authors have not observed any clinical symptoms in cattle, anorexia and weight loss were recorded in naturally infected bulls in the former Czechoslovakia.

(3) Aberrant hosts



Necropsy of goat infected with *F. magna*: fibrin between liver and diaphragm

Aberrant hosts of *F. magna* are sheep and goats. However, the course of infection is similar in guinea pigs, rabbits, bighorn sheep (*Ovis canadensis*) and chamois (*Rupicapra*

rupicapra) that were infected experimentally. Infections in aberrant hosts are characterized by excessive wandering of juvenile flukes and death of the host. Aberrant hosts die usually within 6 months post-infection and the death is associated with acute peritonitis or extensive haemorrhage caused by migrating flukes. In aberrant hosts, flukes do not mature and migrate until the host dies. Occasionally, a few flukes mature and eggs can be found in the faeces. Hepatic lesions in aberrant hosts generally include firm adhesions of the liver to the diaphragm, black pigmentation, hematomas, necroses, and haemorrhagic tracts in which juvenile flukes are located. While a lack of fibrous capsules within hepatic parenchyma has been reported by several authors, flukes in fibrous capsules have also been documented in sheep. However, the wall of the capsule is different from those found in cervids and large bovids. The dominant feature is a diffuse fibrosis throughout the liver and haemorrhagic migratory tracts containing erythrocytes, black pigment, and cell detritus. The liver lesions are infiltrated by eosinophils, plasma cells, and pigment-laden macrophages.

Sheep and goats die acutely without any previous clinical signs. Only elevation of eosinophils and slight increase of γ-globulins were observed in experimentally infected sheep. Recently, several changes in biochemical and haematological profile have been documented in experimentally infected goats. The significant increase of GLDH (glutalaldehyde dehydrogenase) was recorded from 14 week after infection in goats experimentally infected with *F. magna*.

Intermediate hosts

Intermediate hosts of *F. magna* in Europe: freshwater snails, *G. truncatula* (above) and *R. peregra* (below).

Since the presence of an intermediate host is essential to the completion of the life cycle, snails occupy the important role in the epidemiology of *F. magna*. The intermediate hosts of the giant liver fluke belong to the family Lymnaeidae. In North America, a total of 10 lymnaeid snails were reported as intermediate hosts of *F. magna*. 6 of 10 North American snail species were found naturally infected and the other four were infected only under experimental conditions. In addition, the Australian species *Austropeplea* (*Lymnaea*) *tomentosa* was exposed to the North American isolate of *F. magna* and the parasite was able to complete its development. The most common North American natural snail hosts

of the fluke are *Fossaria* (*Galba*) *modicella*, *Stagnicola* (*Lymnaea*) *caperata* and *Fossaria* (*Galba*) *bulimoides techella*. In Europe, an intermediate host had not been known until 1960's. At the beginning, Ślusarski assumed that *Lymnaea stagnalis* could act as an intermediate host of *F. magna* in Europe. His assumption, however, has been neither confirmed by positive findings in the field nor by experimental infection. In 1961, Dr. Erhardová described the life cycle of *F. magna* based on observations of experimentally and naturally infected snails. She confirmed that *Galba truncatula* is an intermediate host of the giant liver fluke in Europe. In later works, the author studied another lymnaeid species in the former Czechoslovakia. However, *G. truncatula* was repeatedly confirmed as the only snail host of *F. magna*. In 1979, Chroustová reported successful experimental infection of *Stagnicola* (*Lymnaea*) *palustris* with *F. magna*. She considered that this species might serve as an intermediate host of the fluke in the environment. Nevertheless, no naturally infected snails were found. Recent studies indicate that another lymnaeid snail, *Radix peregra*, may be also involved in the transmission of *F. magna* in Europe. This opinion is supported by successful experimental infection of *R. peregra* in the lab as well as by findings of naturally infected *R. peregra* in the environment. These findings suggested that the intermediate host spectrum of *F. magna* should be, similarly to North America, diverse in Europe. The list of intermediate hosts of *F. magna* is presented in following table.

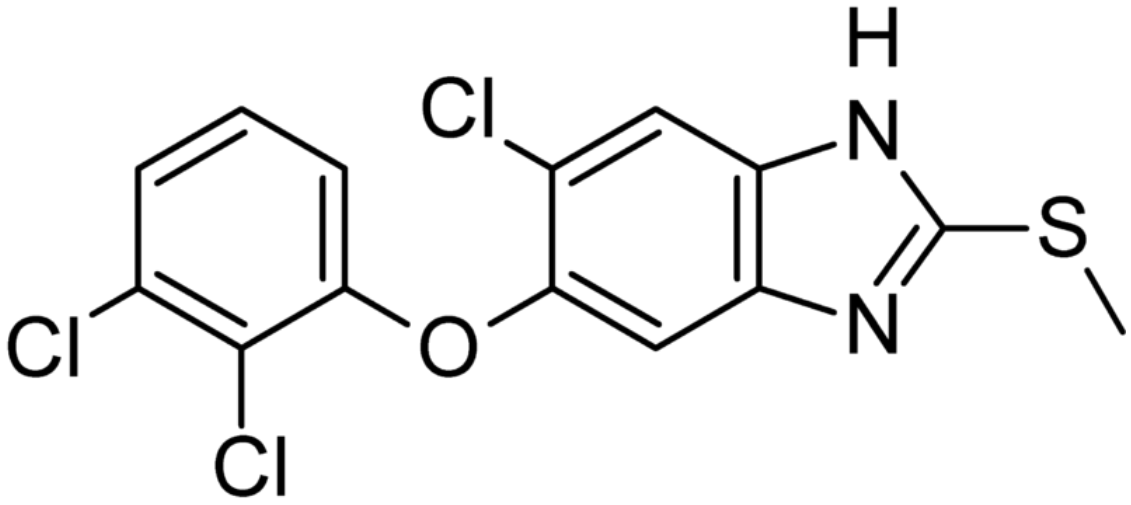
Snail species	Naturally infected	Experimentally infected	Country
NORTH AMERICA			
Fossaria (*Galba*) *bulimoides techella*	yes	yes	United States
Fossaria (*Galba*) *modicella*	yes	yes	United States, Canada
Pseudosuccinea columella	yes	yes	United States
Fossaria (*Galba*) *parva*	yes	yes	Canada
Stagnicola palustris nuttalliana	yes	yes	Canada
Lymnaea stagnalis	no	yes	United States
Stagnicola palustris	no	yes	United States
Stagnicola (*Lymnaea*) *caperata*	yes	yes	United States
Lymnaea ferruginea	no	yes	United States
Austropeplea (*Lymnaea*) *tomentosa*	no	yes	Australia*
Lymnaea umbrosa	no	yes	United States
EUROPE			
Galba truncatula	yes	yes	Czech Republic
Stagnicola (*Lymnaea*) *palustris*	no	yes	Czech Republic
Omphiscola glabra	no	yes	France**
Radix peregra	yes	yes	Czech Republic

(*) Snails originated from Australia infected with United States isolate of *F. magna*
(**) Snails originated from France infected with Czech isolate of *F. magna*

Diagnosis

While the eggs of *F. magna* resemble those of *F. hepatica*, this similarity is of limited use; eggs usually are not passed in cattle and sheep. Recovery of the parasites at necropsy, as well as proper identification of *F. hepatica* or *F. gigantica* is necessary for definite diagnosis. When domestic ruminants and deer share the same grazing areas, the presence of disease due to *F. magna* should be kept in mind. Mixed infections with *F. hepatica* occur in cattle.

Control of F. magna *and prevention*



Formula of triclabendazole

For control of fascioloidosis in wild ruminants, successful application of anthelminthics in feed is necessary. The drug has to have flavour and smell that do not prevent animals from eating medicated feed. In addition, there should adequate therapeutic scope, i.e. span between therapeutic and minimal toxic dose. Therefore, only some of anthelminthics that are efficient in domestic ruminants have been tested in wild ruminants infected with *F. magna*. Several drugs, namely oxyclosanide, rafoxanide, albendazole, diamphenetide, closantel, clorsulon, and triclabendazole, have been used in control of *F. magna* infection in cervids. However, the results have differed between different authors. In the same way as for *F. hepatica*, triclabendazole seems to be the most effective against *F. magna*. Fascioloidosis of cervids was successfully controlled with triclabendazole in USA, and triclabendazole with levamizole in Hungary, Canada, Austria, and Croatia. In contrast, rafoxanide is commonly used in treatment in Czech Republic Slovakia and Hungary. Nevertheless, recent studies suggested that use of rafoxanide in control of *F. magna* infection should be considered. Unfortunately, rafoxanide in a commercial drug called Rafendazol Premix is the only registered drug for wild ruminants. Triclabendazole and others are produced as drugs for domestic animals and it can be used in free-living animals only with special permit.

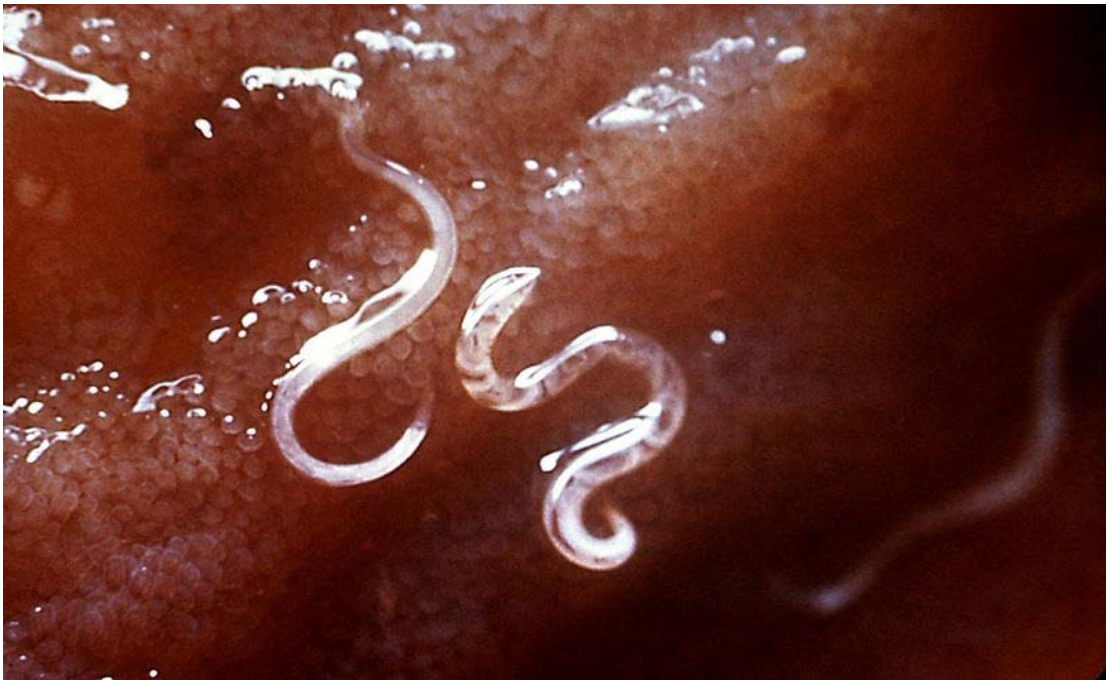
Chapter- 11

Parasitic Worm

Parasitic worms or **helminths** are a division of eukaryotic parasites that, unlike external parasites such as lice and fleas, live inside their host. They are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease. Those that live inside the digestive tract are called intestinal parasites. They can live inside humans as well as other animals. Approximately 3 billion people globally are infected with helminths.

Helminthology is the study of parasitic worms and their effect on their hosts. The word helminth comes from Greek hélmins, a kind of worm.

Categorization



Hookworms attached to the intestinal mucosa

Scolex (head) of *Taenia solium*, a tapeworm

Two pinworms

Parasitic worms are categorized into three groups: cestodes (tapeworms), nematodes (roundworms), and trematodes (flukes). The following table shows the principal morphologic differences of these different families of helminths:

	Cestodes (tapeworms)	Trematodes (flukes)	Nematodes (roundworms)
Shape	Segmented plane	Unsegmented plane	Cylindrical
Body cavity	No	No	Present
Body covering	Tegument	Tegument	Cuticle
Digestive tube	No	Ends in cecum	Ends in anus
Sex	Hermaphroditic	Hermaphroditic, except schistosomes which are dioecious	Dioecious
Attachment organs	Sucker or bothridia, and rostellum with hooks	Oral sucker and ventral sucker or acetabulum	Lips, teeth, filariform extremities, and dentary plates
Example diseases in humans	Tapeworm infection	Schistosomiasis, swimmer's itch	Ascariasis, dracunculiasis, elephantiasis, enterobiasis (pinworm), filariasis, hookworm, onchocerciasis, trichinosis, trichuriasis (whipworm)

Note that ringworm (dermatophytosis) is actually caused by various fungi and not by a parasitic worm.

Reproduction and proliferation

Parasitic worms are sequential hermaphrodites and reproduce depending on the species of worm, either with the presence of a male and female worm, joining sperm and eggs, producing fertile eggs, such as hookworms, or by breaking off segments that contain both male and female sex organs that are able to produce fertile eggs without the presence of a male or female (e.g., tapeworms).

All worm offspring are passed on through poorly-cooked meat, especially pork, wild fish, and beef, contaminated water, feces and mosquitoes. However, it is estimated that 40 million Americans are infected with the most common roundworm in the United States, the pinworm.

Worm eggs or larvae or even adults enter the human body through the mouth, anus, nose, or skin, with most species attaching themselves to the intestinal tract. With the presence of digestive enzymes, worm egg shells are dissolved, releasing a brand-new worm; unlike its egg shell, the parasitic worm is protected from the body's powerful digestive enzymes by producing a protective keratin layer.

Acquisition

Helminths often find their way into a host through mosquito transmission, eating infected food, drinking contaminated water, and walking on infected soil. This is especially a problem in the developing world where food and water is usually unclean, and many people simply do not own shoes. Many walk miles barefoot only to collect contaminated water for their families, and as a result contract diseases and helminths.

Immune response

Response to worm infection in humans is a Th2 response in the majority of cases. Inflammation of the gut may also occur, resulting in cyst-like structures forming around the egg deposits throughout the body. The host's lymphatic system is also increasingly taxed the longer helminths propagate, as they excrete toxins after feeding. These toxins are released into the intestines to be absorbed by the host's bloodstream. This phenomenon makes the host susceptible to more common diseases such as seasonal viruses and bacterial infections.

Intestinal helminths

Intestinal helminths are a type of intestinal parasite that reside in the human gastrointestinal tract. They represent one of the most prevalent forms of parasitic disease. Scholars estimate that over a quarter of the world’s population is infected with an intestinal worm of some sort, with roundworm, hookworm, and whipworm infecting 1.47 billion people, 1.05 billion people, and 1.30 billion people, respectively. Furthermore, the World Bank estimates that 100 million people may experience stunting or wasting as a result of infection.

Because of their high mobility and lower standards of hygiene, school-age children are particularly vulnerable to these parasites. Overall, it is estimated that 400 million, 170 million, and 300 million children are infected with roundworm, hookworm, and whipworm. Children may also be particularly susceptible to the adverse effects of helminth infections due to their incomplete physical development and their greater immunological vulnerability.

Costs of intestinal helminth infection

Symptoms

In patients with a heavy worm load, parasite infection is frequently symptomatic. Conditions associated with intestinal helminth infection include intestinal obstruction, insomnia, vomiting, weakness, and stomach pains; while the natural movement of worms and their attachment to the intestine may be generally uncomfortable for their hosts. The migration of Ascaris larvae through the respiratory passageways can also lead to temporary asthma and other respiratory symptoms.

In addition to the low-level costs of chronic infection, helminth infection may be punctuated by the need for more serious, urgent care; for example, the World Health Organization found that worm infection is common reason for seeking medical help in a variety of countries, with up to 4.9% of hospital admissions in some areas resulting from the complications of intestinal worm infections and as many as 3% of hospitalizations attributable to ascariasis alone.

Also worth considering is the fact that the immune response triggered by helminth infection may drain the body's ability to fight other diseases, making affected individuals more prone to co-infection. There are reasonable evidences indicating that helminthiasis is responsible for the unrelenting prevalence of AIDS and tuberculosis in developing, particularly African, countries. A review of several data clearly revealed that effective treatment of helminth infection reduces HIV progression and viral load, obviously by improving helminth-induced immune suppression.

Nutrition

One way in which intestinal helminths may impair the development of their human hosts is through their impact on nutrition. Intestinal helminth infection has been associated with problems such as vitamin deficiencies, stunting, anemia, and protein-energy malnutrition, which in turn affect cognitive ability and intellectual development. This relationship is particularly alarming because it is gradual and often relatively asymptomatic.

Parasite infection may affect nutrition in several ways. On the one hand, some scholars argue that worms may compete directly with their hosts for access to nutrients; both whipworm and roundworm are believed to impact their hosts in this way. Nonetheless, Watkins and Pollitt argue that the magnitude of this effect is likely to be minimal; after all, the nutritional requirements of these intestinal worms is small when compared with that of their host organism.

A more probable source of infection-induced malnutrition is the nutrient malabsorption associated with parasite presence in the body. For example, in both pigs and humans, Ascaris has been tied to temporarily induced lactose intolerance and Vitamin A, nitrogen, and fat malabsorption. Impaired nutrient uptake may result from direct damage to the intestine's mucosal walls as a result of the worms' presence, but it may also be a

consequence of more nuanced changes such as chemical imbalances caused by the body's reaction to the helminths. Alternately, Watkins and Pollitt suggest that the worms' release of protease inhibitors to defend against the body's digestive process may impair the breakdown of other, nutritious substances as well. Levinger mentions this briefly in the case of whipworm. Finally, worm infections may also cause diarrhea and speed "transit time" through the intestinal system, further reducing the body's opportunity to capture and retain the nutrients in food.

Worms may also contribute to malnutrition by creating anorexia. A decline in appetite and food consumption due to helminthic infection is widely recognized by the literature, with a recent study of 459 children in Zanzibar reporting that even mothers noticed spontaneous increases in appetite after their children underwent a deworming regime. Although the exact cause of such anorexia is not known, researchers believe that it may be a side effect of body's immune response to the worm and the stress of combating infection. Specifically, some of the cytokines released in the immune response have been tied to anorexic reactions in animals.

Helminths may also affect nutrition by inducing iron-deficiency anemia. This is most severe in heavy hookworm infections, as N. Americanus and A. Duodenale feed directly on the blood of their host. Although the impact of individual worms is limited (each consumes about .02-.07 ml and .14-.26 ml of blood daily, respectively), this may nonetheless add up in individuals with heavy infections, since they may carry hundreds of worms at a given time. One scholar went so far as to predict that "the blood loss caused by hookworm was equivalent to the daily exsanguination of 1.5 million people," while a study in Zanzibar showed that a 15¢ triannual application of Mebendazole could avert 0.25 l of blood loss per child per year. Although whipworm is milder in its effects, it may also induce anemia as a result of the bleeding caused by its damage to the small intestine.

The connection between worm burden and malnutrition is further supported by studies indicating that deworming programs lead to sharp increases in growth; the presence of this result even in older children has lead some scholars to conclude that "it may be easier to reverse stunting in older children than was previously believed." Other, less clearly causal studies also show a strong correlation between worm burden and malnourishment among school-age children.

Delayed intellectual development

Once the links between helminth infection and various forms of malnutrition are established, there are a number of pathways by which parasite burden may affect cognition. For example, poor performance on normal growth indicators appears to be correlated with lower school achievement and enrollment, worse results on some forms of testing, and a decreased ability to focus; on the other hand, iron deficiency may result in "mild growth retardation," difficulty with abstract cognitive tasks, and "lower scores...on tests of mental and motor development...[as well as] increased fearfulness, inattentiveness, and decreased social responsiveness" among very young children.

Anemia has also been associated with reduced stamina for physical labor, a decline in the ability to learn new information, and "apathy, irritability, and fatigue."

These connections are supported by a number of deworming studies. For example, using 47 students from the Democratic Republic of the Congo, Boivin and Giardani (1993) found that iron supplements acted as a complement to deworming medication, producing better effects on mental cognition when they were applied in conjunction than when they were individually administered. He hypothesized that this result was due to the fact that iron supplements may "improve [students'] physical well-being to the point of enhancing attentional or arousal mechanisms influential in learning and cognitive performance," with deworming medication only acting to extend these benefits by further reducing the tendency to anemia.

Perhaps even more fascinating are a number of papers that take the study of intestinal helminth beyond the malnutrition-cognition link to focus on the connections between worm infections and memory formation. For example, Nokes et. al. (1992) find that interventions to reduce whipworm infection in 159 Jamaican schoolchildren led to better "auditory short-term memory" and "scanning and retrieval of long-term memory;" particularly fascinating was his discovery that a nine-week period was all that was necessary for dewormed students to "catch up" to their worm-free peers in test performance. Nokes' optimistic conclusion that "whipworm infection['s]...adverse effect on certain cognitive functions...is reversible by therapy" is particularly significant because it suggests that the effects of worms on intellectual performance may not be restricted to the mechanism of long-term malnutrition, since the physical and developmental effects of such malnutrition would theoretically be irreversible.

Also worth noting are the studies of Ezeamama et. al. (2005) and Sakti et. al. (1999), which studied worm burden in the Philippines and Indonesia, respectively. Both authors found significant negative impacts of helminthic infection on memory and fluency, findings that are particularly meaningful because they included controls for socioeconomic status, hemoglobin levels, and proxies of nutrition (nutritional status and stunting, respectively). As Ezeamama observes, these studies suggest "that undernutrition is not the primary mediator of the observed relationships" between worm infection and intellectual performance, particularly because their findings were significant in aspects of intellect that went beyond mere cognition and reaction time.

Finally, Watkins and Pollitt observe that, much as physical activity is "nutritionally mediated" as patients with heavy worm burden struggle to preserve energy and fight malnutrition, so too could "the poorly nourished mind similarly adapt...by reducing mental effort in the form of arousal and sustained attention." While they find little evidence that this adaptation would provide benefits in the form of energy conservation, it is clear that the active course of ongoing parasitic disease could impose other, more direct limitations on an individual's attention span.

School attendance and outcomes

The day-to-day costs of illness provide a strong explanation for yet another negative consequence of helminth infection, or the observation that it acts as “a very real barrier to children’s progress in school” as quantified by “outcome measures such as absenteeism, under-enrollment, and attrition.” Parasite-heavy students may be too weak to attend classes, or their families may be too indebted by medical bills and low worker productivity to pay for school enrollment fees. This effect may be conceptually distinct from previous findings about the impact of parasitism on cognition and learning; for example, Miguel and Kremer (2004) find that deworming programs improve school attendance by 25% without affecting test outcomes at all. Nonetheless, these effects may also be related: Bleakley (2007) found that school attendance and enrollment grew significantly in the school-age populations that benefited most from the Rockefeller Foundation’s deworming programs, leading to a long-term increase in income as well as a rise in literacy rates.

Prevention and control

History

Public health campaigns to reduce helminth infections in the US may be traced as far back as 1910, when the Rockefeller Foundation began the fight against hookworm – the so-called “germ of laziness” – in the American South. This campaign was enthusiastically received by educators throughout the region; as one Virginian school observed: “‘children who were listless and dull are now active and alert; children who could not study a year ago are not only studying now, but are finding joy in learning...for the first time in their lives their cheeks show the glow of health.’” From Louisiana, a grateful school board added:" As a result of your treatment...their lessons are not so hard for them: they pay better attention in class and they have more energy...In short, we have here in our school-rooms today about 120 bright, rosy-faced children, whereas had you not been sent here to treat them we would have had that many pale-faced, stupid children."

Similar (albeit somewhat more imperialist) reports emerged from various other regions of the developing world at the time; for example, two scholars in Puerto Rico found that: "Over all the varied symptoms with which the unfortunate jibaro [peasant], infected by uncinaria [hookworm], is plagued, hangs the pall of a drowsy intellect, of a mind that has received a stunning blow...There is a hypochondriacal, melancholy, hopeless expression, which in severe cases deepens to apparent dense stupidity, with indifference to surroundings and lack of all ambition.’

Such observations made an intuitive connection between worm burden and intellectual performance, but even today this link is anything but well-established. While it seems that worms may impair cognition in some way, the mechanisms driving this relationship are still hotly debated.

Current efforts

One popular approach to intestinal helminth control is school deworming programs. These programs have a number of advantages. On the one hand, they allow health policymakers to take advantage of existing infrastructure and institutions for the dispensation of medical treatment; students already plan to attend school on a somewhat regular basis, and teachers can easily distribute the medication to their students without receiving any medical training.

School deworming programs have also been shown to have strong positive externalities. Miguel and Kramer (2004) used a difference-in-difference model to prove that deworming programs in some schools reduced the burden of disease in neighboring, untreated schools; other evidence suggests that deworming children also has strong benefits for adult infection rates, since children are a significant source of transmission.

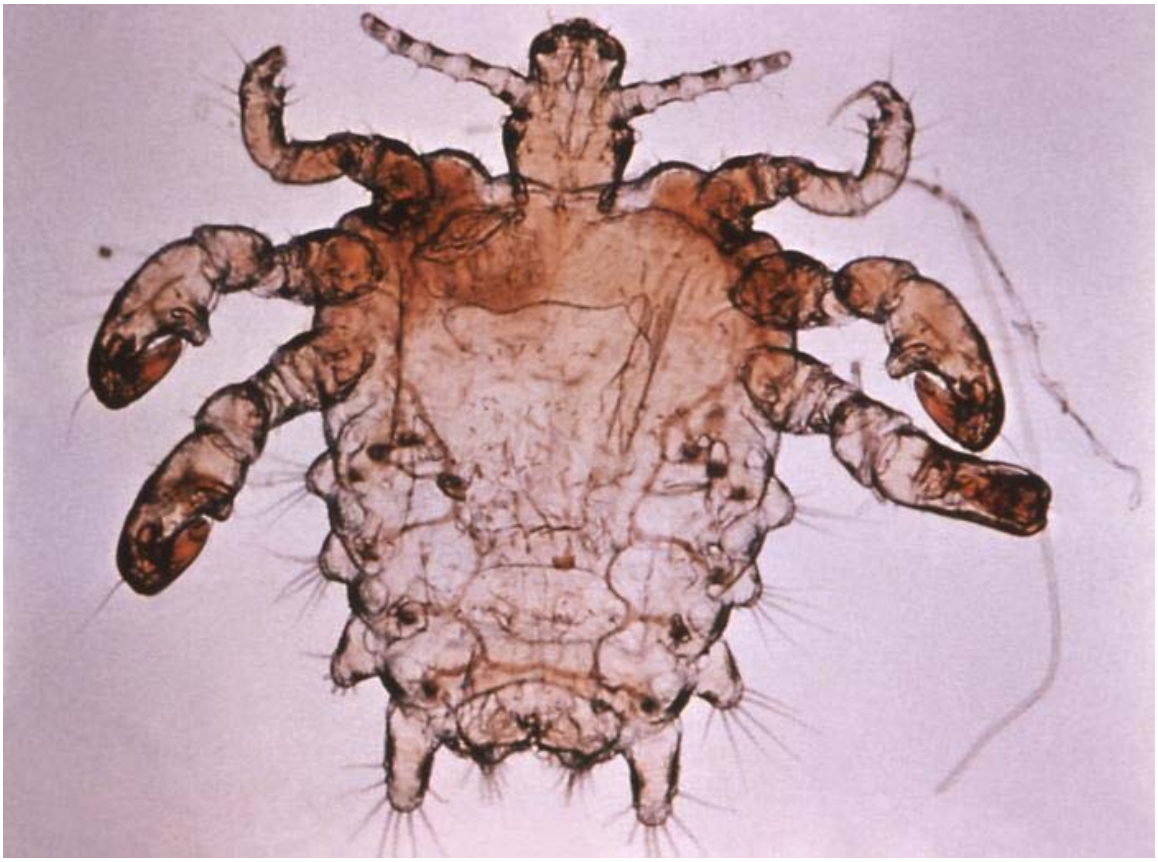
The nature of the intestinal helminths and the medications available to treat them also favor universal deworming programs. Infection is generally diffuse, so it is worth treating a wide sample of the population; furthermore, a drug like albendazole is a cheap, safe intervention that is not particularly specific, and so can be used fairly effectively against all three of the main intestinal helminths (or any coinfection of them). Finally, because these worms cannot replicate inside of their hosts, reducing transmission may be the best way to reduce prevalence, and mass interventions on an annual or biannual basis may in fact be a reasonable means of achieving this goal.

Use in medicine

Parasitic worms have been used as a medical treatment for various diseases, particularly those involving an over active immune response. As humans have evolved with parasitic worms, proponents argue that they are needed for a healthy immune system. Scientists are looking to see if there is a connection between the prevention and control of parasitic worms and the increase in allergies such as hay-fever in developed countries. Parasitic worms may be able to damp down the immune system of their host, making it easier for them to live in the intestine without coming under attack. This may be one mechanism for their proposed medicinal effect.

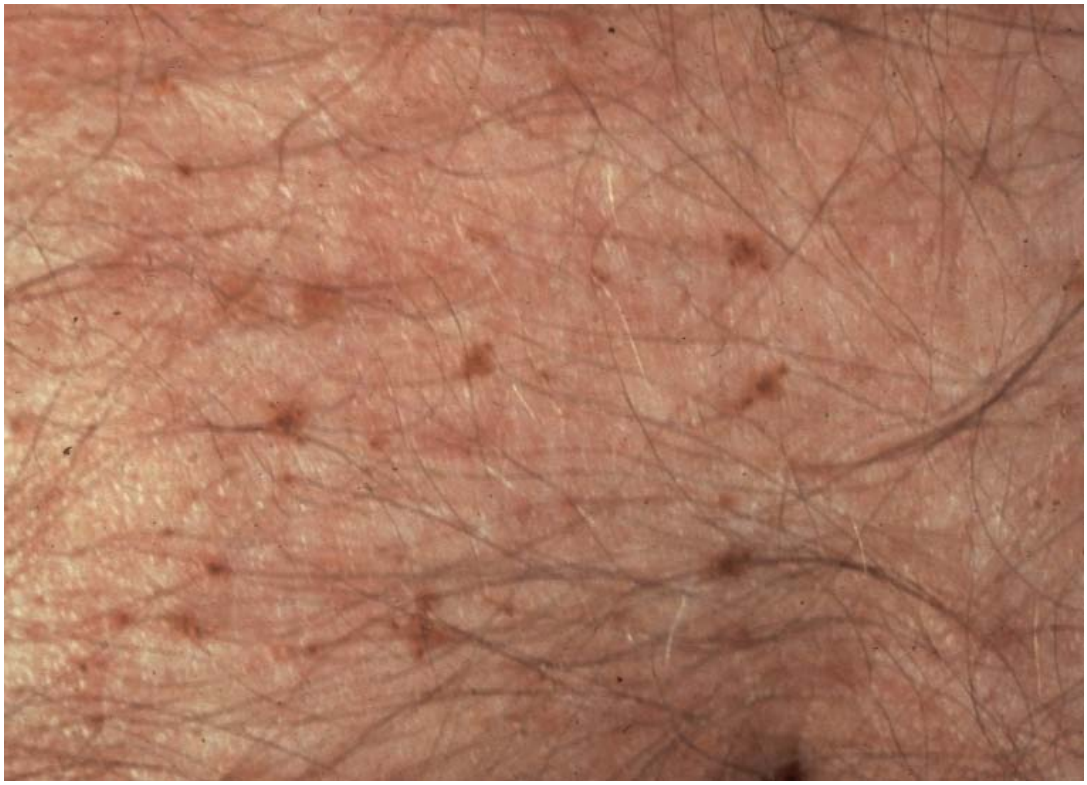
Chapter- 12

Crab Louse

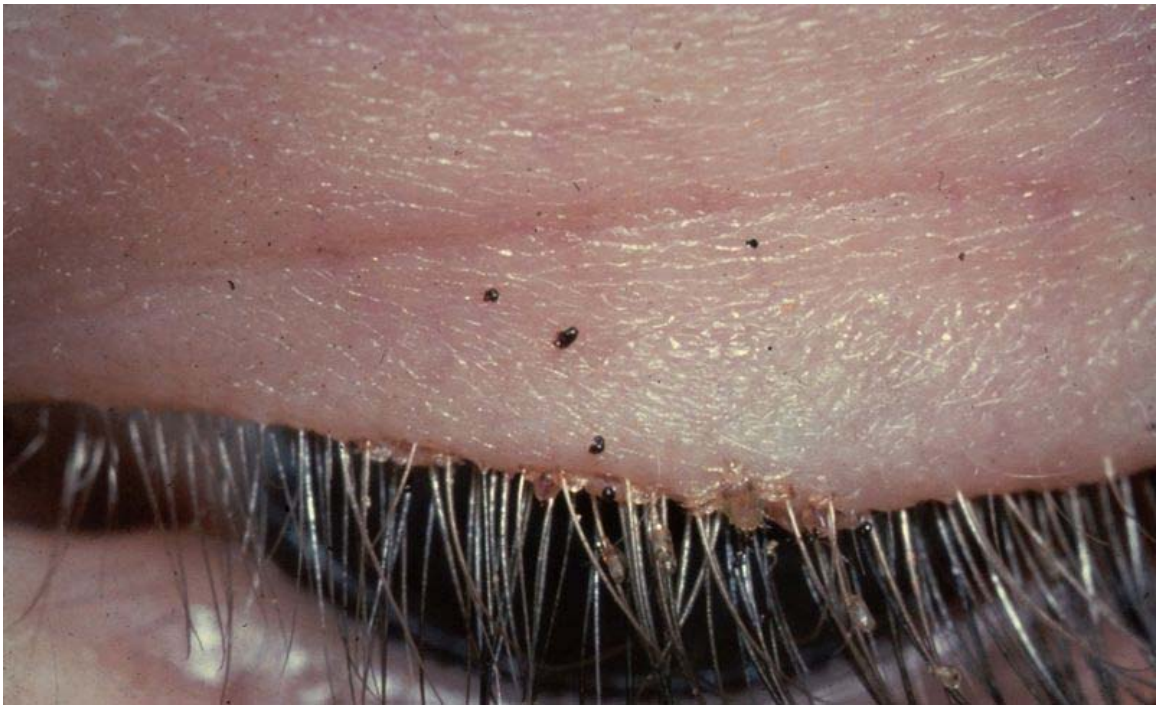


Crab louse

Pubic lice in genital area



Pubic lice on abdomen



Pubic lice on eyelashes

Crab lice (also known as "**crabs**", ***Phthirus pubis***, ***Pthirus pubis***, and "**pubic lice**") are parasitic insects notorious for infesting human genitals. The species may also live on other areas with hair, including the eyelashes. They feed exclusively on blood. Humans are the only known hosts of this parasite, although a closely related species, *Pthirus gorillae*, infects gorilla populations.

Classification

Infestation with pubic lice is called phthiriasis pubis, while infestation of eyelashes with pubic lice is called phthiriasis palpebrarum.

Signs and symptoms

The main symptom is itching, usually in the pubic-hair area, resulting from hypersensitivity to louse saliva, which can become stronger over two or more weeks following initial infestation. In some infestations, a characteristic grey-blue or slate coloration appears (*maculae caeruleae*) at the feeding site, which may last for days.

Transmission

Pubic lice usually infect a new host only by close contact between individuals, usually through sexual intercourse. Parent-to-child infestations are more likely to occur through routes of shared towels, clothing, beds or closets. Adults are more frequently infested

than children. As with most sexually transmitted pathogens, they can only survive a short time away from the warmth and humidity of the human body.

Pubic lice are primarily spread through sweat, body contact or sexual contact. Therefore, all partners with whom the patient has had sexual contact within the previous 30 days should be evaluated and treated, and sexual contact should be avoided until all partners have successfully completed treatment and are thought to be cured. Because of the strong association between the presence of pubic lice and classic sexually transmitted infections (STIs), patients diagnosed with pubic lice should undergo evaluation for other STIs.

Infection in a young child or teenager is not necessarily indicative of sexual abuse, although this possibility should be kept in mind.

Diagnosis

A pubic louse infestation is usually diagnosed by carefully examining pubic hair for nits, nymphs, and adult lice. Lice and nits can be removed either with forceps or by cutting the infested hair with scissors (with the exception of an infestation of the eye area). A magnifying glass or a stereo-microscope can be used for the exact identification. If lice are detected in one family member, the entire family needs to be checked and only those who are infested with living lice should be treated.

Treatment

Crab lice can be treated with Permethrin 1% cream rinse and pyrethrins. They can be used for this purpose and are the drugs of choice for pregnant or lactating women. These agents should be applied to the affected areas and washed off after 10 minutes. Shaving off or grooming any hair in the affected areas with a fine-toothed comb is necessary to ensure full removal of the dead lice and nits. Resistance of pubic lice to pyrethroids must be, if at all, very rare. A second treatment after 10 days is recommended. It is also crucial to make sure that all the bed sheets are changed. The sheets used before the first application of the treatment must be put away in a plastic bag, without air and well shut. They should be left alone for 15 days before washing to avoid the reproduction and survival of lice eggs that may have been left on the sheets and lead to reinfestation.

Pubic lice on the eyelashes can be treated with a permethrin formulation by applying the solution to the infested hair with an applicator.

Lindane shampoo (1%), a pediculicide, although banned in more than 50 countries, is approved by the U.S. Food and Drug Administration (FDA) as safe and effective when used as directed for the second-line treatment of pubic lice ("crabs"). While serious side effects have been reported, they are considered to be rare and have almost always resulted from misuse of medication, such as excessive application and oral ingestion. To minimize this risk, Lindane medications are now dispensed in small single-use bottles. The Centers for Disease Control and Prevention (CDC) notes that Lindane should not be used immediately after a bath or shower, and it should not be used by persons who have

extensive dermatitis, women who are pregnant or lactating or children aged under two years. The FDA similarly warns against use in patients with a history of uncontrolled seizure disorders and premature infants, and recommends cautious use in infants, children, the elderly, and individuals with other skin conditions (e.g., atopic dermatitis, psoriasis) and in those who weigh less than 110 lbs (50 kg).

Epidemiology

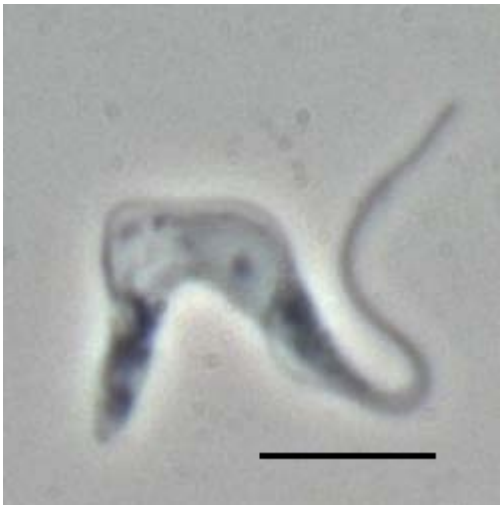
Current worldwide prevalence has been estimated at 2 percent of two human populations, accurate numbers are difficult to acquire, because pubic lice infestations are not considered a reportable condition by many governments, and many cases are self-treated or treated discreetly by personal physicians.

Although any part of the body may be colonized, crab lice favour the hairs of the genital (*Fig. 1*) and peri-anal region. Especially in male patients, pubic lice and eggs can also be found in hair on the abdomen (*Fig. 2*) and under the armpits as well as on the beard and mustache, while in children they are usually found in eyelashes (*Fig. 3*).

Chapter- 13

Trypanosoma Brucei

Trypanosoma brucei



Trypanosoma brucei brucei TREU667
(Bloodstream form, phase contrast picture. Black bar indicates 10 µm.)

Scientific classification	
Kingdom:	Excavata
Phylum:	Euglenozoa
Class:	Kinetoplastea
Order:	Trypanosomatida
Genus:	*Trypanosoma*
Species:	***T. brucei***

Binomial name

Trypanosoma brucei
Plimmer & Bradford, 1899

Subspecies

T. b. brucei

T. b. gambiense
T. b. rhodesiense

False colour SEM micrograph of procyclic form *Trypanosoma brucei* as found in the tsetse fly midgut. The cell body is shown in orange and the flagellum is in red. 84 pixels/μm.

Trypanosoma brucei is a parasitic protist species that causes African trypanosomiasis (or sleeping sickness) in humans and nagana in animals in Africa. There are 3 sub-species of *T. brucei*: *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*.

These obligate parasites have two hosts - an insect vector and mammalian host. Due to the large difference between these hosts the trypanosome undergoes complex changes during its life cycle to facilitate its survival in the insect gut and the mammalian bloodstream. It also features a unique and notable variable surface glycoprotein (VSG) coat in order to avoid the host's immune system. There is an urgent need for the development of new drug therapies as current treatments can prove fatal to the patient as well as the trypanosomes.

The infection: Trypanosomiasis

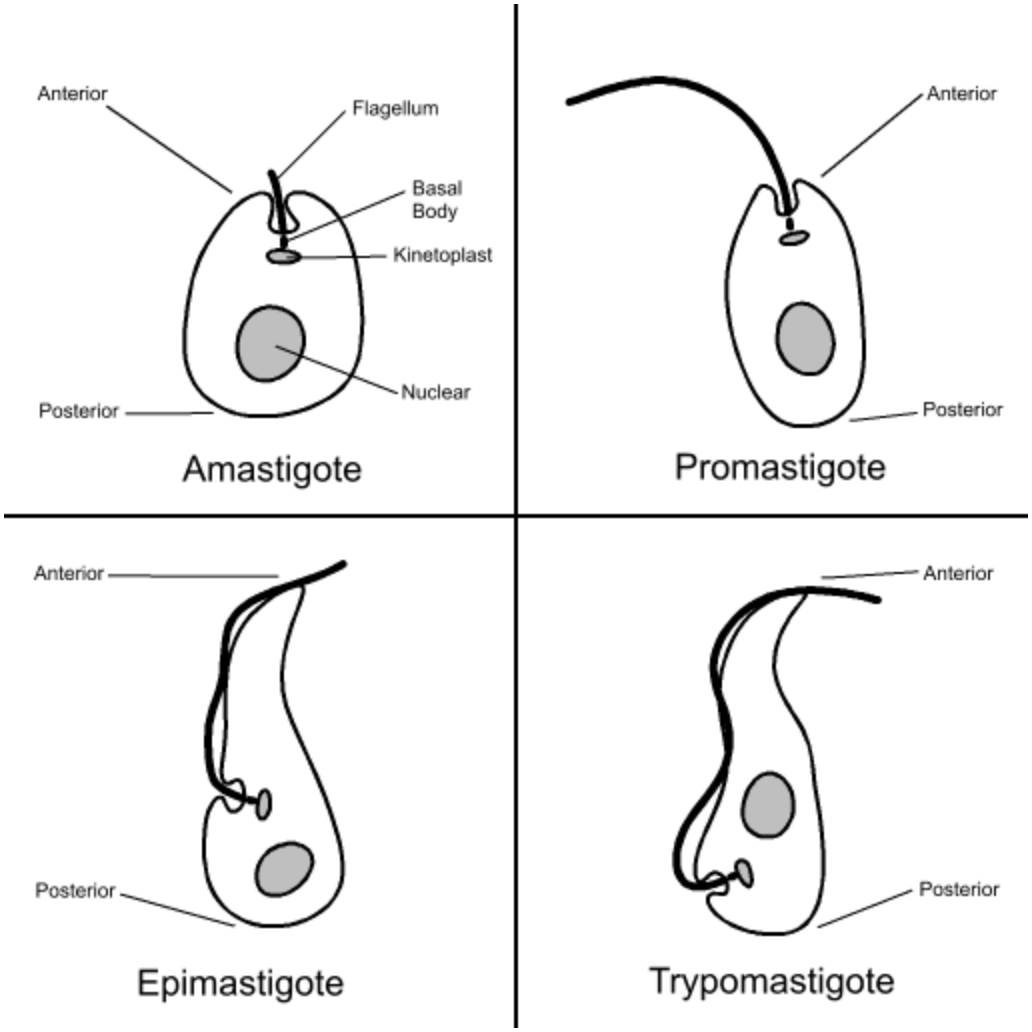
The insect vector for *T. brucei* is the tsetse fly. The parasite lives in the midgut of the fly (procyclic form), whereupon it migrates to the salivary glands for injection to the mammalian host on biting. The parasite lives within the bloodstream (bloodstream form) where it can reinfect the fly vector after biting. Later during a *T. brucei* infection the parasite may migrate to other areas of the host. A *T. brucei* infection may be transferred human to human via bodily fluid exchange, primarily blood transfer.

There are three different sub-species of *T. brucei*, which cause different variants of trypanosomiasis.

- *T. brucei gambiense* - Causes slow onset chronic trypanosomiasis in humans. Most common in central and western Africa, where humans are thought to be the primary reservoir.
- *T. brucei rhodesiense* - Causes fast onset acute trypanosomiasis in humans. Most common in southern and eastern Africa, where game animals and livestock are thought to be the primary reservoir.
- *T. brucei brucei* - Causes animal African trypanosomiasis, along with several other species of trypanosoma. *T. b. brucei* is not human infective due to its susceptibility to lysis by human apolipoprotein L1. However, as it shares many features with *T. b. gambiense* and *T. b. rhodesiense* (such as antigenic variation) it is used as a model for human infections in laboratory and animal studies.

The cell structure

The structure of the cell is fairly typical of eukaryotes. All major organelles are seen, including the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus etc. Unusual features include the single large mitochondria with the mitochondrial DNA structure known as the kinetoplast, and its association with the basal body of the flagellum. The cytoskeleton is made up primarily of microtubules. The cell surface of the bloodstream form features a dense coat of variable surface glycoproteins (VSGs) which is replaced by an equally dense coat of procyclins when the parasite differentiates into the procylic in the tsetse fly midgut.



Trypanosomatid cellular forms

Trypanosomatids show specific cellular forms:

- Amastigote - Basal body anterior of nucleus, with a short, essentially non-functional, flagellum.
- Promastigote - Basal body anterior of nucleus, with a long detached flagellum.
- Epimastigote - Basal body anterior of nucleus, with a long flagellum attached along the cell body.
- Trypomastigote - Basal body posterior of nucleus, with a long flagellum attached along the cell body.

These names are derived from the Greek *mastig*- meaning whip, referring to the trypanosome's whip-like flagellum.

T. brucei is found as a trypomastigote in the slender, stumpy, procyclic and metacyclic forms. The procylic form differentiates to the proliferitive epimastigote form in the

salivary glands of the insect. Unlike some other trypanosomatids, the promastigote and the amastigote form do not form part of the *T.brucei* life cycle.

The genome

The genome of *T. brucei* is made up of:

- 11 pairs of large chromosomes of 1 to 6 megabase pairs.
- 3-5 intermediate chromosomes of 200 to 500 kilobase pairs.
- Around 100 mini chromosomes of around 50 to 100 kilobase pairs. These may be present in multiple copies per haploid genome.

The large chromosomes contain most genes, while the small chromosomes tend to carry genes involved in antigenic variation, including the VSG genes. The genome has been sequenced and is available online.

The mitochondrial genome is found condensed into the kinetoplast, an unusual feature unique to the kinetoplastea class. It and the basal body of the flagellum are strongly associated via a cytoskeletal structure.

VSG surface coat

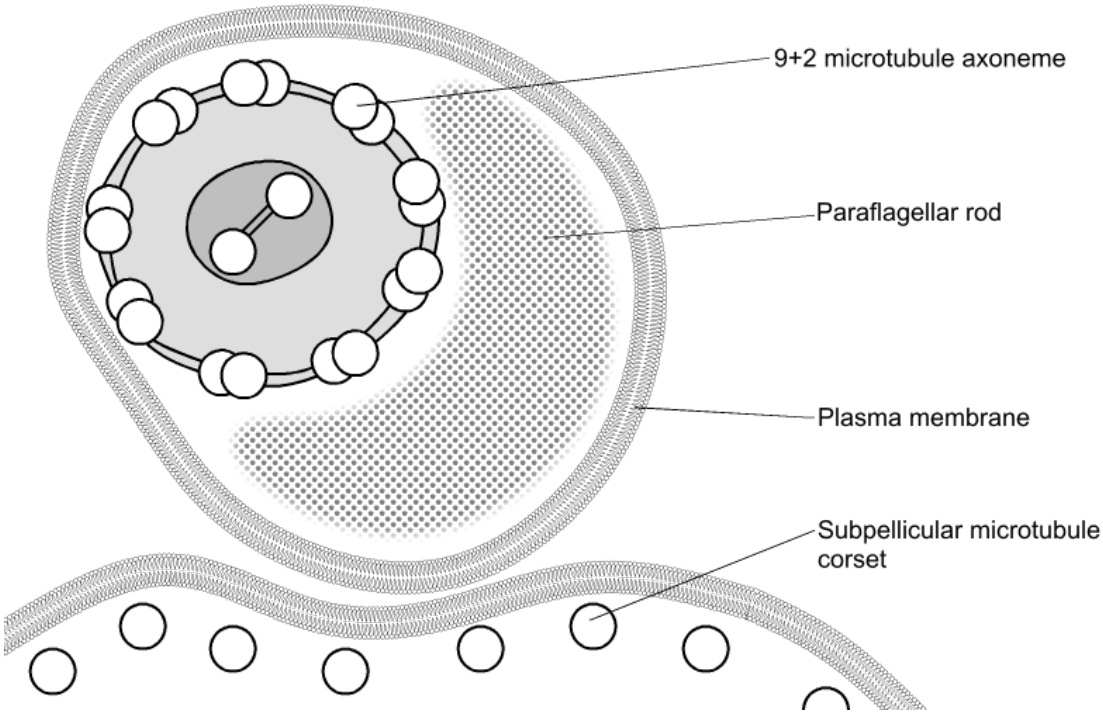
The surface of the trypanosome is covered by a dense coat of Variable Surface Glycoprotein (VSG), which allows persistence of an infecting trypanosome population in the host.

The cytoskeleton

The cytoskeleton is predominantly made up of microtubules, forming a subpellicular corset. The microtubules lie parallel to each other along the long axis of the cell, with the number of microtubules at any point roughly proportional to the circumference of the cell at that point. As the cell grows (including for mitosis) additional microtubules grow between the existing tubules, leading to semiconservative inheritance of the cytoskeleton. The microtubules are orientated + at the posterior and - at the anterior.

Microfilament and intermediate filaments also play an important role in the cytoskeleton, but these are generally overlooked.

Flagellar structure



Trypanosoma Brucei flagellar structure

The trypanosome flagellum has two main structures. It is made up of a typical flagellar axoneme which lies parallel to the paraflagellar rod, a lattice structure of proteins unique to the kinetoplastida, euglenoids and dinoflagellates.

The microtubules of the flagellar axoneme lie in the normal 9+2 arrangement, orientated with the + at the anterior end and the - in the basal body. The a cytoskeletal structure extends from the basal body to the kinetoplast. The flagellum is bound to the cytoskeleton of the main cell body by four specialised microtubules, which run parallel and in the same direction to the flagellar tubulin.

The flagellar function is twofold - locomotion via oscilations along the attached flagellum and cell body, and attachment to the fly gut during the procyclic phase.

The VSG coat

The surface of the trypanosome is covered by a dense coat of ~$1x10^7$ molecules of Variable Surface Glycoprotein (VSG). This coat enables an infecting *T. brucei* population to persistently evade the host's immune system, allowing chronic infection. The two properties of the VSG coat that allow immune evasion are:

- Shielding - the dense nature of the VSG coat prevents the immune system of the mammalian host from accessing the plasma membrane or any other invariant

surface epitopes (such as ion channels, transporters, receptors etc.) of the parasite. The coat is uniform, made up of millions of copies of the same molecule; therefore the only parts of the trypanosome the immune system can 'see' are the N-terminal loops of the VSG that make up the coat.
- Periodic antigenic variation - the VSG coat undergoes frequent stochastic genetic modification - 'switching' - allowing variants expressing a new VSG coat to escape the specific immune response raised against the previous coat.

Antigenic variation

Sequencing of the *T. brucei* genome has revealed a huge *VSG* gene archive, made up of thousands of different *VSG* genes. All but one of these are 'silent' VSGs, as each trypanosome expresses only one *VSG* gene at a time. VSG is highly immunogenic, and an immune response raised against a specific VSG will rapidly kill trypanosomes expressing this *VSG*. This can also be observed in vitro by a complement-mediated lysis assay. However, with each cell division there is a possibility that one or both of the progeny will switch expression to a silent *VSG* from the archive (see below). The frequency of such a switch has been measured to be approximately 1:100. This new VSG will likely not be recognised by the specific immune responses raised against previously expressed VSGs. It takes several days for an immune response against a specific to develop, giving trypanosomes which have undergone VSG coat switching some time to reproduce (and undergo further VSG coat switching events) unhindered. Repetition of this process prevents extinction of the infecting trypanosome population, allowing chronic persistence of parasites in the host. The clinical effect of this cycle is successive 'waves' of parasitaemia (trypanosomes in the blood).

VSG structure

VSG genes are hugely variable at the sequence level. However, for them to fulfil their shielding function, different VSGs have strongly conserved structural features. VSGs are made up of a highly variable N terminal domain of around 300 to 350 amino acids, and a more conserved C terminal domain of around 100 amino acids. The C terminal domain forms a structural bundle of four alpha helices, while the N terminal domain forms a 'halo' around the helices. The tertiary structure of this halo is well conserved between different VSGs (in spite of wide variation in amino acid sequence) allowing different VSGs to form the physical barrier required to shield the trypanosome's surface. VSG is anchored to the cell membrane via a glycophosphatidylinositol (GPI) anchor - a covalent linkage from the C terminus, to approximately four sugars, to a phosphatidylinositol phospholipid acid which lies in the cell membrane. VSGs form homodimers.

VSG archive structure

The *VSG* gene archive is the collection of silent VSGs in the *T. brucei* genome. Some of these are full-length, intact genes; others are pseudogenes) typically with omitted sections or premature stop codons. Expression of an antigenically novel VSG can occur by simply switching to a different full-length VSG gene. However, only 5% of the archive is made

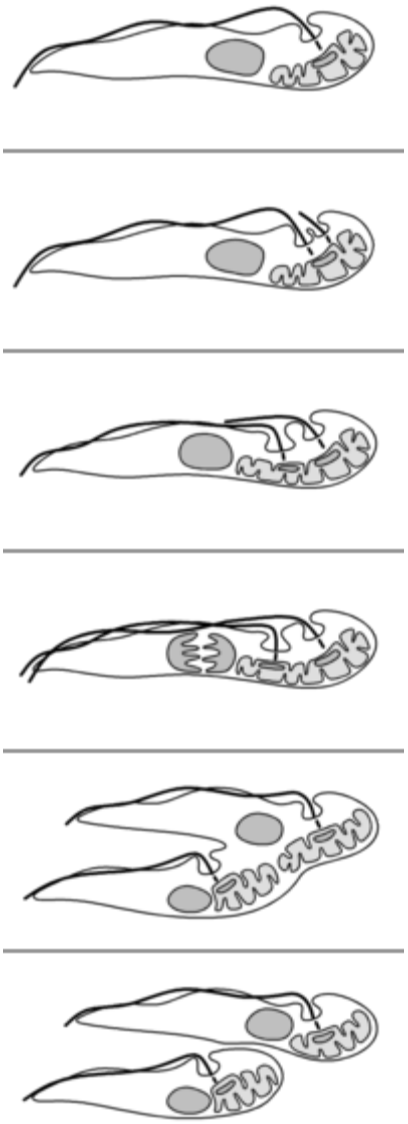
up of such complete silent VSGs. To utilise the rest of the silent VSG archive, ‘mosaic’ VSGs can be formed by replacing part of the expressed VSG with a structurally homologous region from the archive. The combinatorial nature of mosaic formation in conjunction with the huge silent VSG archive gives the parasite a theoretically limitless VSG library, and is the major barrier to vaccine development.

VSG expression

One major focus in trypanosome research is how the majority of VSG genes are kept silent, and how these genes are switched. The expressed VSG is always located in an Expression Site - found at the telomeres of the large and intermediate chromosomes. Each is a polycistronic unit, containing a number of Expression Site-Associated Genes (ESAGs) all expressed along with the active VSG. While there are at least 20 known expression sites, only a single one is ever active at one time. A number of mechanisms appear to be involved in this process, but the exact nature of the silencing is still unclear.

The VSG can be switched either by changing the active expression (from the active to a previously silent site) or by changing the VSG gene in the active site. The genome contains many copies of possible VSG genes, both on minichromosomes and in repeated sections in the interior of the chromosomes. These are generally silent, typically with omitted sections or premature stop codons, but are important in the evolution of new VSG genes. It is estimated up to 10% of the *T.brucei* genome may be made up of VSG genes or pseudogenes. Any of these genes can be moved into the active site by recombination for expression. Again, the exact mechanisms that control this are still only partially known.

Mitotic process



Trypanosome cell cycle (procyclic form)

The mitotic division of *T.brucei* is unusual in terms of the cytoskeletal process. The basal body, unlike a centrosome of most eukaryotic cells, plays an important role in the organisation of the spindle.

Stages of mitosis:

1. The basal body replicates, both remaining associated with the kinetoplast.
2. The kinetoplast undergoes replication, and the daughter kinetoplasts are separated by the basal bodies.

3. The second flagellum grows while the nucleus undergoes replication.
4. The mitochondria divides, and cytokinesis progresses from the anterior to posterior end.
5. The division resolves. The daughter cells may stay connected for a significant length of time after cytokinesis is complete.

Chapter- 14

Kleptoparasitism

Great Frigatebirds chasing a Red-footed Booby in order to steal its food

Kleptoparasitism or **cleptoparasitism** (literally, parasitism by theft) is a form of feeding in which one animal takes prey or other food from another that has caught, collected, or otherwise prepared the food, including stored food (as in the case of cuckoo bees, which

lay their eggs on the pollen masses made by other bees). The term is also used to describe the stealing of nest material or other inanimate objects from one animal by another.

The kleptoparasite gains either by obtaining prey or other objects that it could not obtain itself, or by saving the time and effort required to obtain it. However, the kleptoparasite may run the risk of injury from the victim if it is able to defend its property.

Kleptoparasitism may be *intraspecific* (the parasite is the same species as the victim) or *interspecific* (the parasite is a different species). In the latter case, the parasites are commonly close relatives of the organisms they parasitize ("Emery's Rule").

Animals that have extraordinarily specialized feeding methods are often targets of kleptoparasitism. For example, oystercatchers are unusual in being able to break through the shells of mussels; adult oystercatchers suffer intraspecific kleptoparasitism from juveniles that are not yet strong or skillful enough to open mussels easily. Diving birds that bring their prey to the surface suffer interspecific kleptoparasitism from gulls, which are unable to fetch fish from the sea floor themselves. Chinstrap penguins also actively engage in kleptoparasitism, being known to steal rocks and other nest materials from members of their colony for use in their own nest.

Arthropods

A cuckoo bee from the genus *Nomada*

Bees and wasps

There are many different lineages of cuckoo bees, all of which lay their eggs in the nest cells of other bees. There is also a family of cuckoo wasps, many of which lay their eggs in the nests of potter and mud dauber wasps; many other lineages of wasps in various families have evolved similar habits. These insects are normally referred to as "cleptoparasites," rather than as "brood parasites." The distinction is that the term "brood parasite" is generally restricted to cases where the immature parasite is fed directly by the adult of the host, and raised as the host's offspring (as is common in birds). Such cases are virtually unknown in bees and wasps, which tend to provide all of the food for the larva before the egg is laid; in only a few exceptional cases (such as parasitic bumblebees) will a bee or wasp female feed a larva that is not her own species. The difference is only in the nature of the interaction by which the transfer of resources occurs (tricking a host into handing over food rather than stealing it by force or stealth), which is why brood parasitism is considered a special form of kleptoparasitism.

Flies

The Craticulina sp. fly is a parasite of the sand wasp, depositing its larvae on the food reserved for the larvae of the wasp.

Some flies are kleptoparasites. This includes several flies of the Chloropidae and Milichiidae families. Some adult milichiids, for example, visit spider webs where they scavenge on half-eaten stink bugs. Others are associated with robber flies (Asilidae), or

Crematogaster ants. Flies in the genus *Bengalia* (Calliphoridae) steal food and pupae transported by ants and are often found beside their foraging trails.

True bugs

Velia caprai (Ardennes, Belgium)

Many semiaquatic bugs (Heteroptera) are known to engage in kleptoparastism of prey. In one study, whenever the bug *Velia caprai* (Water Cricket) took prey heavier than 7.9 g, other bugs of the same species joined it and successfully ate parts of the prey.

Spiders

Kleptoparasitic spiders, which steal or feed on prey captured by other spiders, are known to occur in five families:

- Theridiidae (*Argyrodes* species)
- Dictynidae (*Archaeodictyna ulova*)
- Salticidae (species of *Portia* and *Simaetha*)
- Symphytognathidae (*Curimagua bayano*)
- Mysmenidae (*Isela okuncana*, *Kilifia inquilina*, and *Mysmenopsis* species).

Vertebrates

This gull is probably attempting to steal food from a sea otter

Birds

Kleptoparasitism is relatively uncommon in birds; most species do not practice such piracy. However, some non-passerine groups, such as skuas, jaegers and frigatebirds, rely extensively on such behavior to obtain food, and others—including raptors, gulls, terns, coots, and some ducks and shorebirds—will do so opportunistically. Among opportunistic species such as the Roseate Tern, research has found that parent birds involved in kleptoparasitism are more successful in raising broods than non-kleptoparasitic individuals. Bald Eagles can also be seen attacking smaller raptors, such as Ospreys, to steal fish away from them. Among passerine birds, there are fewer known examples of kleptoparasitism, though Masked Shrikes have been recorded stealing food from wheatears, and Eurasian Blackbirds have been seen stealing smashed snails from other thrushes.

Western Gull (*Larus occidentalis*) in pursuit of Elegant Tern (*Thalasseus elegans*)

Skuas (including the smaller species known as jaegers in North America) are masters of piracy. Their victims are typically gulls and terns, though other fish-eating species (including auks) are also pursued until they disgorge their catches. The fact that skuas are swift and agile fliers—and that they sometimes gang up on a single victim—aid in their success rate.

During seabird nesting seasons, frigatebirds will soar above seabird colonies, waiting for parent birds to return to their nests with food for their young. As the returning birds approach the colony, the frigatebirds (which are fast and agile) drop down and pursue them vigorously; they have been known to seize tropicbirds by their long tail plumes. Many of the frigatebirds' colloquial names, including *Man-o'-War Bird* and *Pirate of the Sea*, are a clear reference to this kleptoparasitic behaviour. A study of kleptoparasitism in the Magnificent Frigatebird suggests that the amount of food obtained by kleptoparasitism may be marginal.

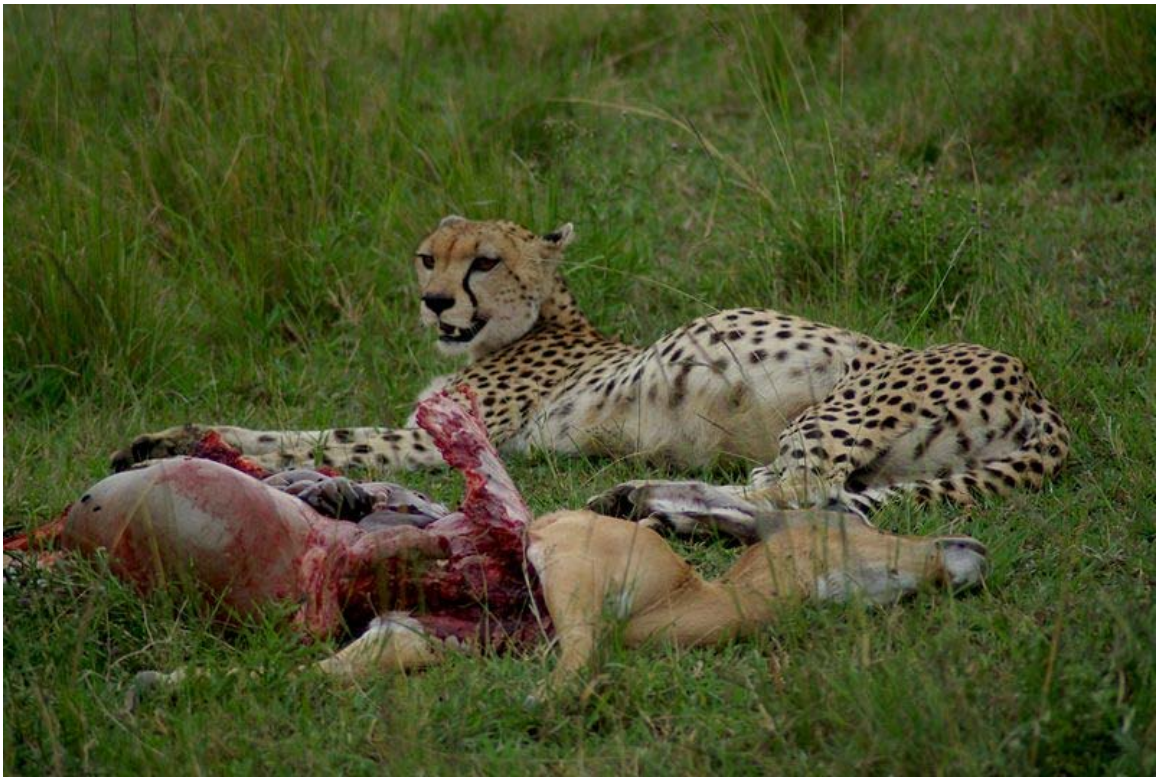
Intraspecific kleptoparasitism often occurs between gulls

Gulls are the perpetrators as well as the victims of kleptoparasitism, with some species frequently exhibiting the behavior, particularly during the breeding season. While the victim is most often another member of the same species, other (principally smaller) gulls and terns are also targeted. In the Americas, Heermann's Gulls and Laughing Gulls are known to steal food from Brown Pelicans; as the pelicans surface and empty the water from their bills, the gulls lurk nearby and grab escaping food items.

Several species of coots and gallinules have been recorded engaging in kleptoparasitism. American Coots often feed in the company of other waterfowl species, and occasionally will rob diving ducks—including Ring-necked Ducks, Redheads and Canvasback—when they surface with food. Eurasian Coots steal from conspecifics, as well as from diving and dabbling ducks, and swans. Allen's Gallinules rob both conspecifics and African Pygmy Geese.

Mammals

The relationship between Spotted Hyenas and Lions, in which each species steals the other's kills, is a form of kleptoparasitism. All hyena species engage in this behavior when they can, and jackals also steal from other carnivores' kills. Sperm Whales sometimes steal fish from fishermen's lines, making them the largest of all kleptoparasites.



A cheetah has killed an Impala. Natural predator-prey relationship

Three minutes later: a Spotted Hyena and another are running toward the kill.

38 seconds later: The cheetah has fled without resistance. The vultures will also engage in kleptoparasitism: When the hyenas move a piece of the carcass, the vultures will take scraps from the ground.

Humans

Humans have been found to chase away lions from their captured prey and consume the meat themselves. A researcher noted the behaviour in Waza National Park, Cameroon in 2006 and other ecologists have suggested that the behaviour may be widespread but often goes unrecognised. Interviews with Bororo herdsmen suggested that they often chase lions away from their prey. This kleptoparasitism may be contributing to the decline of lion populations within the national park.

Chapter- 15

Mosquito

Mosquito Temporal range: 79–0 Ma

A female mosquito *Culiseta longiareolata*.
Scientific classification
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Suborder: Nematocera
Infraorder: Culicomorpha
Superfamily: Culicoidea
Family: **Culicidae**

Meigen, 1830

Subfamilies
Anophelinae
Culicinae
Toxorhynchitinae

Diversity
41 genera
See: List of mosquito genera

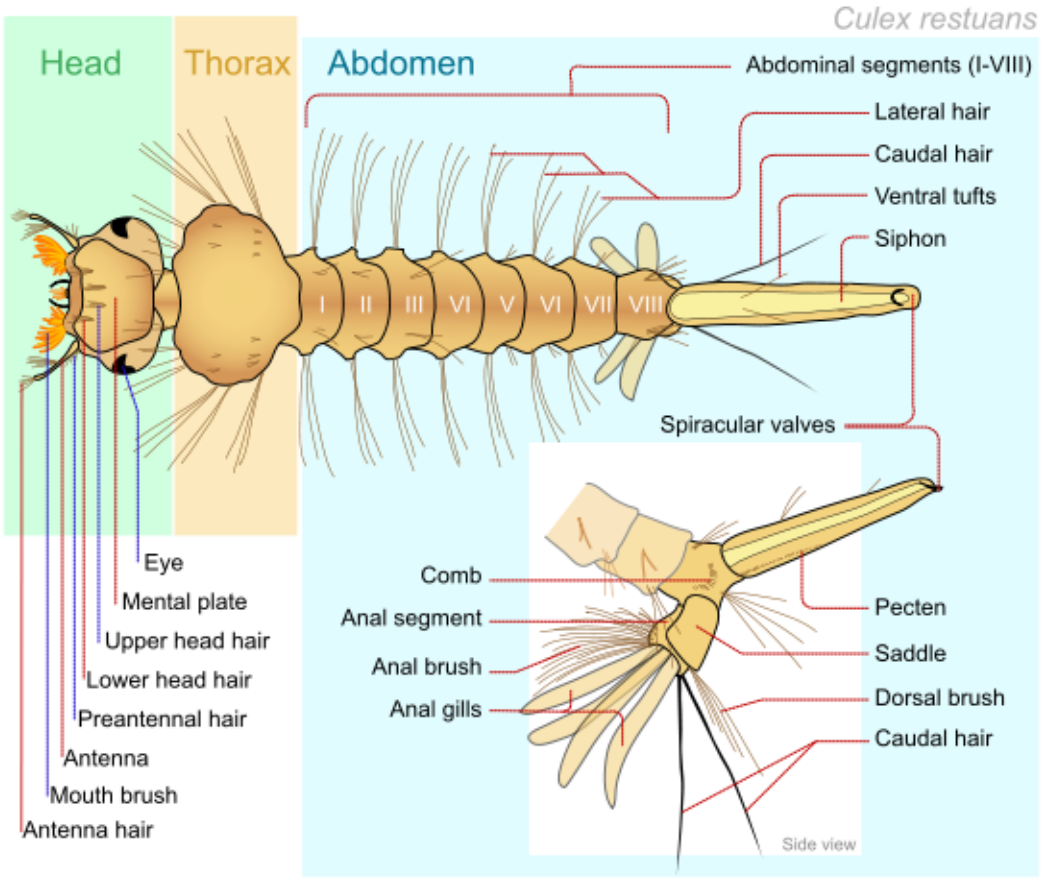
Mosquito (from the Spanish or Portuguese word for *little fly*) is a common insect in the family **Culicidae** (from the Latin *culex* meaning *midge* or *gnat*). Mosquitoes resemble crane flies (family Tipulidae) and chironomid flies (family Chironomidae), with which they are sometimes confused by the casual observer.

Mosquitoes go through four stages in their life-cycle: egg, larva, pupa, and adult or imago. Adult females lay their eggs in standing water, which can be a salt-marsh, a lake, a puddle, a natural reservoir on a plant, or an artificial water container such as a plastic bucket. The first three stages are aquatic and last 5–14 days, depending on the species and the ambient temperature; eggs hatch to become larvae, then pupae. The adult mosquito emerges from the pupa as it floats at the water surface. Adults live for 4–8 weeks.

Mosquitoes have mouthparts that are adapted for piercing the skin of plants and animals. While males typically feed on nectar and plant juices, the female needs to obtain nutrients from a "blood meal" before she can produce eggs.

There are about 3,500 species of mosquitoes found throughout the world. In some species of mosquito, the females feed on humans, and are therefore vectors for a number of infectious diseases affecting millions of people per year. Some scientists believe that eradicating mosquitos would not have serious consequences for any ecosystems.

Life cycle



Anatomy of a *Culex* larva

Anopheles larva from southern Germany, about 8 mm long

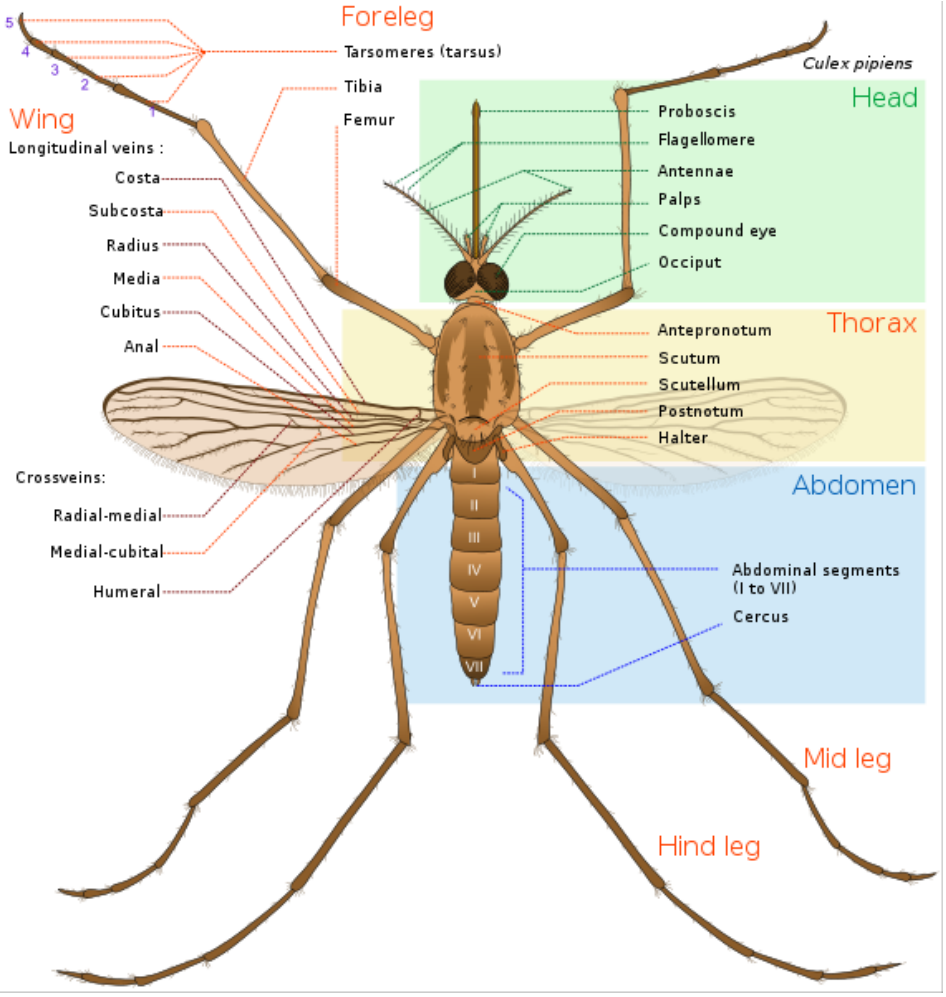
Larva

Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax with no legs and a segmented abdomen.

Larvae breathe through spiracles located on the eighth abdominal segment, or through a siphon, and therefore must come to the surface frequently. The larvae spend most of their time feeding on algae, bacteria, and other micro-organisms in the surface microlayer. They dive below the surface only when disturbed. Larvae swim either through propulsion with the mouth brushes,or by jerky movements of the entire body, giving them the common name of "wigglers" or "wrigglers".

Larvae develop through four stages, or instars, after which they metamorphose into pupae. At the end of each instar, the larvae molt, shedding their skin to allow for further growth.

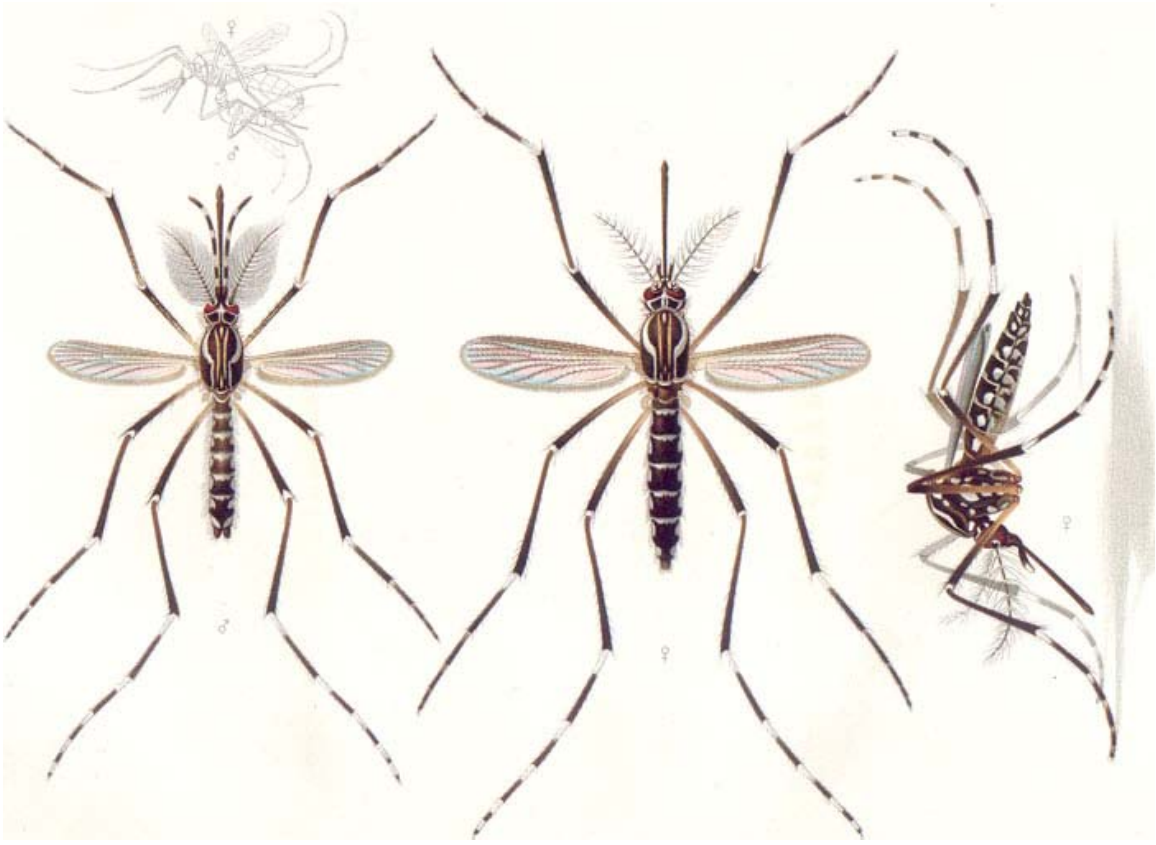
Pupa



Anatomy of an adult mosquito

The pupa is comma-shaped, as in *Anopheles* when viewed from the side, and is commonly called a "tumbler". The head and thorax are merged into a cephalothorax with the abdomen circling around underneath. As with the larvae, pupae must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax. However, pupae do not feed during this stage. After a few days, the pupa rises to the water surface, the dorsal surface of the cephalothorax splits and the adult mosquito emerges. The pupa is less active than larva.

Adult



Adults of the yellow fever mosquito *Aedes aegypti*, a typical member of the subfamily Culicinae. The male on the left, females on the right. Note the bushy antennae and longer palps in the male.

The duration from egg to adult varies among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as five days but usually take 40 – 42 days in tropical conditions. The variation of the body size in adult mosquitoes depends on the density of the larval population and food supply within the breeding water. Adult flying mosquitoes frequently rest in a tunnel that they build right below the roots of the grass.

Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate.

Males live for about a week, feeding on nectar and other sources of sugar. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2–3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host seeking. The females are the only ones who actually use blood as a meal, but they feed on nectar as well.

The cycle repeats itself until the female dies. While females can live longer than a month in captivity, most do not live longer than 1–2 weeks in nature. Their lifespan depends on temperature, humidity, and also their ability to successfully obtain a blood meal while avoiding host defenses.

Length of the adult varies but is rarely greater than 16 mm (0.6 in), and weight up to 2.5 mg (0.04 grain). All mosquitoes have slender bodies with three sections: head, thorax and abdomen.

The head is specialized for acquiring sensory information and for feeding. The head contains the eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odors as well as odors of breeding sites where females lay eggs. In all mosquito species, the antennae of the males in comparison to the females are noticeably bushier and contain auditory receptors to detect the characteristic whine of the female. The compound eyes are distinctly separated from one another. Their larvae only possess a pit-eye ocellus. The compound eyes of adults develop in a separate region of the head. New ommatidia are added in semicircular rows at the rear of the eye; during the first phase of growth, this leads to individual ommatidia being square, but later in development they become hexagonal. The hexagonal pattern will only become visible when the carapace of the stage with square eyes is molted. The head also has an elongated, forward-projecting "stinger-like" proboscis used for feeding, and two sensory palps. The maxillary palps of the males are longer than their proboscis whereas the females' maxillary palps are much shorter. (This is typical for representatives of subfamilies.) As with many members of the mosquito family, the female is equipped with an elongated proboscis that she uses to collect blood to feed her eggs.

The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The insect wing is an outgrowth of the exoskeleton. The *Anopheles* mosquito can fly for up to four hours continuously at 1 to 2 kilometres per hour (0.62–1.2 mph) travelling up to 12 km (7.5 mi) in a night.

The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal. The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen.

Feeding habits of adults

Aedes aegypti vector of dengue fever and yellow fever

Both male and female mosquitoes are nectar feeders, but the females of many species are also capable of drinking blood from many mammals. Females do not require blood for their own survival, but they do need supplemental substances such as protein and iron to develop eggs.

With regard to host location, carbon dioxide and organic substances produced from the host, humidity, and optical recognition play important roles. In *Aedes* the search for a host takes place in two phases. First, the mosquito exhibits a nonspecific searching behavior until the perception of host stimulants then it follows a targeted approach.

Most mosquito species are crepuscular (dawn or dusk) feeders. During the heat of the day most mosquitoes rest in a cool place and wait for the evenings, although they may still bite if disturbed. Some species, like Asian tiger mosquito, are known to fly and feed during daytime.

Both male and female are nectar feeders

Mosquitoes are adept at infiltration and have been known to find their way into residences via deactivated air conditioning units.

Prior to and during blood feeding, they inject saliva into the bodies of their source(s) of blood. This saliva serves as an anticoagulant: without it, the female mosquito's proboscis would quickly become clogged with blood clots. Female mosquitoes hunt their blood host by detecting carbon dioxide (CO_2) and 1-octen-3-ol from a distance.

Mosquitoes of the genus *Toxorhynchites* never drink blood. This genus includes the largest extant mosquitoes, the larvae of which prey on the larvae of other mosquitoes. These mosquito eaters have been used in the past as mosquito control agents, with varying success.

Saliva

In order for the mosquito to obtain a blood meal it must circumvent the vertebrate physiological responses. The mosquito, as with all blood-feeding arthropods, has mechanisms to effectively block the hemostasis system with their saliva, which contains a mixture of secreted proteins. Mosquito saliva negatively affects vascular constriction, blood clotting, platelet aggregation, angiogenesis and immunity and creates

inflammation. Universally, hematophagous arthropod saliva contains at least one anticlotting, one anti-platelet, and one vasodilatory substance. Mosquito saliva also contains enzymes that aid in sugar feeding and antimicrobial agents to control bacterial growth in the sugar meal. The composition of mosquito saliva is relatively simple as it usually contains fewer than 20 dominant proteins. Despite the great strides in knowledge of these molecules and their role in bloodfeeding achieved recently, scientists still cannot ascribe functions to more than half of the molecules found in arthropod saliva. One promising application is the development of anti-clotting drugs based on saliva molecules, which might be useful for approaching heart-related disease, because they are more user-friendly blood clotting inhibitors and capillary dilators.

It is now well recognized that the feeding ticks, sandflies, and, more recently, mosquitoes have an ability to modulate the immune response of the animals (hosts) they feed on. The presence of this activity in vector saliva is a reflection of the inherent overlapping and interconnected nature of the host hemostatic and inflammatory/immunological responses and the intrinsic need to prevent these host defenses from disrupting successful feeding. The mechanism for mosquito saliva-induced alteration of the host immune response is unclear, but the data has become increasingly convincing that such an effect occurs. Early work described a factor in saliva that directly suppresses TNF-α release, but not antigen-induced histamine secretion, from activated mast cells. Experiments by Cross et al. (1994) demonstrated that the inclusion of *Ae. aegypti* mosquito saliva into naïve cultures led to a suppression of interleukin (IL)-2 and IFN-γ production, while the cytokines IL-4 and IL-5 are unaffected by mosquito saliva. Cellular proliferation in response to IL-2 is clearly reduced by prior treatment of cells with SGE. Correspondingly, activated splenocytes isolated from mice fed upon by either *Ae. aegypti* or *Cx. pipiens* mosquitoes produce markedly higher levels of IL-4 and IL-10 concurrent with suppressed IFN-γ production. Unexpectedly, this shift in cytokine expression is observed in splenocytes up to 10 days after mosquito exposure, suggesting that natural feeding of mosquitoes can have a profound, enduring, and systemic effect on the immune response.

T cell populations are decidedly susceptible to the suppressive effect of mosquito saliva, showing enhanced mortality and decreased division rates. Parallel work by Wasserman et al. (2004) demonstrated that T- and B-cell proliferation was inhibited in a dose dependent manner with concentrations as low as 1/7th of the saliva in a single mosquito. Depinay et al. (2005) observed a suppression of antibody-specific T cell responses mediated by mosquito saliva and dependent on mast cells and IL-10 expression.

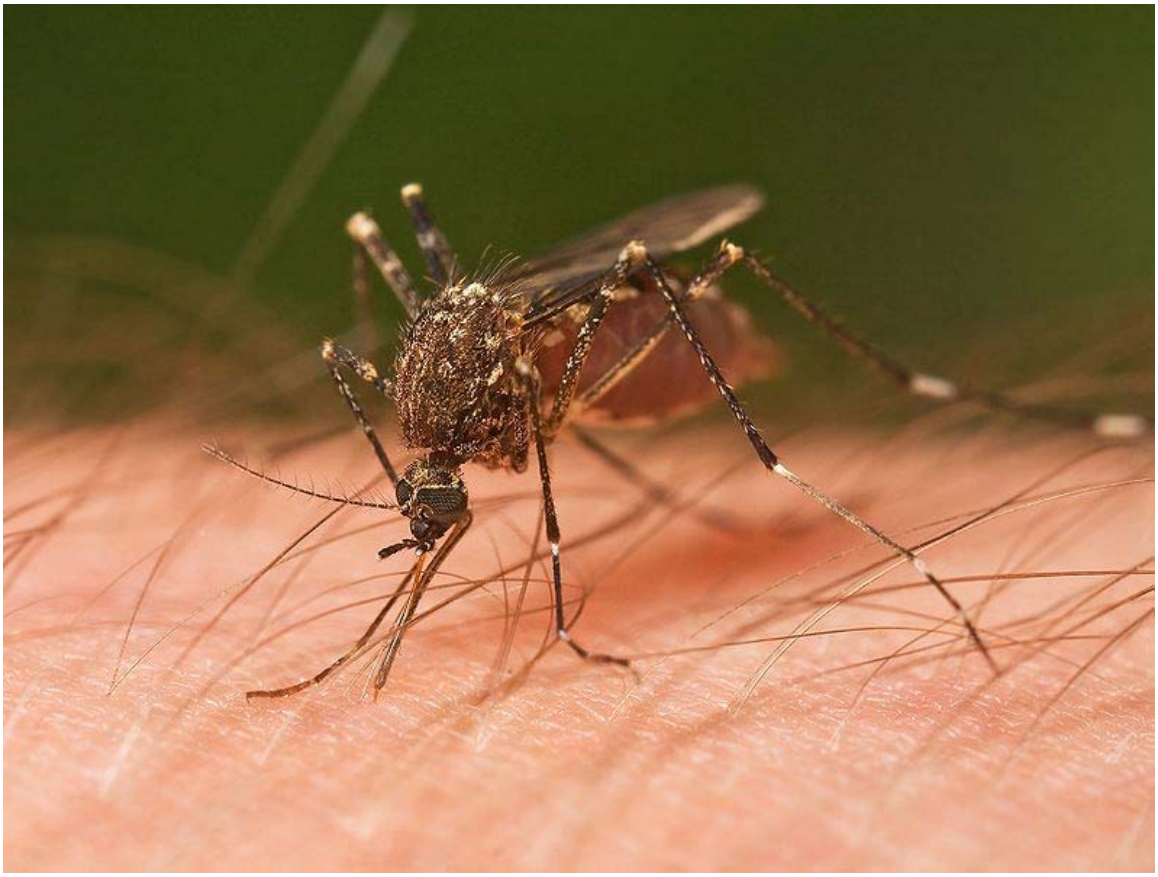
A recent study suggests that mosquito saliva can also decrease expression of interferon−α/β during early mosquito-borne virus infection. The contribution of type I interferons (IFN) in recovery from infection with viruses has been demonstrated *in vivo* by the therapeutic and prophylactic effects of administration of IFN-inducers or IFN, and recent research suggests that mosquito saliva exacerbates West Nile virus infection, as well as other mosquito-transmitted viruses.

Egg development and blood digestion

Two important events in the life of female mosquitoes are egg development and blood digestion. After taking a blood meal the midgut of the female synthesizes proteolytic enzymes that hydrolyze the blood proteins into free amino acids. These are used as building blocks for the synthesis of egg yolk proteins.

In the mosquito *Anopheles stephensi* Liston, trypsin activity is restricted entirely to the posterior midgut lumen. No trypsin activity occurs before the blood meal, but activity increases continuously up to 30 hours after feeding, and subsequently returns to baseline levels by 60 hours. Aminopeptidase is active in the anterior and posterior midgut regions before and after feeding. In the whole midgut, activity rises from a baseline of approximately 3 enzyme units (EU) per midgut to a maximum of 12 EU at 30 hours after the blood meal, subsequently falling to baseline levels by 60 hours. A similar cycle of activity occurs in the posterior midgut and posterior midgut lumen, whereas aminopeptidase in the posterior midgut epithelium decreases in activity during digestion. Aminopeptidase in the anterior midgut is maintained at a constant low level, showing no significant variation with time after feeding. alpha-glucosidase is active in anterior and posterior midguts before and at all times after feeding. In whole midgut homogenates, alpha-glucosidase activity increases slowly up to 18 hours after the blood meal, then rises rapidly to a maximum at 30 hours after the blood meal, whereas the subsequent decline in activity is less predictable. All posterior midgut activity is restricted to the posterior midgut lumen. Depending upon the time after feeding, greater than 25% of the total midgut activity of alpha-glucosidase is located in the anterior midgut. After blood meal ingestion, proteases are active only in the posterior midgut. Trypsin is the major primary hydrolytic protease and is secreted into the posterior midgut lumen without activation in the posterior midgut epithelium. Aminopeptidase activity is also luminal in the posterior midgut, but cellular aminopeptidases are required for peptide processing in both anterior and posterior midguts. Alpha-glucosidase activity is elevated in the posterior midgut after feeding in response to the blood meal, whereas activity in the anterior midgut is consistent with a nectar-processing role for this midgut region.

Distribution



Female *Ochlerotatus notoscriptus* feeding on a human arm, Tasmania, Australia

While many species are native to tropical and subtropical regions, some such as *Aedes* have successfully adapted to cooler regions. In the warm and humid tropical regions, they are active the entire year long; however, in temperate regions they hibernate over winter. Eggs from strains in the temperate zones are more tolerant to the cold than ones from warmer regions. They can even tolerate snow and sub-zero temperatures. In addition, adults can survive throughout winter in suitable microhabitats.

Means of dispersal

Over large distances the worldwide distribution is carried out primarily through sea routes, in which the eggs, larvae, and pupae in combination with water-filled used tires and cut flowers are transported around. As with sea transport, the transport of mosquitoes in personal vehicles, delivery trucks, and trains plays an important role.

Disease

Anopheles albimanus mosquito feeding on a human arm. This mosquito is a vector of malaria and mosquito control is a very effective way of reducing the incidence of malaria.

Mosquitoes are a vector agent that carries disease-causing viruses and parasites from person to person without catching the disease themselves.

The principal mosquito borne diseases are the viral diseases yellow fever, dengue fever and Chikungunya, transmitted mostly by the *Aedes aegypti*, and malaria carried by the genus *Anopheles*. Though originally a public health concern, HIV is now thought to be almost impossible for mosquitoes to transmit.

Mosquitoes are estimated to transmit disease to more than 700 million people annually in Africa, South America, Central America, Mexico, Russia and much of Asia with millions of resulting deaths. At least 2 million people annually die of these diseases.

Methods used to prevent the spread of disease, or to protect individuals in areas where disease is endemic include Vector control aimed at mosquito eradication, disease prevention, using prophylactic drugs and developing vaccines and prevention of mosquito bites, with insecticides, nets and repellents. Since most such diseases are carried by "elderly" females, scientists have suggested focusing on these to avoid the evolution of resistance.

Control

Larvae in stagnant water

There are many methods used for mosquito control. Depending on the situation, source reduction (e.g. removing stagnant water), biocontrol (e.g. importing natural predators such as dragonflies), trapping, and/or insecticides to kill larvae or adults may be used.

Natural predators

The dragonfly nymph eats mosquitoes at all stages of development and is quite effective in controlling populations. Although bats and Purple Martins can be prodigious consumers of insects, many of which are pests, less than 1% of their diet typically consists of mosquitoes. Neither bats nor Purple Martins are known to control or even significantly reduce mosquito populations.

Some cyclopoid copepods are predators on first instar larvae, killing up to 40 Aedes larvae per day. Larval *Toxorhynchites* mosquitoes are known as natural predators of other Culicidae. Each larva can eat an average of 10 to 20 mosquito larvae per day. During its entire development, a *Toxorhynchites* larva can consume an equivalent of 5,000 larvae of the first instar (L1) or 300 fourth instar larvae (L4) (Steffan & Evenhuis, 1981; Focks, 1982). However, *Toxorhynchites* can consume all types of prey, organic debris (Steffan & Evenhuis, 1981), or even exhibit cannibalistic behavior. A number of fish are also

known to consume mosquito larvae, including bass, bluegill, piranha, catfish, fathead minnows, the western mosquitofish (*Gambusia affinis*), goldfish, guppies, and killifish.

Bacillus thuringiensis israelensis has also been used to control them as a biological agent.

Mosquito bites and treatment

Mosquitoes prefer some people over others. The preferential victim's sweat simply smells better than others because of the proportions of the carbon dioxide, octenol and other compounds that make up body odor. The powerful semiochemical that triggers the mosquito's keen sense of smell is nonanal. A large part of the mosquito's sense of smell, or olfactory system, is devoted to sniffing out human targets. Of 72 types of odour receptor on its antennae, at least 27 are tuned to detect chemicals found in perspiration.

Visible, irritating bites are due to an immune response from the binding of IgG and IgE antibodies to antigens in the mosquito's saliva. Some of the sensitizing antigens are common to all mosquito species, whereas others are specific to certain species. There are both immediate hypersensitivity reactions (Types I & III) and delayed hypersensitivity reactions (Type IV) to mosquito bites.

There are several commercially available anti-itch medications, including those taken orally, such as Benadryl, or topically applied antihistamines and, for more severe cases, corticosteroids such as hydrocortisone and triamcinolone. Many effective home remedies exist, including calamine lotion and vinegar. A paste of meat tenderizer containing papain and water breaks down the proteins in the mosquito saliva. By using a brush to scratch the area surrounding the bite and running hot water (around 49 °C) over it can alleviate itching for several hours by reducing histamine-induced skin blood flow. Plain household sudsy ammonia is also a good treatment, ammonia being the main ingredient in Tender's AfterBite remedy, especially as a first wash option if applied immediate after multibite exposure.

Repellents

The chemical DEET repels some mosquitoes and other insects. Other CDC recommended repellents are Picaridin, Oil of Eucalyptus (PMD) and IR3535.

Evolution

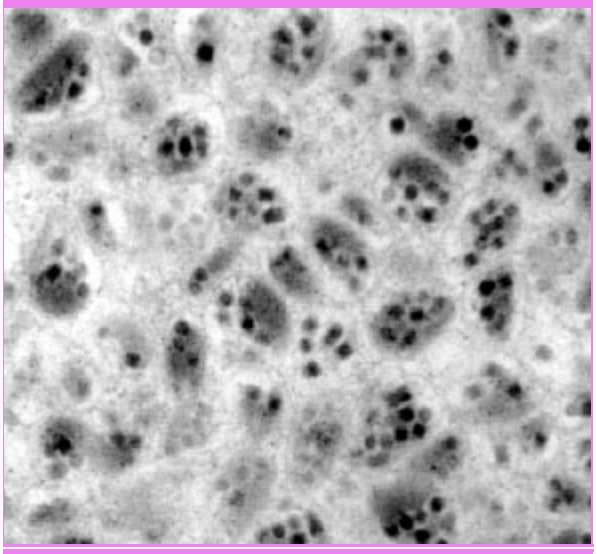
The oldest known mosquito with an anatomy similar to modern species was found in 79-million-year-old Canadian amber from the Cretaceous. An older sister species with more primitive features was found in amber that is 90 to 100 million years old.

Genetic analyses indicate that the Culicinae and Anophelinae clades may have diverged about 150 million years ago. The Old and New World *Anopheles* species are believed to have subsequently diverged about 95 million years ago.

Chapter- 16

Polydnavirus and Sterile Insect Technique

Polydnavirus

Polydnavirus

Virus classification
Group: Group I (dsDNA)
Family: ***Polydnaviridae***
Genera
Ichnovirus
Bracovirus

The **Polydnaviruses** (PDV) are a family of insect viruses that contain two genera: Ichnoviruses (IV) and Bracoviruses (BV). The ichnoviruses occur in ichneumonid wasps species and bracoviruses in braconid wasps. The genome of the virus is composed of multiple segments of double-stranded, superhelical DNA packaged in capsid proteins and

a double layer (IV) or single layer (BV) envelope. Little or no sequence homology exists between BV and IV, suggesting that the two genera evolved independently.

Biology

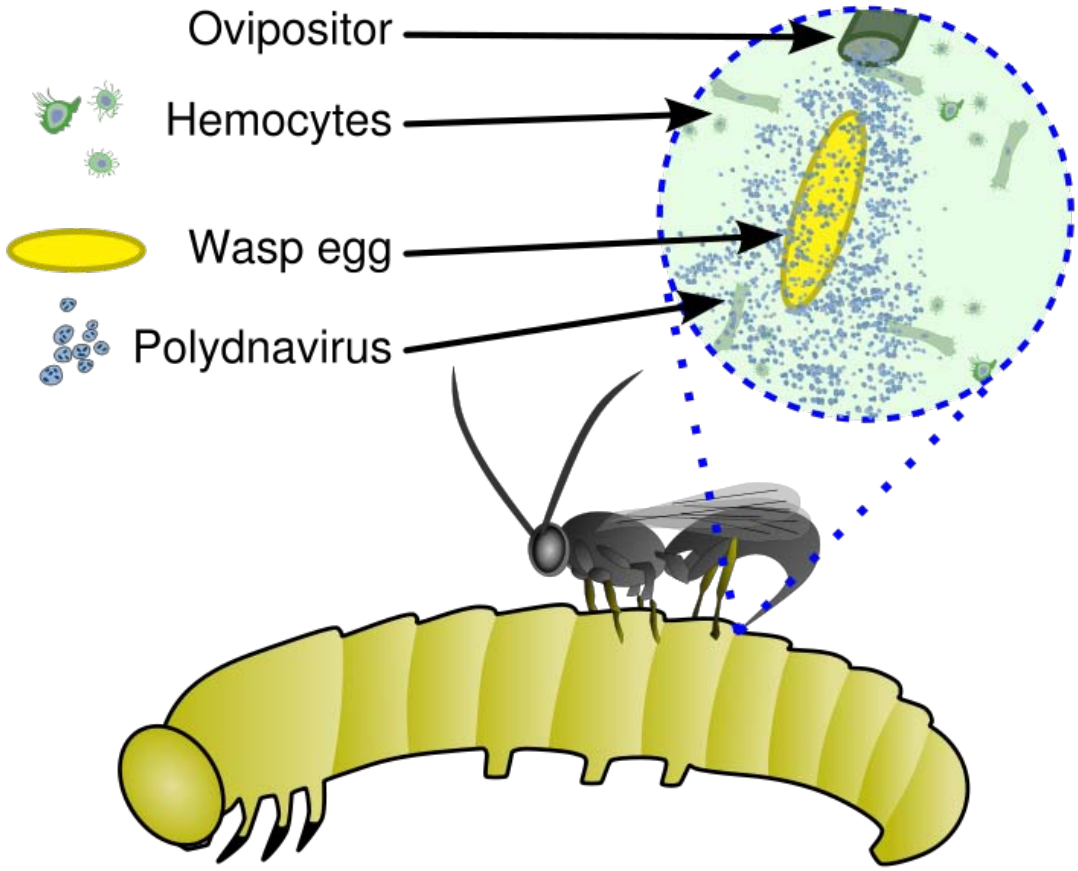


Diagram of a PDV host association

These viruses are part of a unique biological system consisting of an endoparasitic wasp (parasitoid), an insect (usually lepidopteran) larva, and the virus. The full genome of the virus is integrated into the genome of the wasps and the virus only replicates in specific cells in the female wasp's reproductive system. The virus is injected along with the wasp egg into the body cavity of a lepidopteran host caterpillar and infects cells of the caterpillar. The infection does not lead to replication of new viruses, rather it affects the caterpillar's immune system. Without the virus infection, phagocytic hemocytes (blood cells) will encapsulate and kill the wasp egg but the immune suppression caused by the virus allows for survival of the wasp egg, leading to hatching and complete development of the immature wasp in the caterpillar. Additionally, genes expressed from the polydnavirus in the parasitised host alter host development and metabolism to be beneficial for the growth and survival of the parasitoid larva. Thus, the virus and wasp have a symbiotic (mutualistic) relationship.

Characteristics

Both genera of PDV share certain characteristics:

- the virus particles of each contain multiple segments of dsDNA with each segment containing only part of the full genome (much like chromosomes in eukaryotic organisms);
- the genome of each is integrated into the host wasp genome;
- the virus particles are only replicated (produced) in specific cell types in the female wasp reproductive organs.

However, the morphology of the two genera are different when observed by electron microscopy. Ichnoviruses tend to be ovoid (egg-shaped) while bracoviruses are short rods.

Evolution

Nucleic acid analysis suggests a very long association of the viruses with the wasps (greater than 70 million years).

Two proposals have been advanced for how the wasp/virus association developed. The first suggests that the virus is derived from wasp genes. Many parasitoids that do not use PDVs inject proteins that provide many of the same functions, that is, a suppression of the immune response to the parasite egg. In this model, the braconid and ichneumonid wasps packaged genes for these functions into the viruses – essentially creating a gene-transfer system that results in the caterpillar producing the immune-suppressing factors. In this scenario, the PDV structural proteins (capsids) were probably "borrowed" from existing viruses.

The alternative proposal suggests that ancestral wasps developed a beneficial association with an existing virus that eventually led to the integration of the virus into the wasp's genome. Following integration, the genes responsible for virus replication and the capsids were (eventually) no longer included in the PDV genome. This hypothesis is supported by the distinct morphology differences between IV and BV, suggesting different ancestral viruses for the two families. The IV have remarkable similarities to ascoviruses while BV may be related to baculoviruses

Sterile insect technique

Screwworm was the first pest successfully eliminated from an area through the **sterile insect technique** by the use of an area-wide approach.

Sterile insect technique is a method of biological control, whereby millions of sterile insects are released. The released insects are normally male as it is the female that causes the damage, usually by laying eggs in the crop, or, in the case of mosquitoes, taking a bloodmeal from humans. The sterile males compete with the wild males for female insects. If a female mates with a sterile male then it will have no offspring, thus reducing the next generation's population. Repeated release of insects can eventually wipe out a population, though it is often more useful to consider controlling the population rather than eradicating it.

The technique has successfully been used to eradicate the Screw-worm fly (*Cochliomyia hominivorax*) in areas of North America. There have also been many successes in controlling species of fruit flies, most particularly the Medfly (*Ceratitis capitata*), and the Mexican fruit fly (*Anastrepha ludens*).

Insects are mostly sterilized with radiation, which might weaken the newly sterilized insects, if doses are not correctly applied, making them less able to compete with wild males. However, other sterilization techniques are under development which would not affect the insects' ability to compete for a mate.

The technique was pioneered in the 1950s by American entomologists Dr. Raymond C. Bushland and Dr. Edward F. Knipling. For their achievement, they jointly received the 1992 World Food Prize.

Development of the sterile insect technique

Raymond Bushland and Edward Knipling first developed the technique to eliminate screwworms preying on warm-blooded animals, especially cattle herds. With larvae that invade open wounds and eat into animal flesh, the flies were capable of killing cattle

within 10 days of infection. In the 1950s, screwworms caused annual losses to American meat and dairy supplies that were projected at above $200 million. Screwworm maggots are also known to parasitize human flesh. Since female Screwworm mate only once in her lifetime, this physiological phenomenon has been exploited by biologists in breaking it's life cycle. After mating with Sterile males, screwworm female will not mate again, will not lay any eggs from this mate, eventually resulting in decline of its population.

The quest of Bushland and Knipling to find an alternative to chemical pesticides in controlling the devastation wrought by these insects began in the late 1930s when both scientists were working at the United States Department of Agriculture Laboratory in Menard, Texas. At that time, the screwworm was decimating livestock herds across the American South. Red meat and dairy supplies were also affected across Mexico, Central America, and South America.

While Bushland initially researched chemical treatment of screwworm-infested wounds in cattle, Knipling developed the theory of autocidal control – breaking the life cycle of the pest itself. Bushland's enthusiasm for Knipling's theory sparked both men to intensify the search for a way to rear large numbers of flies in a "factory" setting, and most importantly, to find an effective way to sterilize flies.

Their work in this area was interrupted by World War II, but Drs. Bushland and Knipling resumed their efforts in the early 1950s with their successful tests on the screwworm population of Sanibel Island, Florida. The sterile insect technique worked; near eradication was achieved using X-ray sterilized flies.

In 1954, the technique was used to completely eradicate screwworms from the 176-square-mile (460 km^2) island of Curaçao, off the coast of Venezuela. Screwworms were eliminated in a span of only seven weeks, saving the domestic goat herds that were a source of meat and milk for the island people.

During the 1960s and 1970s, SIT was used to control the screwworm population in the United States. The 1980s saw Mexico and Belize eliminate their screwworm problems through the use of SIT, and eradication programs have progressed through all of Central America, with a biological barrier having been established in Panama to prevent reinfestation from the south. In 1991, Knipling and Bushland's technique halted a serious outbreak in northern Africa. Similar programs against the Mediterranean fruit fly in Mexico and California use the same principles. In addition, the technique was used to eradicate the melon fly from Okinawa and has been used in the fight against the tsetse fly in Africa.

The technique has been able to suppress insects threatening livestock, fruit, vegetable, and fiber crops. The technique has also been lauded for its many environmentally sound attributes: it uses no chemicals, leaves no residues, and has no effect on non-target species.

Proven effective in controlling outbreaks of a wide range of insect pests throughout the world, the technique has been a boon in protecting the agricultural products to feed the world's human population. Both Bushland and Knipling received worldwide recognition for their leadership and scientific achievements, including the World Food Prize. Their research and the resulting Sterile Insect Technique were hailed by former U.S. Secretary of Agriculture Orville Freeman as "the greatest entomological achievement of (the 20th) century."

Sterile fly for African trypanosomiasis

Sleeping sickness or the African trypanosomiasis is a parasitic disease in humans. Caused by protozoa of genus *Trypanosoma* and transmitted by the Tsetse fly, the disease is endemic in certain regions of Sub-Saharan Africa, covering about 36 countries and 60 million people. It is estimated that 50,000 - 70,000 people are infected, and about 40,000 die every year. Three major epidemics have occurred in the past hundred years, in 1896 - 1906, 1920, and 1970.

Studies of the tsetse fly show that females generally only mate once in their lifetimes and very rarely mate a second time. Once a female fly has mated, she can then produce continual offspring throughout her short life.

The **sterile fly** is an innovative solution to the problem of the African trypanosomiasis. Specially bred male Tsetse flies are sterilized through irradiation process. These sterilized male flies are then released into areas where sleeping sickness is prevalent, and then mate with the females. Because the male is sterile, and the females mate only once, the population of Tsetse flies in the affected area will drop. Studies have shown that this process has been very effective in preventing sleeping sickness in people who live in the area.

Since sleeping sickness is fatal without treatment and infected people can be without symptoms for months, the release of sterile flies into affected areas leads to greater levels of health and economic activity.

Current targets

- *Anopheles* mosquito - Malaria vector, example *Anopheles arabiensis*.
- Tsetse fly (*Glossina spp*) - sleeping sickness vector.
- Painted Apple Moth (Lepidoptera: Lymantriidae) in Auckland, New Zealand.
- *Aedes* mosquitoes, vectors for filariasis, Dengue and yellow fever.
- Continuing use across the world against various fruit fly species, including Medfly, Caribbean and Mexican fruit fly (Nth, Sth and Central America), Queensland fruit fly in Australia (*Bactrocera tryoni*) and several other *Bactrocera* sp. across Australia, Asia and Oceania. The IAEA lists 36 different fruit fly SIT facilities across the globe, on all six inhabited continents and provides information on the doses of radiation used in the control of pest insects and mites of agricultural, commercial or quarantine significance. It includes data on both the

radiation dose required for the disinfestation of generic commodity groups, fresh and durable, and also the radiation dose used to induce sterility for pest control through the sterile insect technique.

Drawbacks

- As for insecticide treatment, repeated treatment is sometimes required to suppress the population before the use of sterile insects.
- Sex separation could be difficult for some species, though this can be easily performed on Medfly and screwworm, for example.
- Radiation treatment in some case, over doses, affects the health of males, so sterilized insects in such cases are at a disadvantage when competing for females.
- The technique is species specific, for instance: there are 22 species of Tsetse fly in Africa, and the technique must be implemented separately for each.
- Standard operating procedures of mass rearing and irradiation, do not leave room for mistakes. Since the fifties, when SIT was first used as a means for pest control, several failures have occurred in different places around the world where non-sterilized artificial produced insects were released before the problem was spotted.
- Application to large areas should be long lasting, otherwise migration of wild insects from outside the control area could repopulate.
- The major drawback to this technique is that the cost of producing such a large number of sterile insects is often prohibitive in poorer countries.

Genetic modification

A method using recombinant DNA technology to create genetically modified insects called RIDL (Release of Insects carrying a Dominant Lethal) is under development by a company called Oxitec. The method works by introducing a repressible "Dominant Lethal" gene into the insects. This gene kills the insects but it can be repressed by an external additive, which allows the insects to be reared in manufacturing facilities. This external additive is commonly administered orally, and so can be an additive to the insect food. The insects can also be given genetic markers, such as fluorescence, that make monitoring the progress of eradication easier.

There are potentially several types of RIDL, but the more advanced forms have a female-specific dominant lethal gene. This avoids the need for a separate sex separation step, as the repressor can be withdrawn from the final stage of rearing, leaving only males.

These males are then released in large numbers into the affected region. The released males are not sterile, but any female offspring their mates produce will have the dominant lethal gene expressed, and so will die. The number of females in the wild population will therefore decline, causing the overall population to decline.

Using RIDL means that the males will not have to be sterilized by radiation before release, making the males more healthy when they need to compete with the wild males for mates.

Progress towards applying this technique to mosquitos has been made by researchers at Imperial College London who created the world's first transgenic malaria mosquito.

A similar technique is the daughterless carp, a genetically modified organism produced in Australia by the CSIRO in the hope of eradicating the introduced carp from the Murray River system. As of 2005, it was undergoing tests to assess the risks of releasing it into the wild.

Conclusion and perspectives

Biotechnological approaches based on genetically modified organism (transgenic organisms) are still under development. However, since no legal framework exists to authorize the release of such organisms in the nature, sterilization by irradiation remains the most used technique. A meeting was held at FAO headquarters in Rome, 8 to 12 April 2002 on *"Status and Risk Assessment of the Use of Transgenic Arthropods in Plant Protection"*. The resulting proceedings of the meeting have been used by the North American Plant Protection Organization (NAPPO) to develop NAPPO Regional Standard No. 27 on *"Guidelines for Importation and Confined Field release of Transgenic Arthropods"*, which provides the basis for the rational development of the use of transgenic arthropods.

SIT programs will benefit tremendously if genetic methods can be developed that enable only male insects to be reared as has already been done for the medfly. Also more appropriate artificial diets for larvae, and hormonal, nutritional, microbiological, and semiochemical treatments for adults, could make major contributions through improved economy and insect quality.

Economic benefits of SIT has been demonstrated in various cases. Direct benefits of screwworm eradication to the North and Central American livestock industries are estimated to be over $ 1.5 billion/ year, compared with a total investment over half a century of close to $ 1 billion. Mexico protects a fruit and vegetable export market of over $ 3 billion/year through an annual investment of ca. $ 25 million, and medfly-free status has been estimated to have opened markets for Chile’s fruit exports of up to $ 500 million. Eradication of tsetse has resulted in major socio-economic benefits for Zanzibar. When implemented on an area-wide basis and with economies of scale in the mass rearing process, the use of SIT for suppression is cost competitive with conventional control, in addition to its environmental benefits.