GENERAL LIFE CYCLE PATTERNS AND LARVAL FORMS IN TREMATODES

Digenetic trematodes or flukes are among the most common and abundant of parasitic forms. They are parasites of all classes of vertebrates and nearly every organ of the vertebrate body can be parasitized by some kind of trematode, as adult or juvenile. Digenean development occurs in at least two hosts. The first is a mollusk or very rarely an annelid. Many species include a second and even a third intermediate host in their life cycles.

A typical life cycle of trematodes is as follows:

The zygote, resulting from the fusion of male and female gametes, is encased within an egg shell. The eggs, which are quite numerous become lodged in the long and often coiled uterus. From their in utero position the eggs are released into the host's intestinal lumen through the genital pore and later are passed to the outside in the host's feaces. The eggs are deposited in water and eventually hatch, and from each egg a free- swimming **miracidium** emerges.

The miracidium penetrates the integument of a molluscan host (the first intermediate host) and sheds its ciliated epidermis in the process. The naked miracidium develops into a **sporocyst**, which migrates through blood vessels and other spaces and tissues leading to the digestive gland and eventually becomes lodged in that organ. In some species, the eggs enter the molluscan host passively that is, the eggs are ingested by the host, and the miracidia hatch out within the host's digestive tract and then migrate to the digestive gland.

Although the digestive gland is a common site for further development of the sporocyst, it is not the only one. The gonad, mantle, lymph spaces surrounding the intestine, gill chambers, and other areas may also serve as sites for further development. Once established in a suitable position, the sporocyst increases in size and develops to its mature form within the sporocyst, which is commonly elongate and hollow, germinal cells are present. These cells increase in number by mitotic division and eventually differentiate into germ balls. Each germ ball further increases in size and may differentiate into another larval generation called the **redia**.

As in the sporocyst, certain germinal cells in the brood chamber of redia eventually give rise to tail- bearing **cercariae**, a fourth larval generation. Cercariae escape from their molluscan host and becomes free swimming. In some species, the cercaria never leave the molluscan host and enter the next host only if the infected mollusk is ingested.

When the free swimming cercariae come in contact with a compatible second intermediate host – often an arthropod or some other invertebrate, even a vertebrate- they actively penetrate the host's body and encyst. The encysted larva is known as a **metacercaria**. When the second intermediate host is ingested by the vertebrate definitive host, the encysted metacercaria excysts in the host's intestine and gradually matures into the adult.

During the life cycle of various digeneans, it is not uncommon to find mother and daughter generations of sporocysts and redia. The number of generations of these different larval forms is usually consistent in the members of a specific taxon. Variations of this generalized life history pattern are known. From the foregoing, it is evident that stages in the life cycle of the typical trematode include the adult, egg, miraidium, sporocyst, redia, cercaria and metacercaria.

THE ADULT :

Sexually mature adult digenetic trematodes can be separated into morphologic types.

1. The most frequently encountered morphologic type is the **distome**. In this type, the body is commonly elongate oval. The size depending on the species varies from 30 μm to approximately 30mm in length, as in the liver fluke, *Fasciola hepatica*. In some unusual species, such as those that parasitize scombrid fish, the length may be several inches. Both the anteriorly situated oral sucker and acetabulum are present. The mouth is located in the center of the oral sucker, and the intestinal caeca usually are forked, although the caeca of members of the family Cyclocoeliidae are joined Posteriorly to form a ring.
2. The **amphistome** type is characterized by the position of the acetabulum, which is located at the posterior end of the body and is often referred to as the **posterior sucker**. The ovary is located posterior to the testes, an arrangement also found among other types of trematodes, but almost universal among the amphistomes.
3. The **monostome** type is characterized by the presence of only one sucker – the anteriorly located oral sucker.
4. The **gasterostome** type, which is limited to certain species parasitic in fish, is characterized by the mouth being located not in the center of the anterior sucker but in the middle of the ventral sucker. Thus, in this case the ventral sucker is the oral sucker.
5. The **holostome** type, found primarily in the intestine of birds and less frequently in mammals and other vertebrates is characterized by its elongate body, which is divided into a forebody and a hindbody. The mildly ventrally concave forebody bears the anterior sucker and the acetabulum. Quite commonly, the anterior sucker is provided with auxillary suckers flanking it. A special glandular adhesive organ, called the **tribocytic** organ, is located immediately posterior to the acetabulum.
6. The echinostome type is actually a specialized distome, for the positions of the suckers are comparable to those found in distomes. However, it may be considered a distinct type, because there is a collar of large spines surrounding the oral sucker.

   These six morphologic types do not necessarily indicate six distinct phylogenetic groups but are merely useful in describing adult trematodes.

Structure of the adult

The outer surface of the adult digenean is covered by a syncytium known as the tegument. Electron micrographs have revealed that the tegument can be seen to consist of two zones. The outer zone, which is separated from the environment by a unit membrane, consists of a cytoplasmic syncytium. Embedded in this layer are mitochondria, endoplasmic reticulum, various types of vacuoles and, in some instances, glycogen granules and other types of inclusions. The outer surface is usually thrown into folds to form microvilli. These undulations not only serve to increase the absorptive surface, but pinocytotic vesicles are also formed in the crypts between adjacent microvilli for the intake of large molecules and particulate materials. Also embedded in the outer zone of some species are tegumentary spines. These are overlayed by the surficial plasma membrane and undoubtedly serve as ancillary holdfast mechanisms *in situ*. The outer syncytial zone is connected by cytoplasmic bridges to nucleated bodies, known as **cytons** (**or perikarya**), embedded deeper in the parenchyma. The cytons, collectively designated as the inner tegumentary zone, include vacuoles, endoplasmic reticulum, mitochondria, Golgi bodies, glycogen deposits and various types of vesicles in addition to the nucleus. Specifically, lying immediately mediad to the outer tegumentary zone and separated from it by a unit membrane, is a thin layer of connective tissue fibers known as the basal lamella. Beneath this are a series of circular muscles, under which are the fascicles of longitudinal muscles. The occurrence of acid mucopolysaccharides is of particular significance, since these molecules are known to be capable of inhibiting various digestive enzymes. Thus, their presence and possible secretion onto the body surface may account for why the intestinal trematodes are not digested by the host's enzymes. Digeneans possess an incomplete digestive system. The mouth is located anteriorly in the center of the oral sucker (except in gasterostomes). The mouth leads into a bulbous muscular pharynx via a short prepharynx, which may be absent in some species. The pharynx serves primarily as a masticatory organ, from which the ingested food particles pass into the esophagus. The esophagus is lined with a layer of cells. At the posterior terminal of the esophagus the alimentary tract bifurcates, giving rise to two blind-sac intestinal caeca that terminate posteriorly in the parenchyma. Both the buccal cavity and esophagus may receive secretions of salivary and esophageal glands, respectively, which contribute to the process of digestion. The lengths of the esophagus and intestinal caeca are commonly employed, in combination with other characteristics, as taxonomic diagnostic features. Generally, the cells lining the intestinal caeca (gastrodermis) are described categorically as being epithelial; however they perform more than a lining function. These cells are believed to go through a secretory and an absorptive phase. During the absorptive phase, when nutrient material is present, the cells are short; however, when food materials are absent, they become columnar. During the secretory phase, the cells become filled with secretory materials. When these materials are secreted, the cells collapse, and are later regenerated. The secretions presumably represent digestive enzymes, since others have shown that the caecal cells are rich in a number of enzymes and in ribonucleic acid. The reproductive systems,both male and female of digenetic trematodes are somewhat similar to those found in the Monogenea and Aspidobrothrea. The male reproductive system generally includes two testes, although multitesticular species are known; the members of several families (e.g. Spirorchidae, Schistosomatidae) are multitesticular. Digeneans with a single testis also exist. The testis are located in the parenchyma in rather exact positions. Infact, the positions of these gonads are of considerable importance in the identification of species. Leading from each testis is a vas efferens. These ducts generally unite to form the common vas deferens, which opens into the cirrus pouch. In some species the vasa efferentia enter the cirrus pouch independently. The cirrus pouch (or cirrus sac) is situated at the terminal of the male reproductive system enclosing the seminal vesicle, the prostate glands and the protrusible cirrus. In some trematodes a permanent penis is present. In others, the seminal vesicle is located outside the pouch and is known as an external seminal vesicle. Not all digenetic trematodes possess a cirrus pouch, in some the vas deferens empties directly to the outside via the genital pore. Spermatozoa formed in the testes, pass down the vasa efferentia and vas deferens to the seminal vesicle and are stored therein. During coapulation, sperm are ejected through the eversible cirrus, which is inserted in the genital atriumof the female system. The prostate gland cells, which surround and empty into the cirrus, are believed to secrete a fluid in which spermatozoa survive. The female reproductive system generally includes a single ovary located in the parenchyma either anterior or posterior to the testis, depending on the species. Ova(actually secondary oocytes) formed within the ovary are released from that organ via a short **oviduct** that opensinto a minute chamber, the ootype. Three auxillary organs also empty into the ootype: (1) The Mehlis' gland, which is a cluster of unicellular glands surrounding and independently emptying into the ootype.; (2) **the common vitelline duct**, which receives the materials from the vitelline glands via the left and right ducts and deposits them in the ootype; and (3) the duct from the seminal receptacle (absent in some species), which delivers sperm into the ootype or oviduct. In some species a fourth structure- the vitelline reservoir - is present as a diverticulum of the common vitelline duct. The function of this reservoir is for temporary storage of vitelline materials. The Mehlis gland has several functions:

(1) These glands secrete a fluid that enhances the hardening or tanning process of newly formed eggs by maintaining the desired pH, redox potential and so on.

(2) The secretion of these cells causes release of the shell globules from the vitelline glands.

(3) The secretion forms a thin membrane around the cells forming the egg and the shell globules then build up from within this membrane.

(4) Its secretion lubricates the uterus, facilitating passage of the eggs.

(5) Its secretion activates spermatozoa, which are passed down to the ootype.

The **uterus,** which is connected to the ootype, is the long and often convoluted tube, in which the formed eggs are transported to the exterior. In some species, the distal segment of the uterus is muscular and by peristaltic movement expels eggs. The muscular portion is referred to as the **metraterm.** The terminal portion of the uterine tract empties into the genital atrium which, in most species, is the common chamber into which the cirrus also opens. The atrium opens to the exterior through the common genital pore. During self or cross fertilization (The latter being more common), the cirrus is evaginated – that is, with the inside surface becoming the outside, much in the same fashion as a sock is pulled inside out- and is inserted in the female aperture. Spermatozoa introduced down the uterus are stored in the seminal receptacle, from which they enter the ootype, where fertilization and egg formation occur. In some species, a Laurer canal is present. This tube originates from the ootype and passes dorsally through the parenchyma; it may or may not open to the exterior. If the canal opens to the exterior, it may serve as a vagina . This is believed to be a case in the progenitor species. The **vitelline glands**, collectively known as the vitellaria, are groups of glands (acini) commonly located along the two lateral sides of the body, but in some species they converge along the midline. Arising from each acinus is a vitelline duct, which joins other ducts on the same side to form the left and right vitelline ducts, respectively. The left and right ducts either may converge to form the common vitelline duct, which enters the ootype or may enter the ootype independently. In addition to contributing yolk material for incorporation within the egg, the vitelline glands also secrete large globules, known as **shell globules**, which envelop the developing eggs and eventually coalesce and become hardened to form the shell. This hardening process involves the tanning of the protein or sclerotin present within the coalesced globules by quinone. Thus, the vitelline glands contribute both yolk and shell materials. The excretory or osmoregulatory system of digenetic trematodes id of the protonephritic type. The arrangement of the indivisual flame cells along the collecting tubes is so consistent in a given taxon that their arrangement pattern- known as the flame cell pattern- is employed as a tool to indicate phylogenetic relationships. Each flame cells leads into a minute capillary duct that joins with others to form the anterior and posterior collecting ducts. These two ducts on each side of the body empty into an accessory duct (or tubule). The two accessory ducts on each side unite to form the left and right common collecting tubules. When the common collecting tubules extend to the mid region of the body, they either fuse with the excretory vesicle (mesostomate type) or extend to near the anterior end of the body prior to passing posteriorly to join the excretory vesicle (stenostomate type). It is noted that whether the arrangement of the excretory tubules is of the mesostomate or stenostomate type can be recognized in the cercarial stage. The excretory vesicle is singular and opens to the exterior through the pore located at or near the posterior extremity of the body.

LARVAL FORMS OF DIGENETIC TREMATODES

THE EGG

The ovoid digenean egg is typically operculated i.e., there is a lidlike cap or operculum at one end which, when pushed off, permits the miracidium to escape. Eggs of some species possess a minute projection or boss at the opposite end. The eggs of *Schistososme*, however, are not operculated. The shell is split during the hatching process. Within the shell, the fully or partially developed miracidium is surrounded by yolk globules within the vitelline membrane. The latter is formed during the development of the miracidium by the fusion of certain small, surface somatic cells.

THE MIRACIDIUM

When the egg hatches, a minute, elongate ovoid larva covered with flattened ciliated epidermal plates escapes. This is the miracidium. Beneath the epidermal plates are focused well-developed circular and longitudinal muscles. At the anterior tip of the miracidium is a mobile **apical papilla** or **terebratorium or tactile organ or rostrum**. Rostrum is either flexible or rigid.Rostrum is flexible in Fasciola hepatica and rigid in case of Parorchis acanthus. Apical papilla bears a median aperture , which is considered as mouth. On this papilla are four filaments that are connected with the cerebral ganglion. These filaments are probably chemoreceptors. A multinucleated **apical gland**, located in the parenchyma, empties to the outside near the terebratorium. The secretion of this gland aids in dissolving the host's tissue during the miracidium's penetration process. In addition to the apical gland, additional lateral penetration glands occur. These also deposit lytic enzymes at the base of the papilla. The papilla becomes partially invaginated during penetration and thus functions as a suction cup into which the glandular secretion is secreted. The invagination of the papilla is apparently stimulated by a host factor. The sensory organs of the miracidium also may include two, sometimes three, eyespots and lateral papillae, one on each side of the body between the first and second tiers of ciliated plates. In some species, such as *Parorchis acanthus*, a rectum-dwelling parasite in gulls and flamingos, there are additional sensory papillae that are circularly arranged in the same groove. The brain of the miracidium is a large cephalic ganglion lying in the parenchyma behind the apical region. From this center, nerve fibers innervate various tissues and organs of the body. The flame cell type of excretory system is present in the miracidium. The body fluids containing wastes are collected by two or three pairs of flame cells and are deposited to the exterior through two laterally situated excretory pores. During the differentiation of the miracidium within the egg shell, certain germinal cells become trapped in the parenchyma. These develop into germ balls by increasing in size and number and becoming enveloped. These germ balls eventually differentiate into the next larval generation. They are usually located in the posterior portion of the body. Miracidia of digeneans belonging to the families Bucephalidae and Brachylaemidae are unique in that the body plates are not ciliated. Rather, the cilia are arranged in tufts borne on stalks.

Behaviour:

The behavior of free-swimming miracidia has been studied to some extent. The fact that most miracidia have eyespots suggests that they are phototactic. Indeed, in a number of species either positive or negative phototaxis occurs, more commonly the former. Furthermore, the phototactic response is influenced by the ambient temperature. Similarly, it has been shown that miracidial geotactic responses are influenced by temperature.

Feeding and Metabolism

Miracidia cannot ingest food. They can, however, utilize exogenous glucose. Byrant and Williams(1962) have traced the incorporation of ^{14}C-glucose and succinate in *Fasciola* miracidia. They have reported that the labeled glucose becomes incorporated into hexose phosphate, phosphoenolpyruvic acid, alaine and lactic acid. This suggests that the Embden-Meyerhof glycoticpathway occurs. There is no evidence that the complete Kreb's cycle is operative, although two of the enzymes of this cycle, succinic dehydrogenase and fumarase, occur.

THE SPOROCYST

First generation sporocysts, having differentisted from miracidia, frequently are found between the tubules in the digestive gland of the molluscan host. However, first generation or mother sporocysts may occur elsewhere in the intermediate host's body. Those of plagiorchioids (members of the superfamily Plagiorchioids) are attatched to the snail's alimentary trct, and those of the schistosomes usually occur in the tentacles or foot, depending on the site of entry of the miracidia. The shape of sporocysts ranges from ovoid buds to elongate, sausage-shaped bodies, to branched structures. The branched forms found in the members of the families Bucephalidae, Heronimidae, Brachylaemidae, and to some extent, Dicrocoelidae. In these, the branching arms ramify throughout the host's tissues.

The sporocyst wall varies in thickness, depending on age and species. The outermost layer like all the subsequent stages of the digenean life cycle is comprised of a syncytial tegument observable with the electron microscope. Projecting from the tegument are the microvilli which increase the body surface and hence enhance the absorption of nutrients.

Under the tegument is a thin layer of connective tissue known as the basal lamella. Beneath this layer are found circularly arranged muscles followed by an inner layer of parenchymal cells. The **brood chamber** is a hollow space in the center of the sporocyst within which are found the germ balls that eventually differentiate into the next generation. In living specimens, the brood chamber also contains a fluid. Sporocysts contain no alimentary or reproductive systems. A nervous system has been reported in certain species, such as Schistosomes. Flame cells are generally present. In some, there is inconspicuous birth pore, through which the fully formed larva escape. Larvae of those species without a birth pore escape by rupturing the sporocyst wall.

Redia

In some species that include a redial stage, these larvae either develop directly from the miracidium or are found within the brood camber of sporocysts. They eventually escape into the hosts tissues, usually in the digestive gland . In echinostomes whose rediae are found in bivalve mollusks, the rediae are commonly found embedded between the inner and outer lamellae of the hosts gills. The rediae is elongate and normally possesses two or four budlike projections- the ambulatory buds or procruscula-two located anterolaterally and two posterolaterally. Unlike the sporocyst the rediae possesses a mouth located at the anterior terminal. The mouth leads into a muscular phyrnyx, which in turn leads into a blind sac caecum. Rediae are capable of motility through the movement of the ambulatory buds and body contractions, and they ingest host cells. The rediae of certain trematodes possess a pair of cephalic ganglia on each side of the phyrynx. From these ganglia nerve fibers radiate in all directions.

The protonephric osmoregulatory (excretory) system is present in most rediae. The excretory system found in the rediae of cotylophoron cotylophorum, an amphistome fluke parasitic in the rumen of cattle. In this species the collecting duct on each side empty into separate excretory vesicles, which in turn empty to the exterior through two laterally located excretory pores.The flame cell pattern found in these larvae is of the same general as that found in the adult, but there are fewer individual flame cells.

The histology of the body wall of rediae , including its fine structure, is identical to that of sporocysts. More often than not, a birth pore is present, lateral to and near the mouth. Within the brood chamber are found germ balls that either differentiate into another generation of rediae (daughter rediae) or into next larval generation (cercariae).

Cercaria

Cercaria are differentiated from the germ balls in the brood chamber of sporocysts or_rediae which ever may be the pattern in the particular species. After escaping from the brood chamber of the preceding generation, cercariae actively leave the molluscan host and

1. Become free swimming in water, actively seeking the next host
2. encyst on vegetation
3. occur in the slime trail of terrestrial snails
4. encyst in the mantle cavity of the mollusk
5. develop into a tailess, nonencyted matacercaria within the molluscan host.

Thus, except in the first instance, these larvae must await ingestion by the definitive or another intermediate host in order to continue their development.

Cercarial Types

Before describing the internal anatomy, it appears advisable to consider the types of cercariae as based on their external morphology. The separation of cercariae into the following types, however, by no means indicates that the species belonging to each type are closely related. These categories are purely destructive, although, in many cases, closely related flukes do possess similar cercariae.

1. **Monostome:** cercariae possess one sucker only- the anteriorly situated oral sucker. They possess two eyespots and long simple tails, usually with pointed, locomotor processes directed Posteriorly from the posterior extremity of the body proper. This type of cercariae develops , in rediae and give rise to monostomate adults
2. **Amphistome:** cercariae possess a posterior sucker and eyespots. These cercariae develop in rediae and give rise to amphistome adults (superfamily paraamphistomoidea).
3. **Gasterostome:** cercariae possess a mouth located on the ventral surface of the body, and they eventually develop into gasterostome adults of the family Bucephalidae.
4. **Distome:** cercariae possess two suckers- the anteriorly located oral sucker ant the ventrally located acetabullum. This is by far the most common type of cercariae.

The four types of cercariae listed above are classified according to position and number of suckers. The following descriptive are categorized according to shape and relative size of their tails.

1. **Pleurolophocercous:** possess are characterized by a dorsoventral finlike fold along the length of tail. These possess commonly possess eyespots and an extremely small acetabulum that is readily overlooked. Pleurolophocercous cercariae are produced in rediae encyst in ectothermic vertebrates, and when ingested by the definitive host develop into adults belonging to the super family Opisthochioidea.
2. **Cystocercous** cercariae are characterized by a cavity or cyst at the base of the tail into which the body proper can be withdrawn. These are usually parasites of amphibians as adults and belong to the family Gorgoderidae.
3. **Furcocercous** cercariae are characterized by a forked tail. Some species may possess eyespots. Those with a phyrynx are the larvae of holostomes and strigeids, whereas those without a phyrynx are schistosomes or schistosme- related species.
4. **Microcercous** cercariae are characterized by a small tail that may be knoblike or conical. These nonswimming larvae do not represent any specific taxonomic group.
5. **Gymnophallus** cercariae are characterized by the complete absence of tail.
6. **Rhopalocercous** cercariae are characterized by a broad tail that is as wide as or wider than the body proper.
7. **Leptocercous** cercariae are characterized by a straight tail that is slender and narrower than the body proper.

8. **Trichocrcous** cercariae are characterized by tails armed with spine or bristles. These are marine forms.
9. **Cercariaea** like gymnophallus cercariae, lack a tail but differ from the latter in that they generally do not leave molluscan host. In few marine species that do not leave their hosts, cercariae move by inching along in a wormlike crawl.
10. **Rat-king** cercariae are all amrine and are characterized by their colonial arrangement. The tails of the individuals are joined and the bodies arranged in radial pattern.
11. **Cotylocerous** cercariae are similar to the microcercous type except that the short tail is shaped like a cup, generally with large gland cells lining the concavity.

Cercariae can also be categorized morphologically by specialized structures. Some of these are listed below.

1. **Echinostome** cercariae are characterized by the presence of a collar of spines around the anterior sucker. These develop in rediae and mature into adults belonging to the superfamily Echinostomatoidea.
2. **Gymnocephalous** cercariae are typical distomes which, unlike the echinostome cercariae, lack a spinous collar.
3. **Xipphidiocercariae** posses an anterior stylet in the oral sucker. Penetration glands are exceptionally well developed in these cercariae, which develop in sporocysts. Certain Xipphidiocercariae possess a bipartite, transparent, fluid filled sac that overlaps the oral sucker, known as a virgulate organ. Such cercariae are known as virgulate Xipphidiocercariae. Most, if not all, of these cercariae belong to the family Lecithodendriidae. The material secreted by the virgulate organ aids the cercariae in becoming attached to their hosts while penetrating, and it is also protective.
4. **Opthalmocercariae** include all the forms that possess eyespots.

Metacercaria

The final larval stage of digenetic trematodes is the metacercaria. When the free swimming cercariae cones to rest on suitable vegetation ar penetrates a compatible host, it loses its tail and encysts. Within the cyst wall or walls, the body proper of the cercariae metamorphoses into the metacercaria, which is actually a juvenile replica of the adult. Many cercarial structures, such as stylet, penetration glands, cytogenous and mucoid glands, and eyespots, soon disappear. The genital primordium differentiates into gonads that are usually nonfunctional, however in certain progenetic metacercaria, sterile or even fertile eggs are formed. The excretory vesicles in metacercaria are greatly distended and include varying amounts of refractile globules.