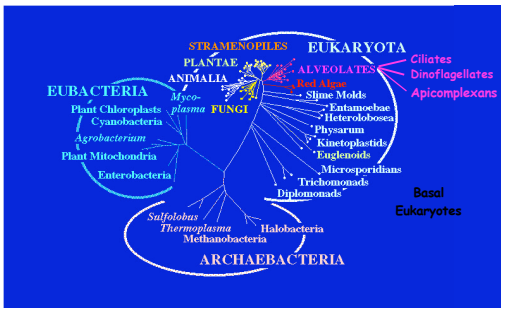
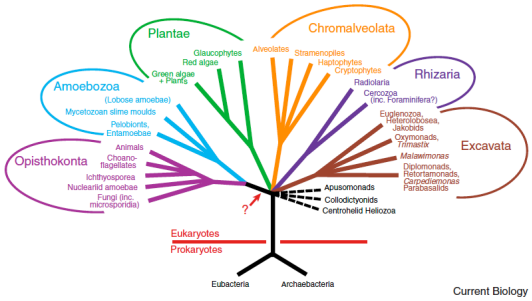
The Three Kingdoms of Life

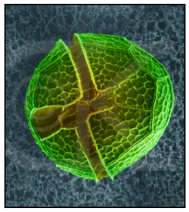


New Eukaryotic Phylogeny



Current Biology

Alveolates - diversity



Ciliates **Dinoflagellates** **Apicomplexans**

Phylum Apicomplexa (sporozoa)

- Large and diverse group (>5000 species)
- All members of this phylum are parasitic
- No cilia or flagella (except for some microgametes)
- Movement by gliding motility
- All members possess an apical complex
- Complex life cycles
  - Spore-like forms - cysts
  - Sexual and asexual stages
  - Intracellular stages
- Class Perkinsasidea
- Class Conoidasida - Coccidia
- Class Aconoidasida - Haemosporidia

Perkinsus marinus

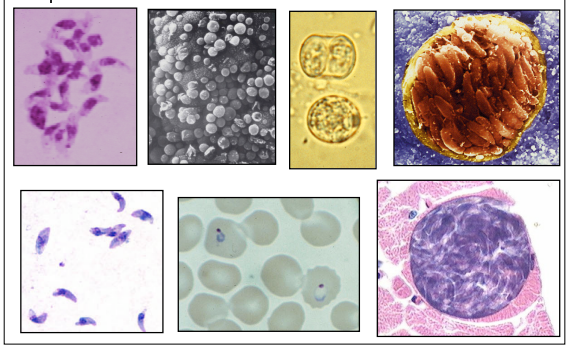
Apicomplexans

- Gregarines
  - parasites of invertebrates, some quite big (used as early research models)
- Coccidians
  - tissue parasites of vertebrates and invertebrates (can have single (e.g. *Eimeria*) or two host (e.g. *Toxoplasma*). Many parasites of medical and veterinary importance. Sex produces a sporelike oocyst
- Haemosporidians
  - (*Plasmodium*) and Piroplasms (*Babesia* & *Theileria*): small parasites of blood cells which are transmitted by arthropods

Important human and animal parasites

- *Plasmodium* - Malaria
- *Toxoplasma* - Toxoplamosis
- *Cryptosporidium* - Cryptosporidiosis
- *Eimeria* - Coccidiosis
- *Sarcocystis* - Sarcocystosis
- *Cyclospora* - Cyclosporosis
- *Isospora* – Isosporiasis - rare
- *Babesia* – Babesiosis - rare

Morphological diversity

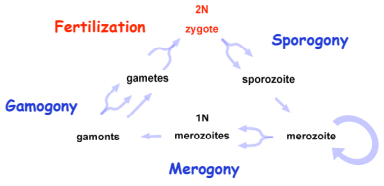


Apicomplexans

- Basic biology and life cycle
- Host cell invasion
- Apicomplexan cell division
- Newly discovered organelles
- Modification of the host cell
- Pathogenesis of disease
- Mechanisms of drug action and resistance
- Why don't we have a malaria vaccine?

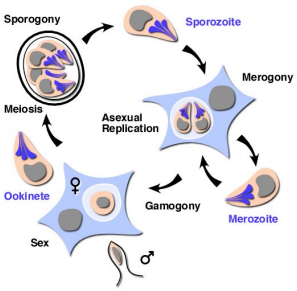
- Will use mainly *Plasmodium*, *Toxoplasma* and *Eimeria* as examples

General Apicomplexan Life Cycle



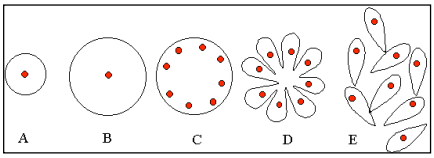
- Sporogony - 1 zygote gives rise to many sporozoites
- Gamogony - gamont gives rise to many gametes
- Merogony - process that increase the number of infective cells

Apicomplexan General Features



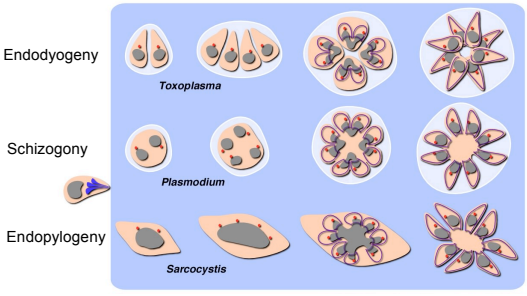
- Apicomplexans are haplonts and meiosis directly follows fertilization
- All replication occurs inside of host cells (with the exception of the conclusion of meiosis in certain species)
- There are several invasive zoite stages

Apicomplexa can separate nuclear division from cytokinesis

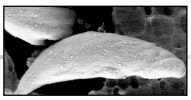


- Growth
- Multiple rounds of nuclear division (B)
- Segregation (C)
- Cytokinesis (D)
- Many progeny from 1 cell (E)

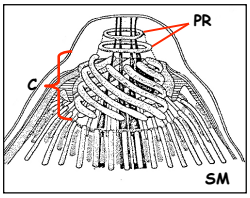
3 modes of intracellular replication

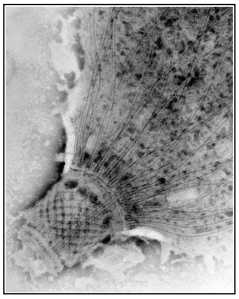


Apical complex



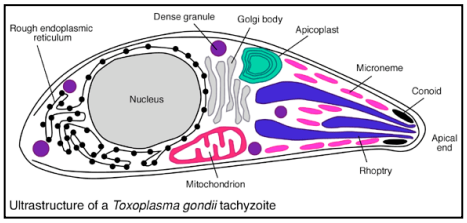
- Ultrastructural complexity at the anterior end
- Electron dense structures
- Concentration of organelles





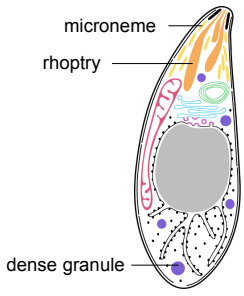
Apicomplexan Ultrastructure

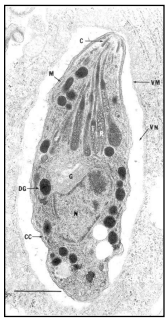
- Apical complex plays a role in invasion
  - Rhoptries and Micronemes - modified secretory organelles
- Apicoplast



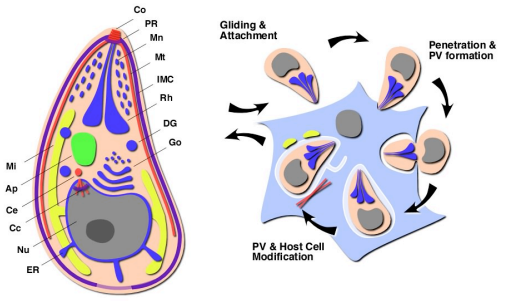
Ultrastructure of a *Toxoplasma gondii* tachyzoite

Specialized Secretory Organelles

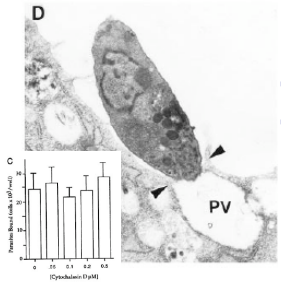




Apicomplexan host cell invasion



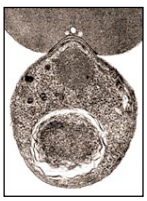
Invasion depends on parasite not host cell actin

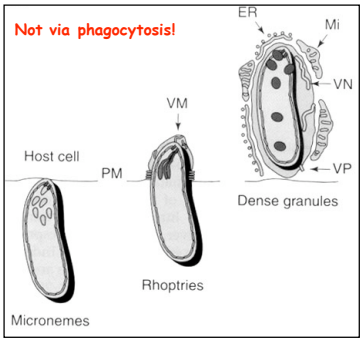


- Cytochalasin treatment does not to appear to inhibit attachment (bar graph in c shows number of parasites bound to cells at different drug doses)
- CytD inhibits the movement of the parasite into the host cell.
- A parasitophorous vacuole (PV) is still set up, however the parasite can not move in, and the moving junction remains at the apical tip of the parasite

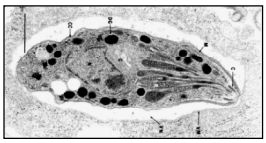
Dobrowolski JM, Sibley LD. Cell. 1996 84(6):933-9.

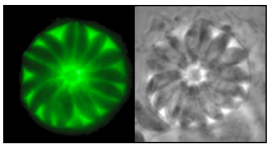
Secretion during invasion is ordered





The Parasitophorous Vacuole



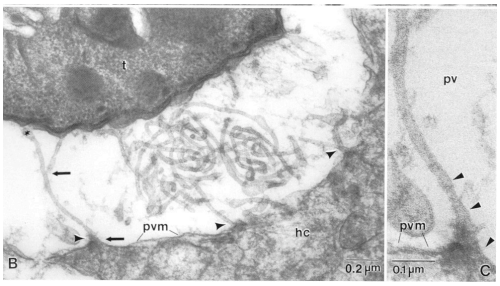


- After invasion parasites reside within a new compartment the PV
- The PV is derived from host cell membrane but behaves different from a phagosome
- The PV membrane is derived from the host cell plasma membrane
- The PV is provided by the parasite (e.g. by secretion)
- Both contribute to the PV

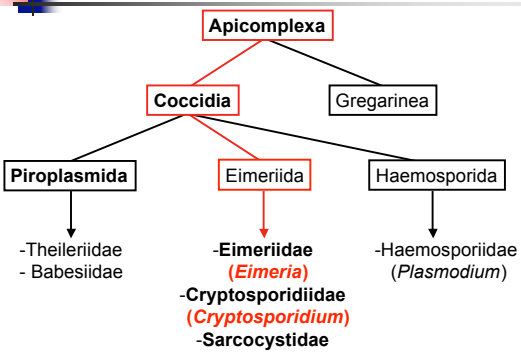
The PV is highly modified to suite the parasite's needs

- Tubular network increases surface (dense granule)
- Sieving pores give access to small nutrient molecules in the host cell cytoplasm (probably dense granule)
- Specific host cell organelles are recruited close to the PV membrane (rhoptry)

Dense granules are involved in establishing the intravacuolar network



Intestinal Coccidian Species



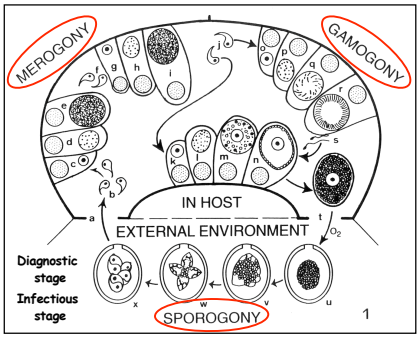
Similarities

- Direct life cycles - no intermediate hosts
  - homoxenous
- Oral-fecal transmission
- Infective stage - oocyst
- Oocysts in contaminated feces are not immediately infective
- Usually contaminated food or water
- Human infections
- Direct Human-Human infection is unlikely
- Oocysts must "mature"
- Of significance as opportunistic infections in immunocompromised people

Eimeria coccidiosis

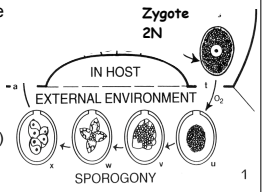
- Disease of chickens
  - other animals as well! (2500 species)
- Can cause high mortality
  - Young birds
- Serious disease causing bloody diarrhea, death
- Parasite replication causes bleeding, and massive swelling in gut
- Once infection is established there is no effective chemotherapy
- In US alone, cost of disease is about $80 million/year including coccidiostats (in the feed).

Eimeria Life Cycle



Sporogony

- Unsporulated cysts are non-infective
  - UV (sunlight) and dessication
  - sensitive
- Sporulation requirements
  - Oxygen
  - Moisture
  - Lowered temperature (exp: 20-23˚C)
  - Sporulation can be fast - 24 hr
- 1st nuclear division is meiosis
- Subsequent divisions are mitotic
- Sporulated cyst
  - 4 sporocysts - each with 2 sporozoites
  - Resistant form - environmental factors
  - Storage in 2% potassium dichromate

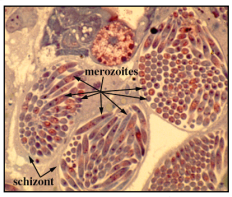


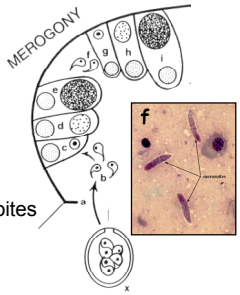
Sporulated

Unsporulated

Merogony - Programmed Amplification

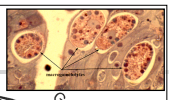
e or i

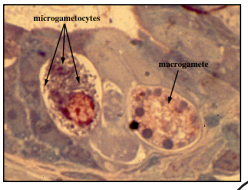


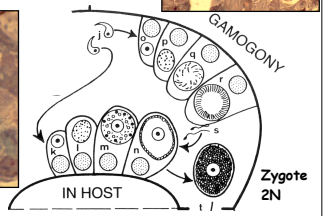


- Excystation - release of sporozoites
  - Mechanical, enzymatic, acid, bile
- Asexual reproduction
  - 1 merozoite produces 2-10,000
- Each *Eimeria* species has a set number of merogonous generations

Gamogony



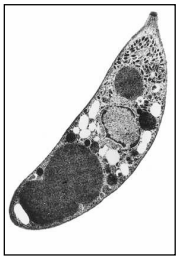




- Sexual reproduction
- Majority of gamonts produced are macrogamonts
  - Macrogamont (F) - unicellular
    - Large number of granules - destined to be oocyst cell wall
  - Microgamont (M)- multiplication, release biflagellated mirogametes

Interesting factoids

- A single oocyst of *Eimeria tenella* will produce 1 million more
- 1 gram of chicken litter (waste) can contain between 100,000 and 200,000 oocysts
- Birds (animals) that are in constant contact with small numbers of oocysts develop immunity to that specific oocyst species
- An ounce of prevention….



Coccidiosis in Chickens