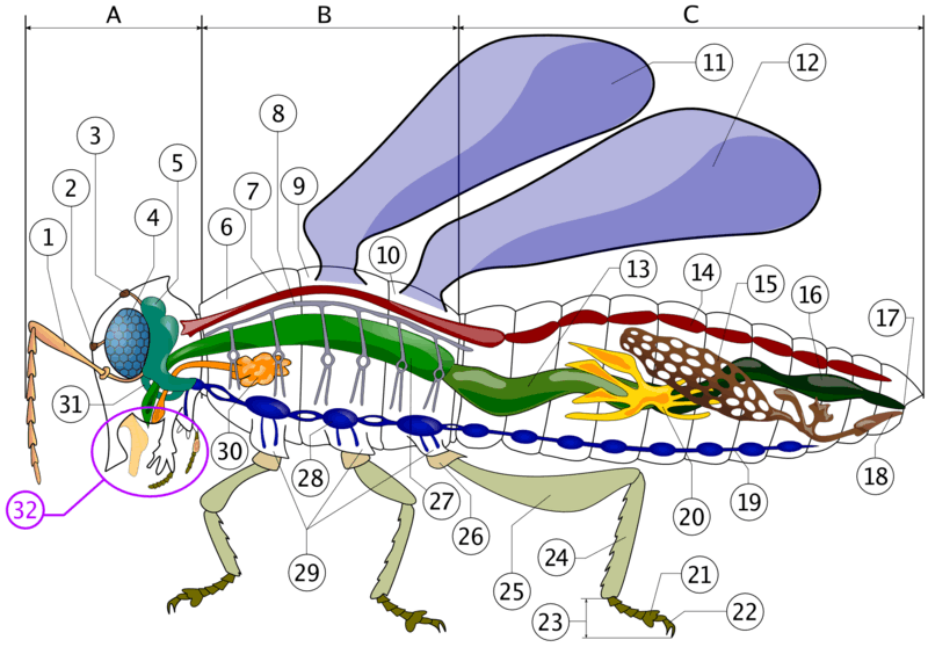
# Chapter 2. Associations between insects and nonpathogenic microorganisms (II)

▲ **The location of mutualistic symbionts associated with insects**

- external
- internal in the digestive tracts and other specialized chambers
- within the hemocoel
- within the cells

☞ in a close association between the insect and microorganism, the anatomy of the insect is usually modified

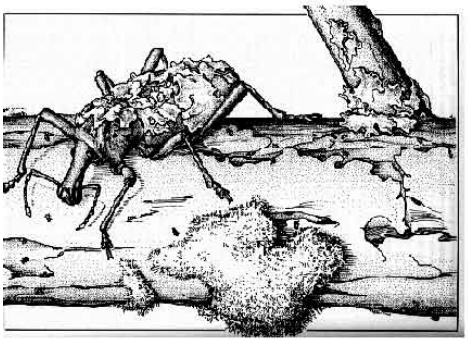



## 1. Extracellular microbiota

- aka **exosymbiont** or **ectosymbiont**
- depend mainly on the habitat of the insect
  ; soil insects will have a large lumber of soil-inhabiting microorganisms
  - external microbiota found on the exterior of the insect's body
  - internal microbiota present in the interior of the insect's body,
    - lumen of the digestive tract
    - lumen of the trachea
    - special cavities and ducts

## 1) External microbiota

- *Gymnopholus lichenifer* (Coleoptera: Curculionidae - weevil) in Papua New Guinea

; carries a garden of lichens on its back

; mites, springtails, psocids (barklice) Xing sloth

- *Hylobius pales* (Coleoptera: Curculionidae)

; fungi, bacteria on surfaces

; inhibits the conidia (=spore) germination of the entomopathogenic fungus, *Metarhizium anisopliae*

⇨ ANTIBIOSIS

- In mosquitoes

; bacteria serve as food or as a stimulant to egg hatching by providing an environment low in DO around the egg


< *Culex* spp. oviposition and egg rafts >

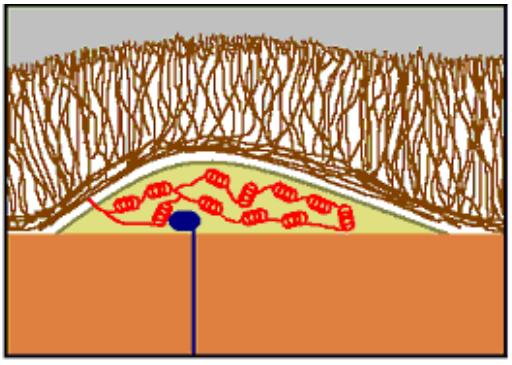
- In onion maggot (Diptera: Anthomyiidae)


; oviposition of the adults is enhanced by microorganism which converting chemical precursors to volatile stimulatory compounds

; fly may help in the dispersal of the bacteria

## 2) Insect-associated fungi

- all major classes of fungi have been reported from the insect's external surface
- *Septobasidium* spp. (Basidiomycota; mushroom)
  - ; tightly associate with scale insects (Hemiptera)
  - ; fungus provides protective home for the scales against predators
  - ; fungus exists superficially and does not penetrate into plant tissues
  - ; parasitizing only a small percentage of individuals
  - ; draw nutrition from the scales
  - ; fungus cannot survive in nature without scales
  - ; scales distribute the fungus
  - ; mutualistic at the population level

< *Septobasidium* sp. on Acacia >

< Brown soft scale >

< Cochineal scale insect >

< *Icerya purchasi* (cottony cushion scale) and *Rodolia cardinalis* >

● **Cottony cushion scale**

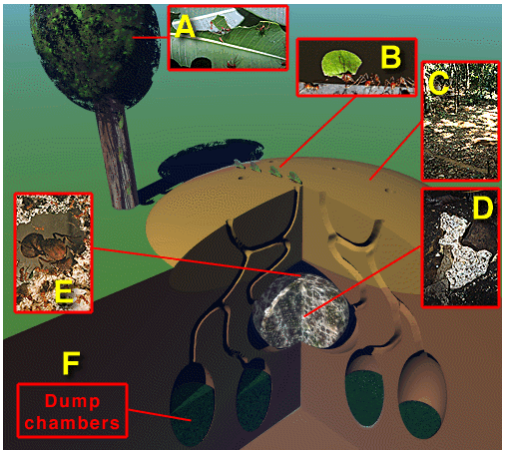
- Introduced to southern California where it caused enough damage in the mid-late 1800s
- In California in 1868, the cottony cushion scale was a new pest attacking citrus, pear, and acacia in southern California threaten the existence of the California citrus industry
- Entomologists guessed that the scale was from Australia, the country from which much of the citrus had been imported
- A **vedalia lady beetle** (*Rodolia cardinalis,* previously known as *Vedalia cardinalis*) was collected and introduced from Australia, the original home of the scale insect
- Vedalia lady beetle is very specific, feeding only on scale insects, and even then, its host range is restricted
- By 1890, all infestations of the cottony cushion scale were completely controlled and citrus industry has yield benefits of millions of dollars annually

### 3) Insect-cultivated fungi

- members of nearly all insect orders are associated with fungi
- some insects, such as beetles, ants, and termites cultivate fungi for food and other purposes, such as maintaining a moist, suitable environment

(1) Fungus-growing ant (Formicidae: Attini: *Atta* spp.)

- aka leaf-cutting ants




< Ant nest and leaf-cutting ant worker >

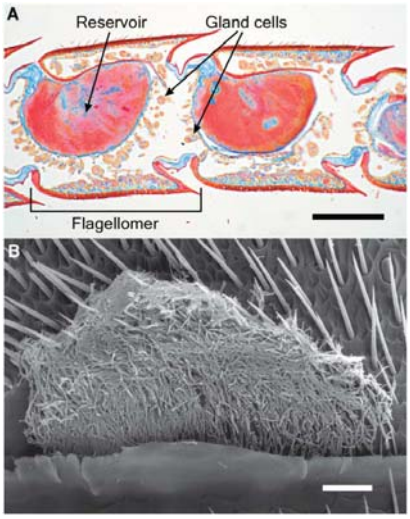
< Leaf-cutting ants tending fungus >

< *Streptomyces* on the cuticle of *Acromyrmex octospinosus* >

- ants cultivate the fungus for food
- fungus can be propagated and protected against other microbial pathogens
- *Streptomyces* (actinomycetes) bacterium produce secondary metabolites which have antibacterial or antifungal properties (=antibiotics) [many are used as **antibiotics for human**]
- bacterium prevents the fungal garden from contaminations

※ Digger wasp females

- European beewolf, *Philanthus triangulum*

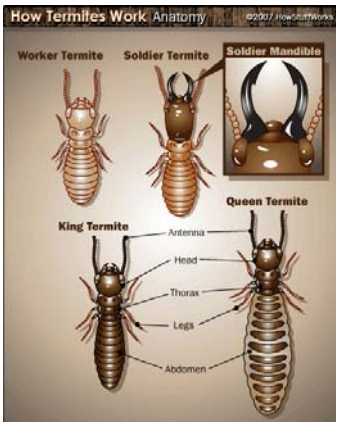

- domesticated a *Streptomyces* bacterium at the specialized antennal "gland" that apparently produces antibiotics to provide long-term antiseptic brood cells for the developing wasp brood (larvae mostly overwinter and emerge next summer, ca. 9mon)

- after having provisioned a nest cell with one to five paralyzed honey bees, a female beewolf pressing a paste of *Streptomyces* out of her antennal cavities just before applying it to the ceiling of a nest cell

- the developing larva stays in close contact with this *Streptomyces* and somehow manages to get the bacteria incorporated on the wall of a silk cocoon that it produces after it has finished feeding

(2) Termites (Isoptera)

- subfamily Macrotermitinae
- cultivate fungi (*Termitomyces*) for food
- construct nest called **"termitaria"**
- termitarium contains special chambers, **"fungus comb"** in which the symbiotic fungus is cultivated on that are derived from termite fecal material with undigested food materials
- The fungus converts undigested woody material in plants into higher quality oligosaccharides and more easily digestible complex sugars for termites
- the combs are constantly replenished and older parts eaten
- termite maintains the fungal garden free of contaminations




< Subterranean termites >

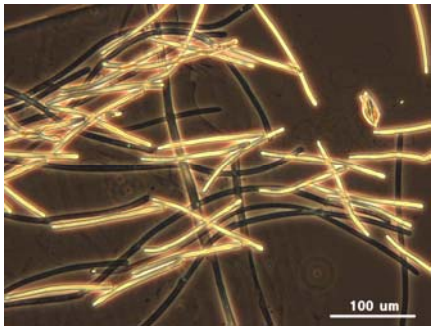
< Termite mound (termitarium) and fungus combs >

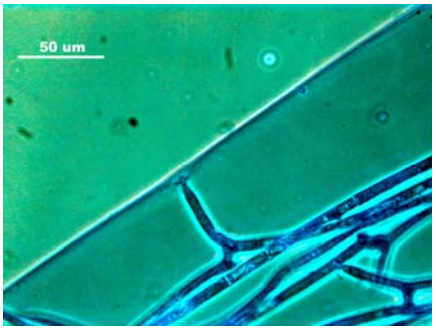
## 4) Internal microbiota

- found in the lumens of the trachea and the digestive tracts
- internal symbionts are basically non-pathogenic but sometimes (such as starvation, high humidity and temperature) they may become pathogenic and cause infections within the digestive tract

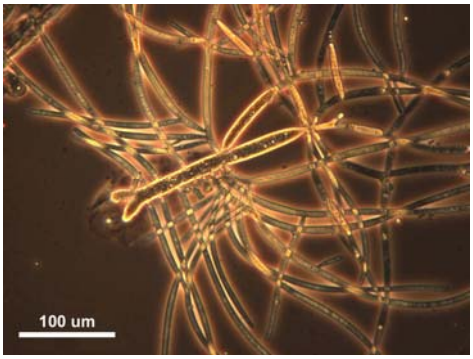
### (1) Trichomycete fungi (Zygomycota: Trichomycetes)

- generally commensalistic to aquatic insect (e.g, mosquitoes, black fly larvae, midge larvae, mayfly nymphs)
- midgut vs. hindgut inhabiting fungi
- some are pathogenic

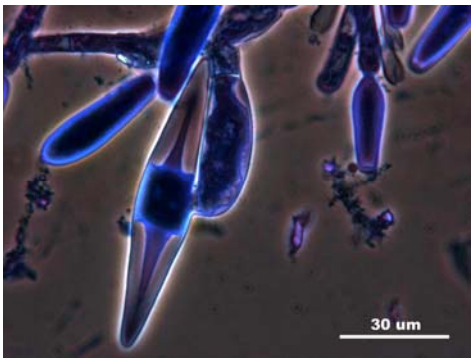


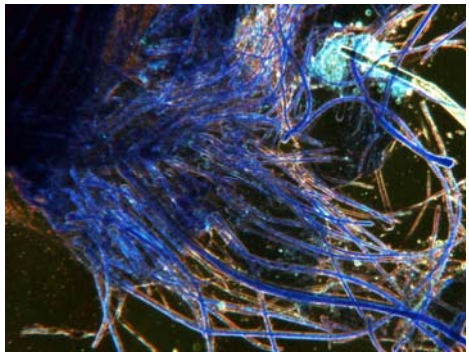

< *Harpella melusinae* >




< *Pennella simulii* >



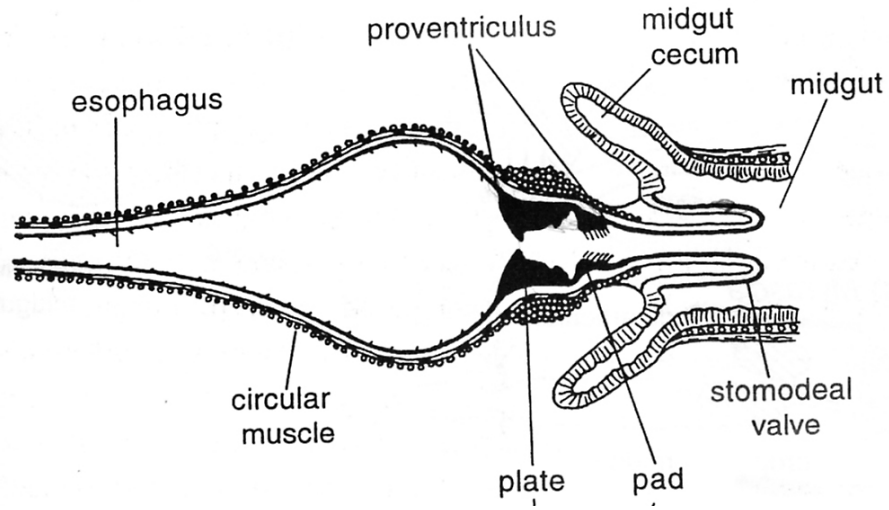

< *Genistellospora homothallica* >



< *Paramoebidium chattoni*, Ichthyosporean protist >

(2) Bacteria

- the most common bacteria found in digestive tract are gram-negative (many are pathogenic)
- specialized appendages, **gastric caeca**, attached to the gut may be filled with a large number of bacteria that produce enzymes and vitamines in some insects




- many species of plant sap feeders (i.e, Hemiptera - plant bugs) tend to have highly modified gastric cacae harboring endosymbionts

- the bacteria appear to play a **mutualistic role** with the insect host by providing the host with nutrients and other substances and obtaining in return a home and means of transmission

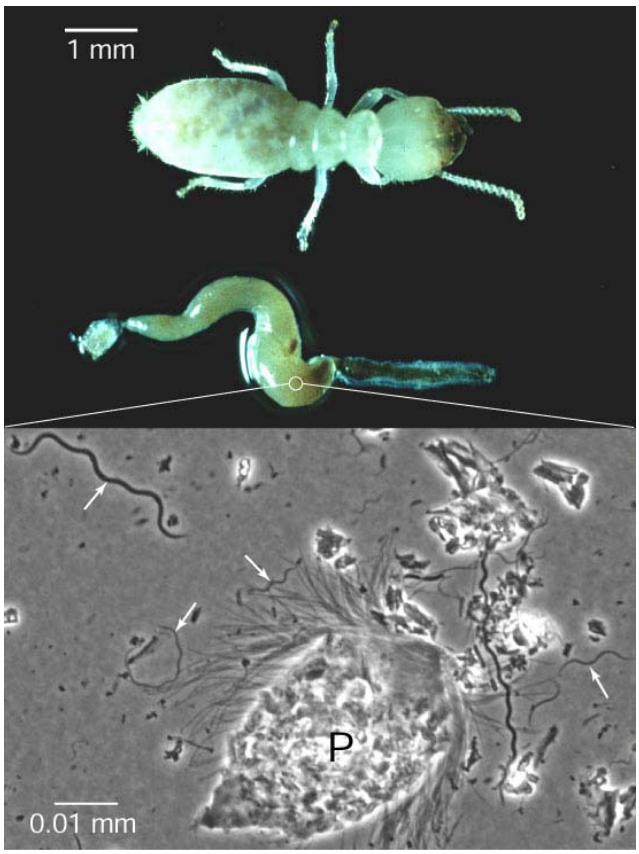
- the internal microbiota that are acquired during the immature stages are not usually retained by the adult insect
  ; in the house fly, *Musca domestica*, some of the newly emerged adults have sterile digestive tracts

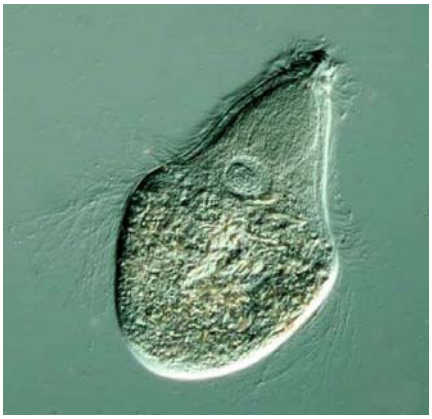

- association between olive fruit fly and *Pseudomonas* spp.
  ; *Pseudomonas* spp. hydrolyzes the protein in the olive and synthesizes methionine and threonine (essential amino acids)


< Olive fruit fly, *Bactrocera oleae* >

< Damaged by *Pseudomonas savastanoi* >

(3) Protozoa
- all families of termites (Isoptera) have members containing protozoa
- since termites cannot digest their own food, they should have colonies of microscopic **bacteria** and **protozoa** in the hindgut
- these **mutualistic symbionts** are able to digest the cellulose of the wood and then excrete carbohydrate, which the termite is able to convert to its own energy
- the protozoa are absent in newly hatched termite nymphs (they are lost at times of each molting, except in the last molt to adults)
  ⇨ **must be re-colonized**
  ; proctodeal feeding (feeding on the exudates from the colonized individual)
  ⇨ **lost in higher termites**
  ; stomodeal feeding (accepting food from nursing workers)



< Subterranean termites and symbiotic protozoa >

- the protozoa cannot survive for long periods in the absence of bacteria that help to maintain conditions favorable for them

## 2. Intrahemocoelic and intracellular microbiota

- endosymbiont ⇨ endosymbiosis or endocytobiosis
- symbionts occur free in the **hemolymph** or attached to structures in the hemocoel or in the **fat body** (in cockroach)
- intracellular mutualism is common in blood-sucking and plant sap feeding insects
- the endosymbionts and host are usually intimately associated so that it is extremely hard to isolate endosymbionts and culture them independently
- absence of the symbionts results in short survival rate, modified behavior, poorly-developed offspring in the cockroaches

▷ **Mycetomes**

; term proposed by Sulc (1910) for the specialized structures containing the microorganism which he believed that the microorganisms in the cells were FUNGI

; individual cells that contain the symbionts are called **"mycetocyete"**

; some scientists differentiate the cells with bacterial symbionts as **"bacteriocytes"** → bacteriomes

⇨ hereditary structure (must be inherited, generally thru maternal line, like mitochondria)

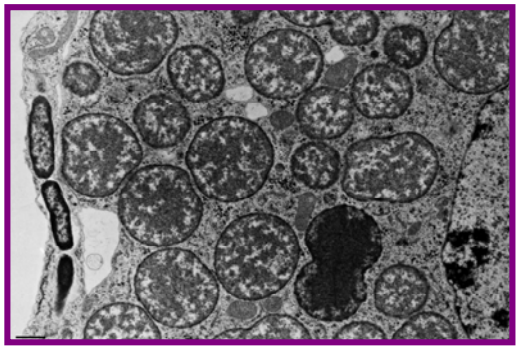
⇨ develop even in the absence of symbionts

### 1) Aphids and their symbionts

- almost all aphids contain the obligate symbiont, *Buchnera aphidicola* **(primary symbiont)**
- many aphid also have facultative relationships with a variety of other symbionts, such as *Serratia symbiotica* and *Hamiltonella defensa* **(secondary symbionts)**

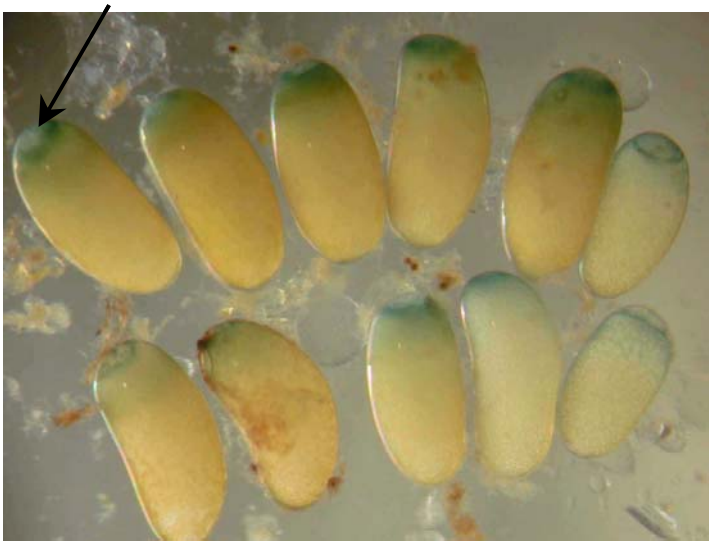
☞ these facultative symbionts have been shown to provide hosts with defense against parasitoids and pathogenic fungi as well as to increase tolerance to heat stress

; *Hamiltonella defensa* contains variable bacteriophages that carry genes encoding eukaryote-targeted toxins which make it possible to subdue parasitic wasp larvae



< Aphids and *Buchnera aphidicola* within bacteriocyte >

< Aphid with maternal bacteriocyte containing *Buchnera* >

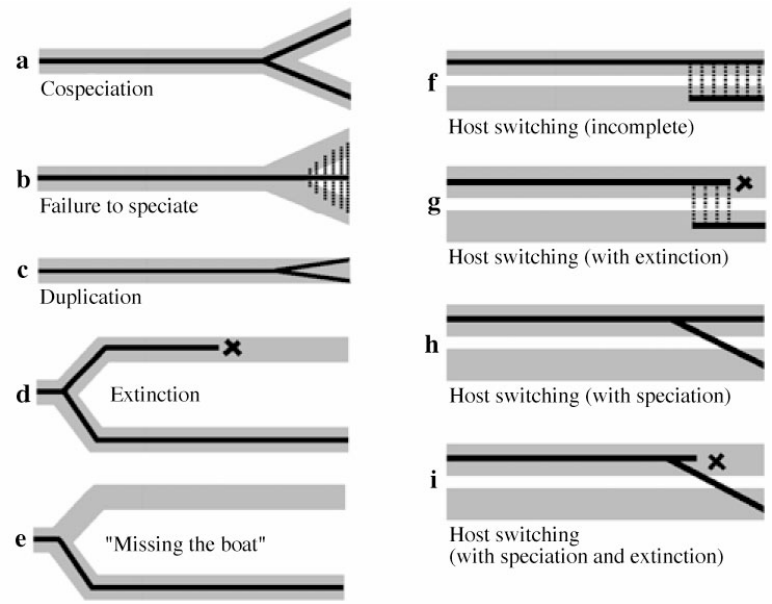


< Aphid eggs with maternally derived *Buchnera* >

- the symbiont has cospeciated with aphids throughout their evolutionary history, through strict maternal transmission (transovum)

- the principal function of *Buchnera* is to provide the insect with 9 essential

amino acids, nutrients in short supply in the aphid diet of plant phloem sap




< Host and symbiont evolutionary relationships >