associations between insects and microbes (microbe/host)

- Commensalism (+/0)
- Parasitism (+/-)
- Mutualism (+/+)

all are forms of symbiosis

Microbial Associates of Insects

- Wolbachia – a diversity of effects on hosts
- Bacteria produce an antibiotic for bee wolves (example of providing a specific product)
- Nutritional assistance: obligate mutualists
- Facultative guests
- A parade of partners
- External mutualists: ants and fungus

Discovery of Wolbachia

- In mosquito breeding experiments in 1924, some crosses were completely sterile
- Due to presence of this bacteria
- Widely occurring in insects (20-75% infection rate by species)
- In most, no obvious effects documented
- Some insects have >1 Wolbachia species

Wolbachia has different types of effects on hosts

- Cause female-biased sex ratios of offspring
- Increase host fitness by sterilizing non-hosts
- Obligate mutualist
- Benign reproductive parasite

Female biased sex-ratios

- Parthenogenesis
- Feminization

Infected hosts more successful

- Cytoplasmic incompatibility

Parthenogenesis-inducing Wolbachia

Encarsia wasp
sex determination in Hymenoptera

- haplodiploid system
- unfertilized eggs (haploid) become males
- fertilized eggs (diploid) become females

Uninfected females produce both males and females
Infected females produce only females

Parthenogenesis-inducing (PI) bacteria increase in frequency in a population by converting males to females

How do they do that? Found only in haplodiploid sex determining systems - Hymenoptera, Thysanoptera, some mites

Parthenogenesis-inducing (PI) bacteria converting males to females

Feminization

- Ostrinia moth
- Males are homogametic sex (ZZ)

feminization

- Wolbachia infected males (ZZ) develop as females
- When these females are treated with antibiotics, and mate with normal males (ZZ) all offspring are male
- maintained at low level in population
Female biased sex-ratios

Infected hosts more successful

- Parthenogenesis
- Feminization
- Cytoplasmic incompatibility

What causes the incompatibility and allows rescue?
- Best current model is “modification-rescue”
- Sperm nucleus modified by Wolbachia so that it cannot successfully fuse with an egg nucleus unless some product in cytoplasm of infected egg “rescues” it

Wolbachia as a mutualist

Wolbachia and Onchocerca volvulus
- black flies transmit Onchocerciasis = river blindness
- nematodes that form nodules, eventually end up in eyes

Wolbachia and Onchocerca volvulus
- treatment first with anti-nematode chemical (ivermectin)
- recently awareness of symbiont led to treatment with antibiotics
- antibiotics kills the worms!
Beewolves - Philanthus
• prey on bees
• nest in sandy soil
• larvae feed on bees
• spin cocoon, overwinter
• moist nest – risk of infection

Beewolves - Philanthus
• bacterial symbionts on antennae
• Streptomyces
• vertically transmitted
• secreted into brood cell and taken up by larvae and incorporated into cocoon*
• each species has its own species of Streptomyces
• abt 26 myo

Many insects have extreme diets - they are very unbalanced in nutrients – lacking proteins, vitamins, etc sap, wood, wool, blood...

They can get away with this because they have microbe mutualists

The Aphid and Buchnera
bacteriome in aphid

- Special host cells in body cavity
- Containing 60-90 cells
- Bacteria are inside these cells in vesicles
- Each aphid has about 5 million Buchnera

Obligate mutualism

- Aphids die without Buchnera
- Show this with antibiotics
- Buchnera can't be cultured outside aphids – very specific needs

Transmission: vertical and horizontal

- Vertical: from one generation to the next
- Horizontal: between individuals that are not parent and offspring and often not even of the same species

Transmission: vertical and horizontal

- Vertical: symbiont expect to be completely faithful to the lineage and co-evolve with host taxon over time
- Horizontal: symbiont 'species' passed easily among individuals and species over time-widely distributed

Vertical

- From one generation to the next
- From mother to offspring

Aphids

- Aphids first appeared about 150-200 million years ago
- This is a partial phylogeny
Aphids and Buchnera

- PERFECT agreement between the trees
- rigorous vertical transmission

Go back to Wolbachia

- What kind of transmission (horizontal or vertical)?

Horizontal or Vertical?

- Horizontal transmission required to explain the distribution of *Wolbachia*
- Even though there is vertical transmission, horizontal transmission must be common over evolutionary time

Typical of ancient, obligate lineages

Compare host and symbiont phylogenies

- Hymenoptera: Torymidae: PI
- Hemiptera: Delphacidae: CI
- Coleoptera: Tenebrionidae: CI
- Hymenoptera: Trichogrammatidae: PI
- Hymenoptera: Encyrtidae: PI
- Hymenoptera: Aphelinidae: PI
- Hemiptera: Delphacidae: CI
- Hymenoptera: Cynipidae: PI
- Hymenoptera: Pteromalidae: PI
- Hymenoptera: Aphelinidae: PI
- Hymenoptera: Eucoilidae: PI
- Diptera: Culicidae: CI
- Diptera: Drosophilidae: CI
- Crustacea: sowbugs: Fem

Horizontal

- Occasional transfer between insects
Implications of isolation

Mutations will accumulate
= Functions of some genes will be lost
Selection FOR maintaining genes that will compensate for mutations
Tend to lose genes for which host can take over function
Selection FOR keeping genes that help host fitness (making essential amino acids)
Genomes (# of functioning genes) will tend to decrease in size

Buchnera’s genome

• About 640,000 bp
• Compared to 4.6 million bp in E. coli

A major *Buchnera* contribution

• essential amino acids
• especially heavy synthesis of tryptophan
• has 16 copies of tryptophan gene

Some aphids have other symbionts

The Cast

The Pea Aphid

• parthenogenic during the summer (females produce females = clonal)
The secondary symbiont -

- Secondary symbiont is a younger associate
- Facultative – not found in all populations
- 3 types identified so far
- What are their functions?

Secondary Symbionts

![Secondary Symbionts](image)

The Parasitoid Wasp

- wasps lay eggs in 2nd instar aphids

The experiment

- infect SecondarySymbiont-free aphids with the SS* by injection
- allow wasps to parasitize aphids uninfected and infected lines
- Are there differences?
  - Hamiltonella

What happened to the wasp eggs/larvae?

- parasitism rates reduced significantly by 2 of the 3 SS strains
- (eggs laid in equal numbers on all types)

![Graph](image)

- young aphids had healthy wasp larvae
- older larvae contained many dead wasp larvae
Noticed variation in resistance

• There are different strains (genetic differences) of both aphids and Hamiltonella.
• How do aphid and SS strains interact with respect to anti-wasp effect?

Different SS strains in same aphid strain

• all 4 SS strains gave resistance to aphids BUT varies from 29-82%
• It is something characteristic of the SS strain that determines effect level

What does phage do for Hamiltonella?

• allow H. to establish a stable infection of host cells by distending cell membranes
• protect its aphid host and therefore its own environment

Does the same NY1 SS lineage give same resistance in DIFFERENT aphid lines?

• Resistance varies 9% in first experiment and 3% in second
• = same

WHAT causes resistance?

• amplification of genome of Hamiltonella showed at least two phages (viruses) associated with it
• the phages encode a gene for cdtB, cytolethal distending factor, a toxin
• Toxin is the likely agent of wasp death
Sap feeding insects

• “fairy land of symbiosis” (Paul Buchner)
• individuals can have up to 6 types of obligate symbionts, each transmitted in its own way
• symbionts evolve in parallel with their host
• pattern of symbiont associates over time

Genomes of obligate microbial mutualists decay

genes are damaged over time
numerous chaperonins to make damaged proteins work
smallest genome of a symbiont contains only 182 genes
some genes have been transferred to host genome
what happens when they can’t be repaired? Stop being useful?

Relationships between microbes and other organisms have been going on since the beginning

alliances between different forms of life

The mutualism: attine ants and their fungi

• worker ants harvest huge amounts of leaves and other plant material for their fungus gardens
• in tropical America, they are the single most important herbivore
• can be a major pest as they harvest the plants we nurture in our ‘gardens’
the assembly line

- leaves are cut into smaller and smaller pieces
- and eventually ‘fed’ to the fungus
- … and the ants eat the fungus

Cultivation

- cut leaves and render them into small pieces
- transplant tufts of older fungus to new gardens
- space it out so there is room for growth
- apply fecal manure

Cultivation

- groom the fungus - remove contaminating spores and parasites
- after about 5 weeks ready to harvest for several months - easily harvestable knobs
- Protein/fat/carbs are about 24%-2%-27% of dry weight
- YUM!

Ant-fungal phylogeny

- 50 mya origin of cultivation by ants
- Higher attines
- Lower attines

Higher Attines

- Phylogenies on the same track
Lower Attines

- Free living (black)
- Cultivars (red)
- 3 additional domestications
- Almost all ants cultivate more than one type
- New associations being added
- Similar species, different cultivars and vice versa

All is not so simple and peaceful as it seems

- A line of parasitic fungi was discovered that can quickly kill the true mutualist
- *(Escovopsis)* - detectable in most gardens

Garden 10 days after infected with *Escovopsis*

Some researchers noticed white crud on some of the workers in the fungus gardens

Actinomycete bacteria
found mainly on garden tending ants
produces antibiotic that controls *Escovopsis!*

Ants-fungi-parasitic fungi-bacteria