INTIMATE STRANGERS:
MICROBIAL PARTNERS IN THE NATURAL WORLD

OLLI

SPRING 2020

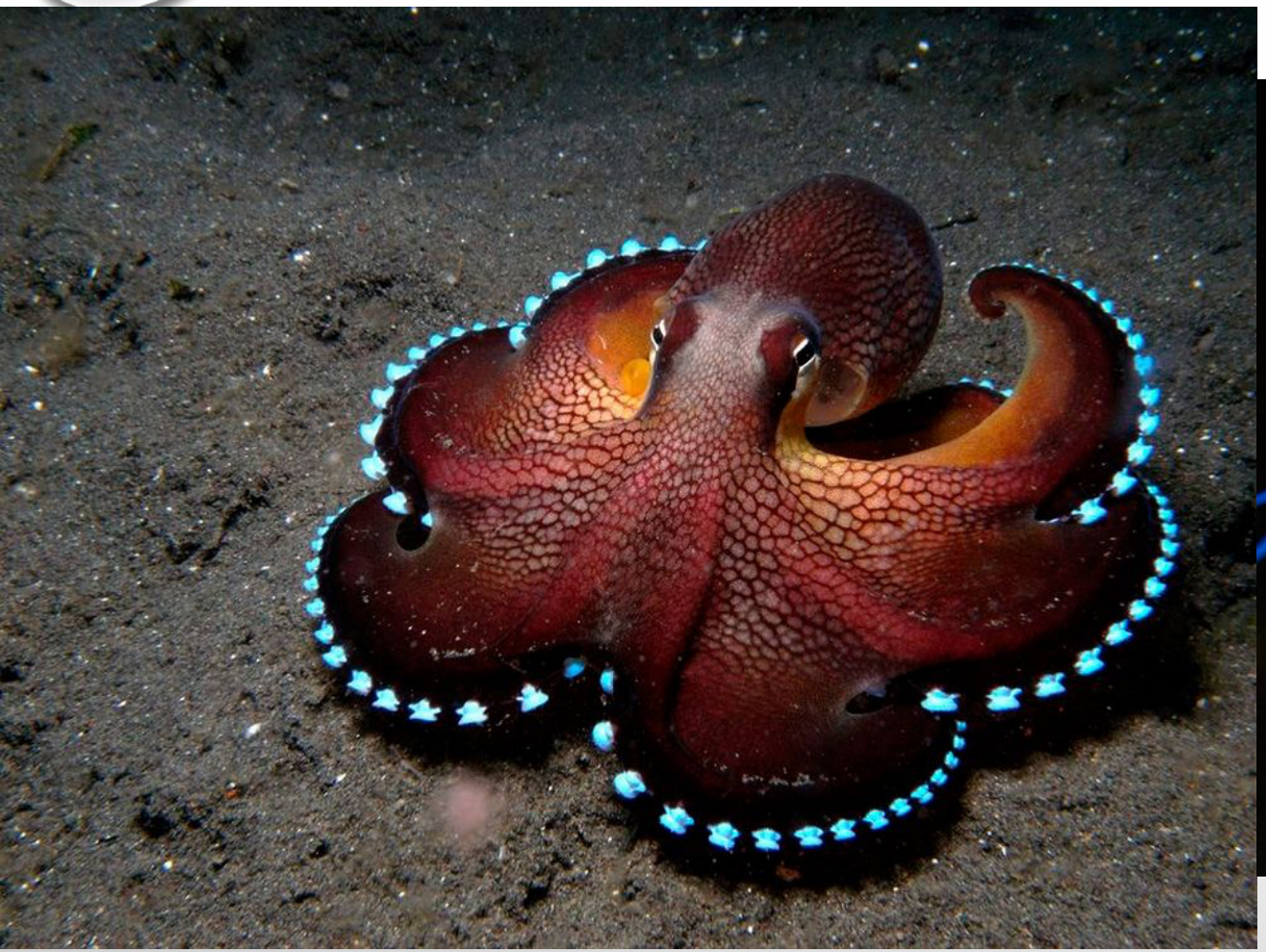
LECTURE 5:
EXPLORING MICROBIAL INTERACTIONS – II

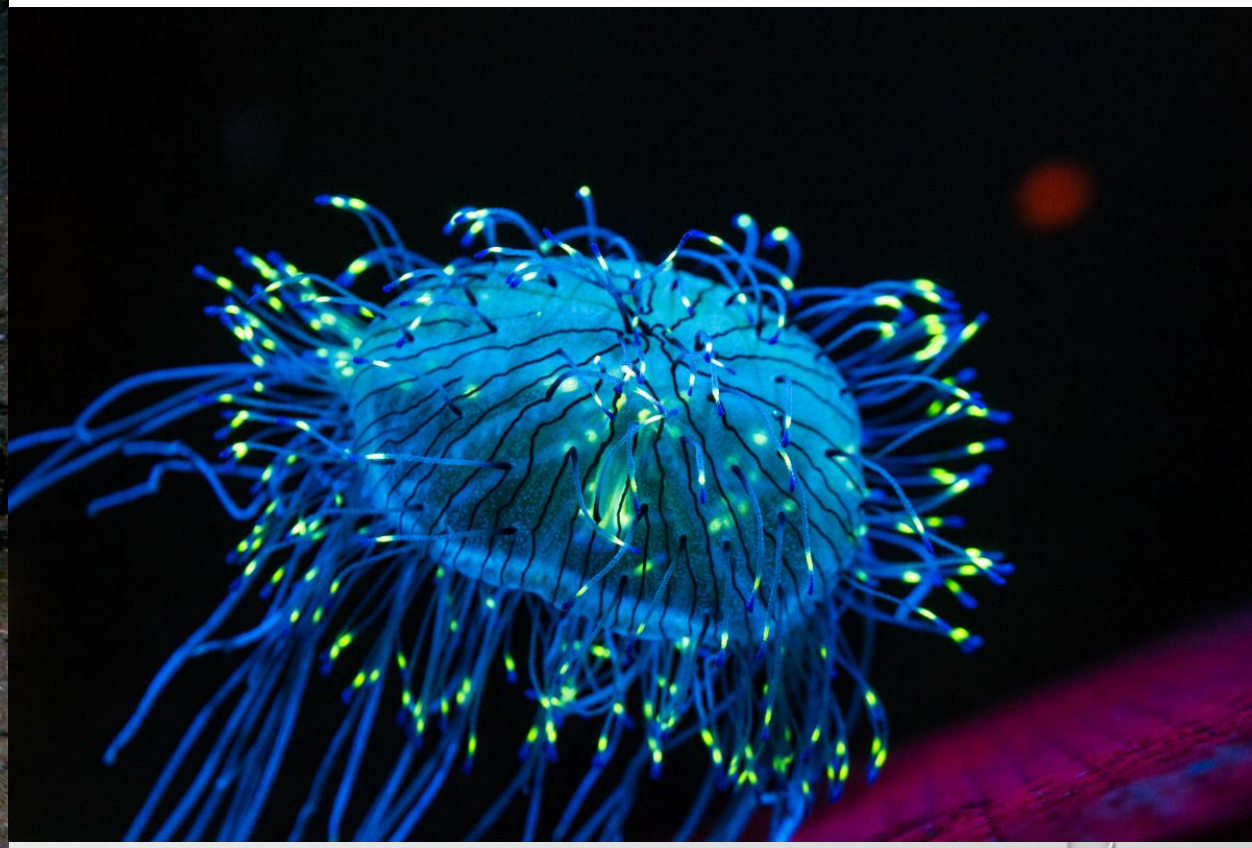
BIOLUMINESCENCE AND THE SQUID

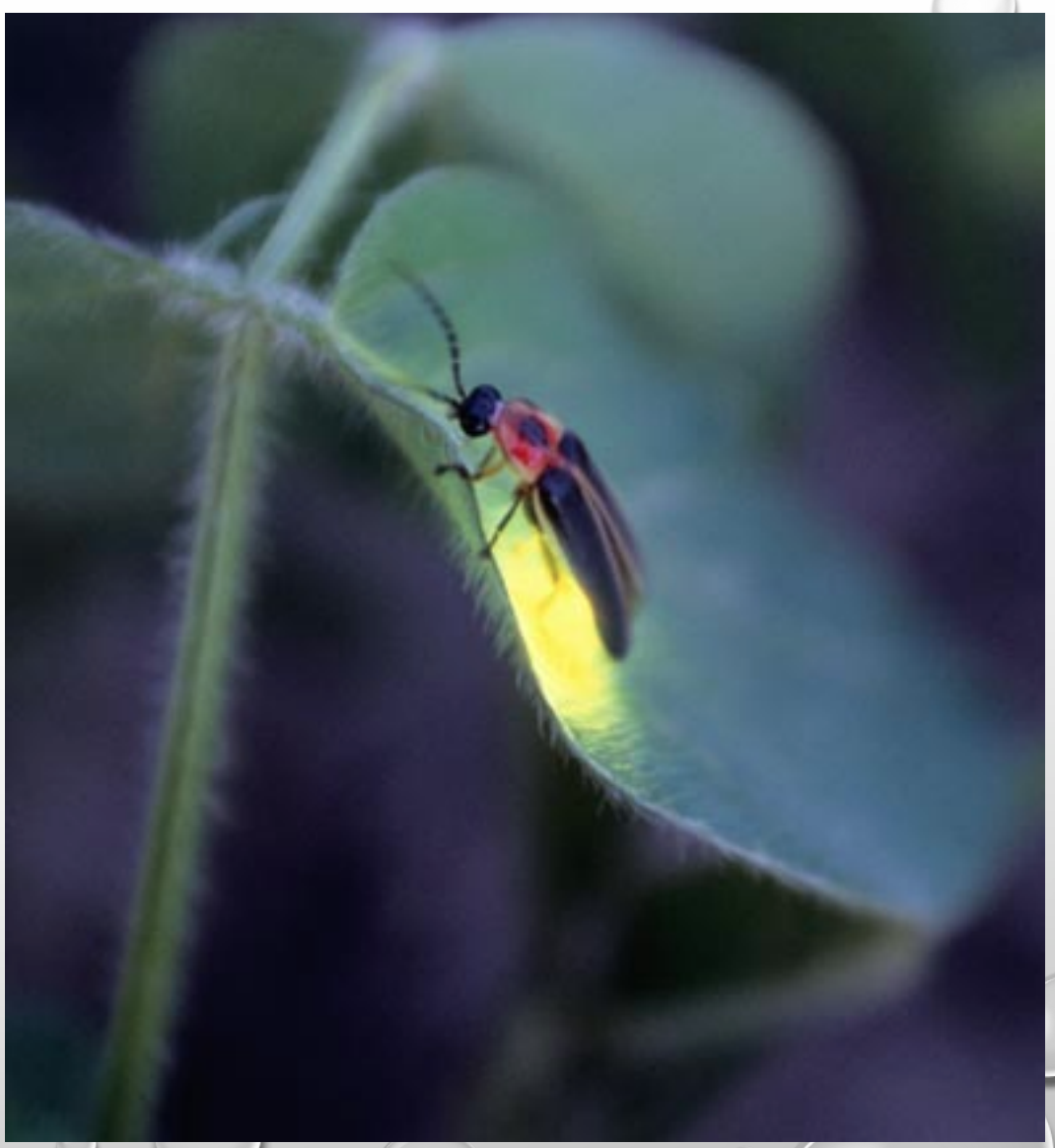
ENDOSYMBIONTS: LESSONS FROM INSECTS

BIOLUMINESCENCE: PROVIDING COVER AND MORE

- PRODUCTION AND EMISSION OF LIGHT BY A LIVING ORGANISM
- IN MARINE VERTEBRATES AND INVERTEBRATES, TERRESTRIAL ARTHROPOD SUCH AS FIREFLIES SOME FUNGI, AND MICROORGANISMS. IN SOME ANIMALS, THE LIGHT IS BACTERIOGENIC, PRODUCED BY SYMBIOTIC BACTERIA; IN OTHERS, IT IS AUTOGENIC, PRODUCED BY THE ANIMALS THEMSELVES
- GENERAL CHEMICAL REACTION:
  - LUCIFERIN (LIGHT-EMITTING PIGMENT) + O_2 → OXIDIZED LUCIFERIN + ENERGY IN THE FORM OF LIGHT
- USES: ILLUMINATION, CAMOUFLAGE, MIMICRY, SIGNALING TO POTENTIAL MATES







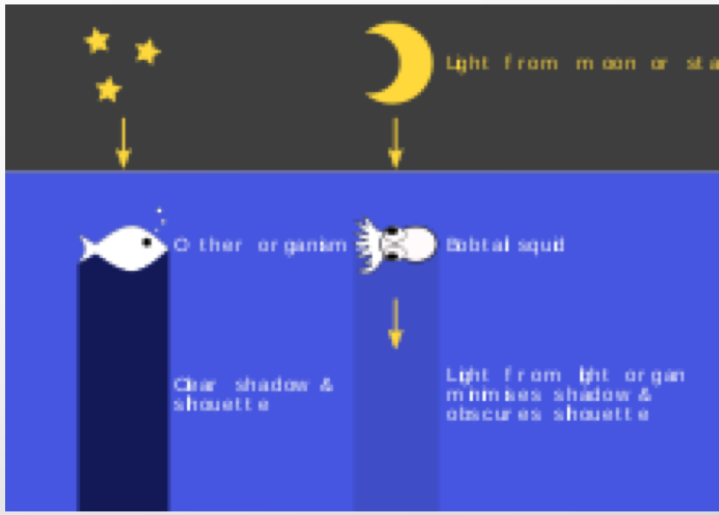
DINOFLAGELLATES

HAWAIIAN BOBTAIL SQUID

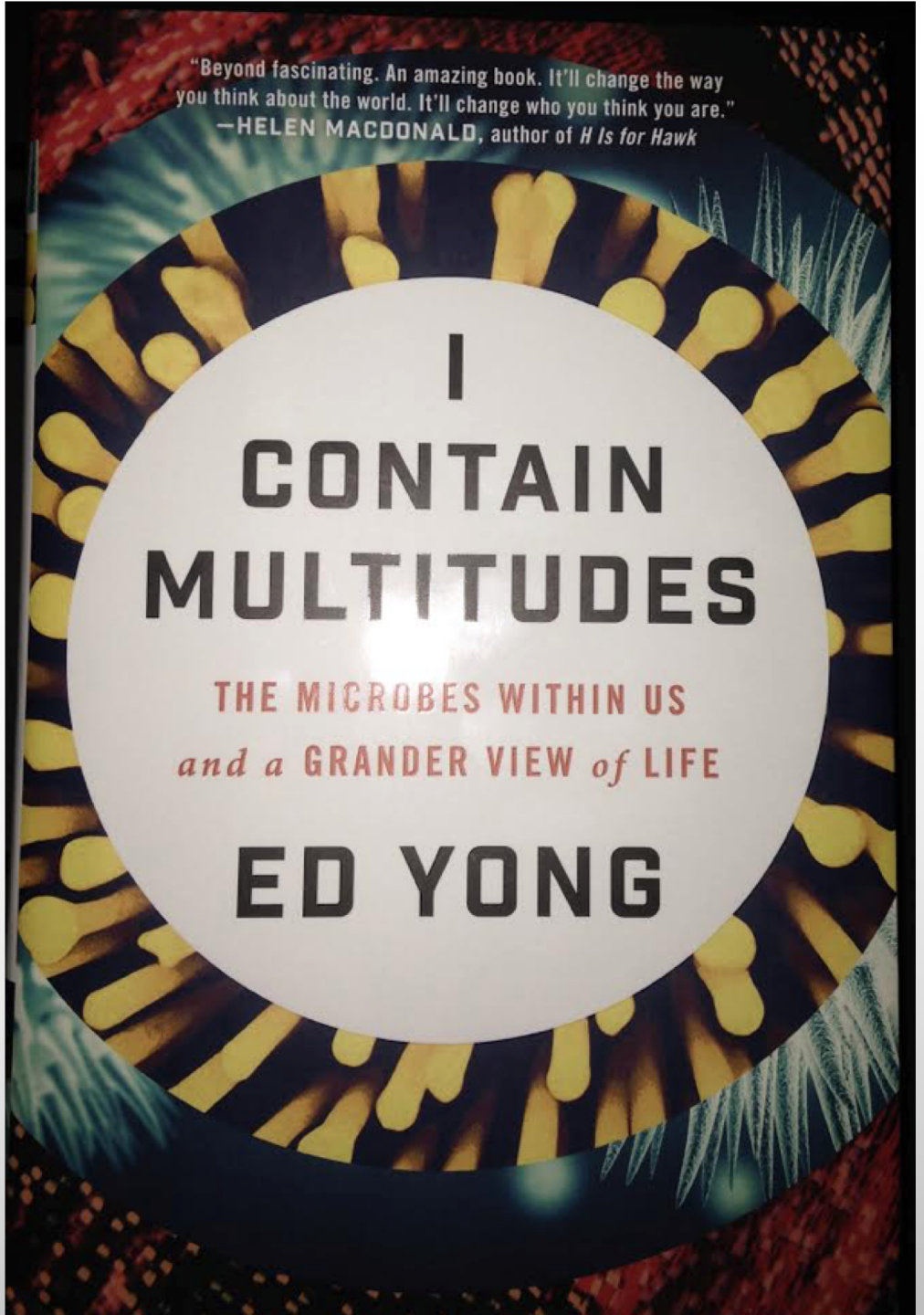
AND GLOWING

SOME FACTS ABOUT THE BOBTAIL SQUID

- RELATED TO CUTTLEFISH
- EIGHT SUCKERED ARMS AND TWO TENTACLES
- GENERALLY QUITE SMALL (TYPICAL MALE LENGTH BETWEEN 1 AND 8 CM)
- HABITAT: SHALLOW COASTAL WATERS
- IN A SYMBIOTIC RELATIONSHIP WITH BIOLUMINESCENT BACTERIA (*VIBRIO FISCHERI*) WHICH INHABIT A SPECIAL LIGHT ORGAN IN THE SQUID'S MANTLE
- THE BACTERIA ARE FED A SUGAR AND AMINO ACID SOLUTION BY THE SQUID
- IN RETURN THEY HIDE THE SQUID'S SHADOW WITHIN THE WATER COLUMN WHEN VIEWED FROM BELOW, PROVIDING CAMOUFLAGE AND PROTECTION FROM PREDATORS → **COUNTERILLUMINATION**

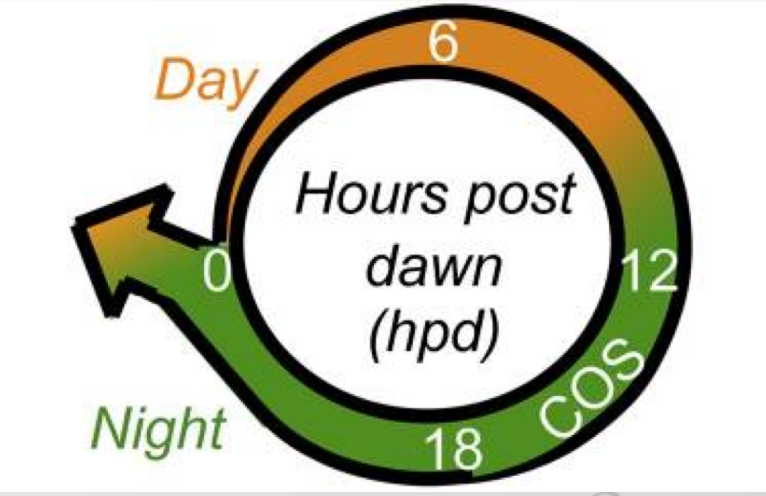


HTTPS://WWW.BIOINTERACTIVE.ORG/CLASSROOM-RESOURCES/NATURES-CUTEST-SYMBIOSIS-BOBTAIL-SQUID



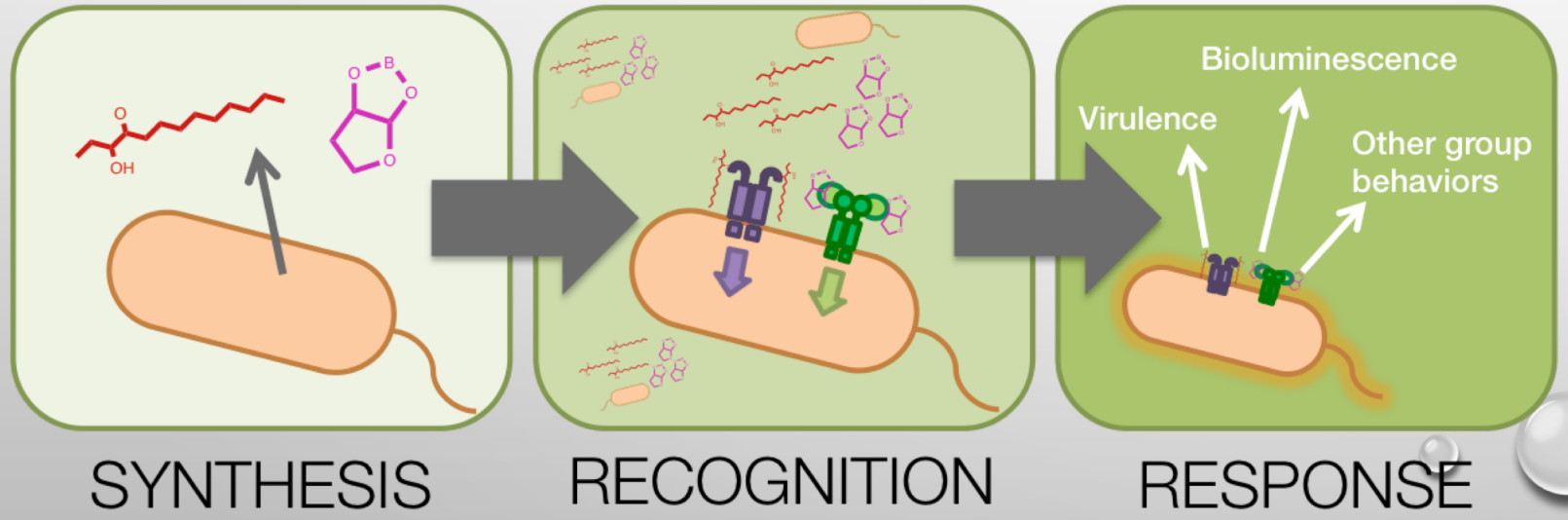
THERE IS MORE TO THE STORY

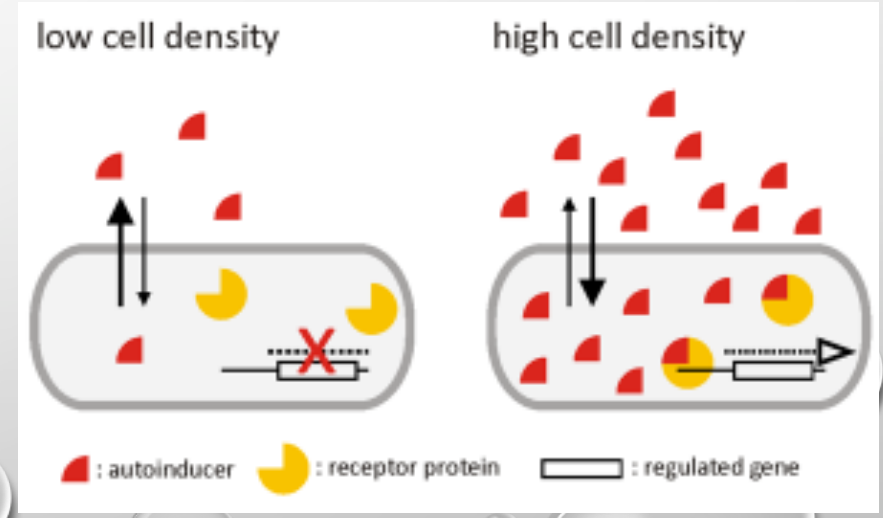
- WHAT WE HAVE SEEN:
- THE SYMBIONTS ARE OBTAINED THROUGH HORIZONTAL TRANSMISSION FROM THE AMBIENT SEAWATER BY EACH GENERATION OF JUVENILE SQUID. THE CHEMICAL ATTRACTANT FOR THE VIBRIO IS A COMPONENT OF THE SQUID MUCUS
- THEY RESIDE IN THE LIGHT ORGAN
- AND NOW THE MORE:
- THE SQUID–VIBRIO ASSOCIATION IS CHARACTERIZED BY DAILY RHYTHMS OF SYMBIONT GROWTH AND BIOLUMINESCENCE
- EACH MORNING AT DAWN, AFTER ACTIVE FORAGING IN THE WATER COLUMN, THE HOST EXPELS THE CONTENTS OF THE LIGHT-ORGAN, INCLUDING 95% OF THE SYMBIONT POPULATION, INTO THE SURROUNDING SEAWATER
- THE REMAINING SYMBIONT CELLS REPOPULATE THE LIGHT ORGAN WITHIN HOURS, EVENTUALLY GLOWING AND PROVIDING THE SQUID'S NOCTURNAL BIOLUMINESCENCE



HOW DOES VIBRIO KNOW WHEN TO START GLOWING – QUORUM SENSING

- AN INTERCELLULAR FORM OF COMMUNICATION THAT BACTERIA USE TO COORDINATE GROUP BEHAVIORS
- DEPENDS ON THE SYNTHESIS AND DIFFUSION OF SIGNALING MOLECULES INTO THE ENVIRONMENT
- UPON REACHING A THRESHOLD CONCENTRATION, THESE MOLECULES WILL TRIGGER CELLULAR RESPONSES, TYPICALLY BY ALTERING GENE EXPRESSION ACROSS THE ENTIRE POPULATION





SYMBIONT SETS THE HOST'S BIOLOGICAL CLOCK

- CIRCADIAN RHYTHM IS GOVERNED BY AN INTERNAL OR "BIOLOGICAL CLOCK" WITH A CYCLE OF ABOUT 24 HOURS AND SEEMS TO BE REGULATED LARGELY BY EXPOSURE TO LIGHT AND DARKNESS. IT IS RESPONSIBLE FOR SLEEP CYCLES AND OTHER PHYSIOLOGICAL AND METABOLIC FUNCTIONS. DISRUPTION OF CIRCADIAN RHYTHMS HAVE SERIOUS HEALTH CONSEQUENCES
- LIGHT GENERATED BY THE VIBRIO SYMBIONT TRIGGERS A GENETIC CASCADE IN THE CELLS OF THE SQUID LIGHT ORGAN, WHICH, IN TURN, CONTROL THE DAILY CYCLE OF BIOLOGICAL ACTIVITY TYPICALLY SYNCHRONIZED BY ENVIRONMENTAL CUES SUCH AS SUNLIGHT
- "INSTEAD OF ENVIRONMENTAL LIGHT, THIS ANIMAL RESPONDS AND CYCLES IN RESPONSE TO THE LUMINESCENCE FROM ITS OWN LIGHT ORGAN"

INSECTS AS SYMBIONT HOSTS

- INSECTS ARE THE MOST SUCCESSFUL ANIMALS, ACCOUNTING FOR >90% OF KNOWN ANIMAL SPECIES AND DOMINATING A VARIETY OF TERRESTRIAL HABITATS

- MANY INSECT LIFESTYLES ARE FOUNDED ON ASSOCIATIONS WITH MICROORGANISMS

- **ALL** INSECTS INVESTIGATED TO DATE BEAR RESIDENT MICROORGANISMS AND, ALTHOUGH SOME ARE NOT OBLIGATELY DEPENDENT ON THEIR MICROBIOTA, THERE IS INCREASING EVIDENCE THAT THESE MICROORGANISMS INFLUENCE MANY INSECT TRAITS

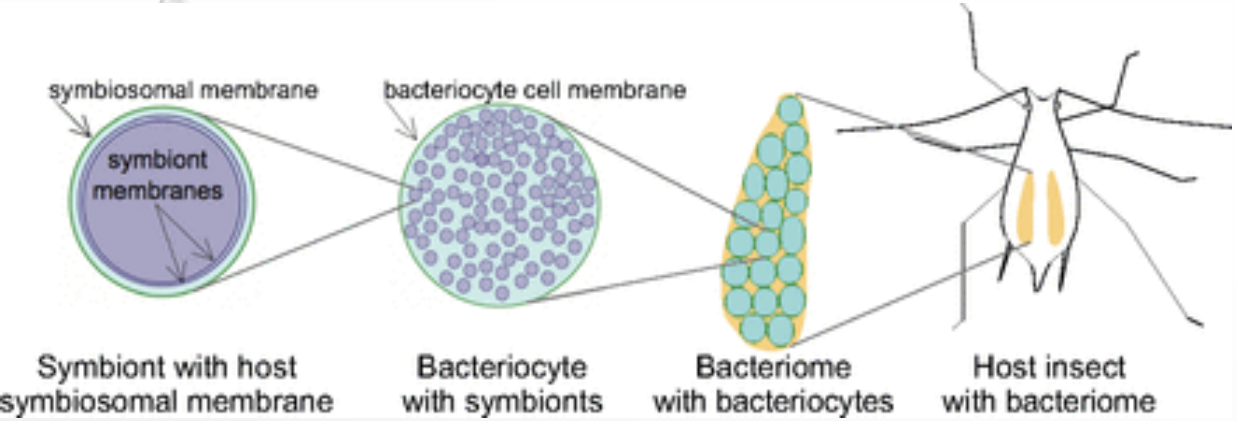
OBLIGATE SYMBIONTS

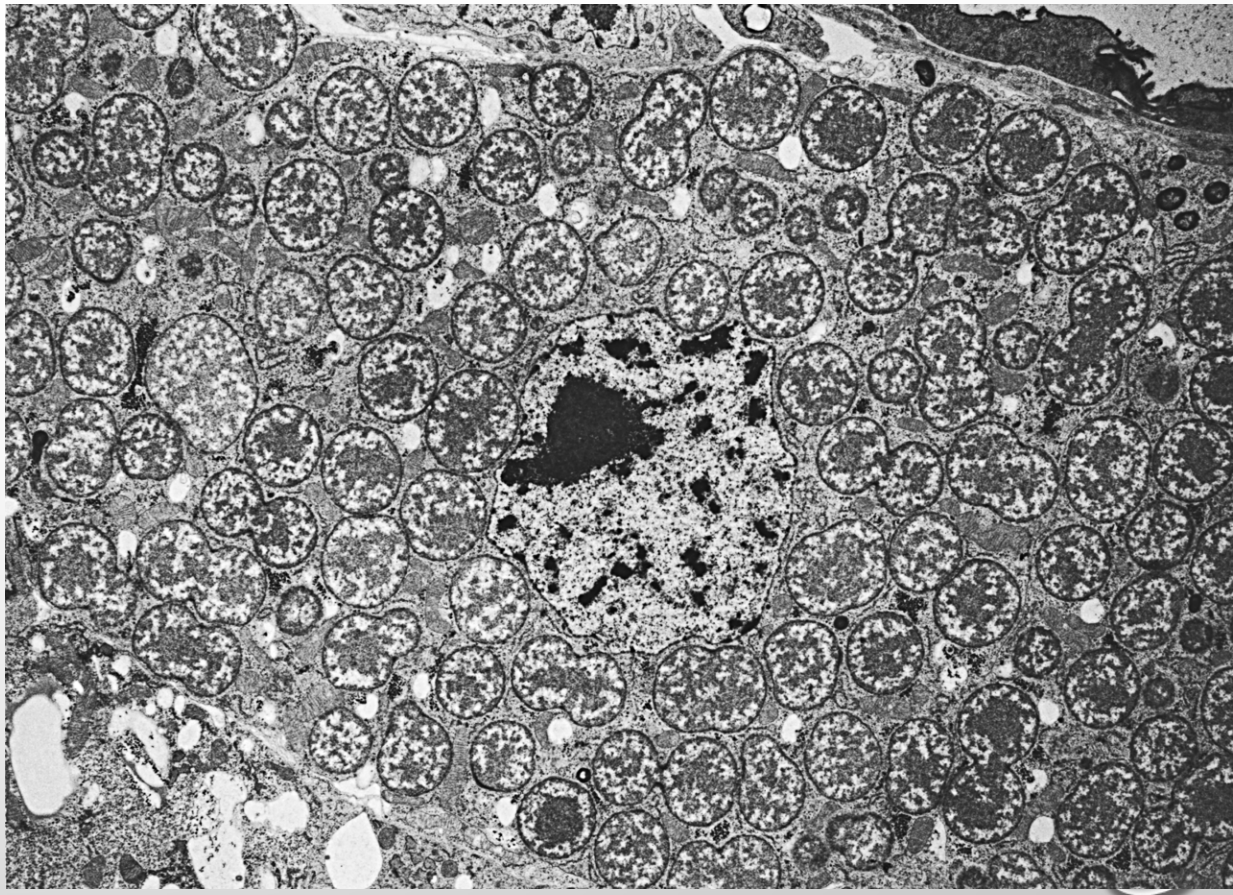
- EXCLUSIVELY OR PREDOMINANTLY VERTICALLY (AND USUALLY MATERNALLY) TRANSMITTED, AND THUS HERITABLE
- TEND TO HAVE A NUTRITIONAL FUNCTION AND TYPICALLY OCCUR IN INSECTS THAT FEED ON IMBALANCED AND POOR DIETS SUCH AS PLANT SAPS OR CELLULOSE, OR BLOOD
- SOME ALSO AFFECT THE REPRODUCTIVE SYSTEM OF THEIR HOSTS BY EITHER CAUSING FEMALE-BIASED SEX RATIOS OR IMPACTING ON THE ABILITY OF PAIRS OF INDIVIDUALS TO PRODUCE OFFSPRING
- IN ADDITION TO THEIR INDIVIDUAL EFFECTS ON HOSTS AND THEIR POSSIBLE CONSEQUENCES ON INSECT COMMUNITIES, INSECT SYMBIONTS MAY THEMSELVES FORM COMMUNITIES → OFTEN SEVERAL BACTERIAL STRAINS OR SPECIES CO-OCCUR WITHIN THE SAME HOST, AND IN SOME CASES, THE PRESENCE OF MULTIPLE SPECIES IS REQUIRED FOR THE SURVIVAL OF THE INSECT

SHARED COOKING: APHIDS AND *BUCHNERA*

- **APHIDS** ARE SMALL SAP-SUCKING INSECTS (CA. 5,000 SPECIES)
- 400 OF THESE ARE FOUND ON FOOD AND FIBER CROPS, AND MANY ARE SERIOUS PESTS
- VERY DESTRUCTIVE: WEAKEN THE PLANT BY SUCKING SAP, VECTORS FOR PLANT VIRUSES
- REPRODUCTION TYPICALLY DOES NOT INVOLVE MALES (PARTHENOGENESIS) → OFFSPRING ARE CLONAL TO THEIR MOTHER AND ARE ALL FEMALE
- SOMETIMES FEMALES PARTHENOGENETICALLY PRODUCE SEXUAL FEMALES AND MALES (FEMALES ARE XX, MALES ARE X0)
- APHIDS HARBOR A VERTICALLY TRANSMITTED (FROM PARENT TO ITS OFFSPRING) OBLIGATE SYMBIONT, *BUCHNERA*

BUCHNERA APHIDICOLA

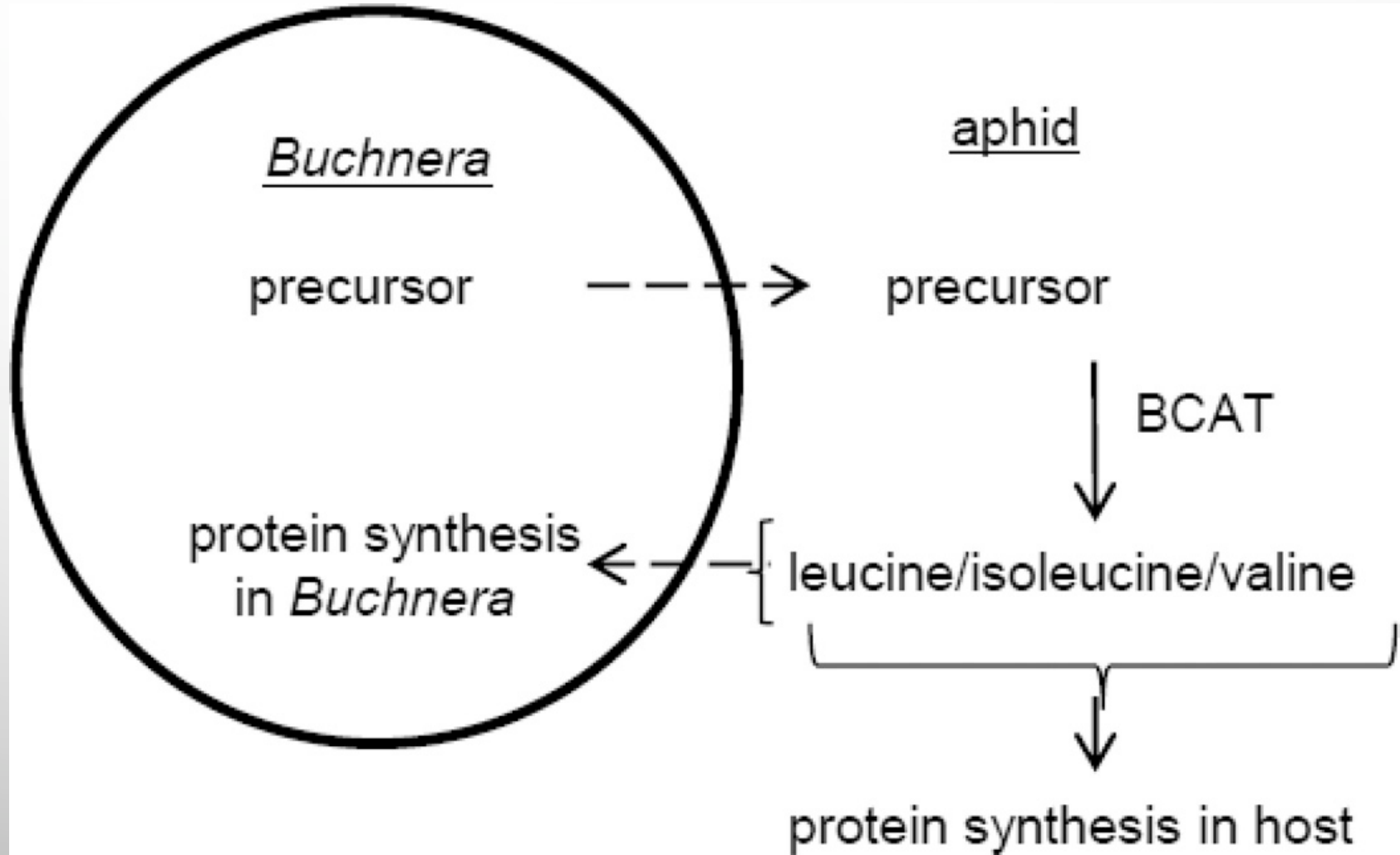




- *BUCHNERA* IS BELIEVED TO HAVE HAD A FREE-LIVING ANCESTOR SIMILAR TO A MODERN *E. COLI*
- THE SYMBIOTIC RELATIONSHIP WITH APHIDS BEGAN BETWEEN 160 MILLION AND 280 MILLION YEARS AGO
- APHIDS HAVE DEVELOPED SPECIAL CELLS (BACTERIOCYTES) AND ORGANS (BACTERIOME) TO HOUSE *BUCHNERA*
- A MATURE APHID MAY CARRY AN ESTIMATED 5.6×10^6 *BUCHNERA* CELLS

TWO COOKS FOR ONE MEAL

- APHIDS FEED ON NUTRIENT-POOR PLANT SAP (DEFICIENT IN ESSENTIAL AMINO ACIDS)
- NUTRITIONAL STATUS OF APHIDS IS COMPROMISED BY EXPERIMENTAL ELIMINATION OF THE MICROORGANISMS
- TWO-WAY TRANSFER OF MULTIPLE METABOLITES BETWEEN THE BACTERIOCYTE CYTOPLASM AND INTRACELLULAR BACTERIA → COUPLED METABOLISM THROUGH SHARED METABOLIC PATHWAYS



MICROBIAL PROTECTION TO HOST

- FROM PARASITIC WASPS (THEY INJECT AN EGG INTO THE **APHID**). WHEN THE EGG HATCHES, THE LARVAE BEGIN TO CONSUME THE APHID FROM INSIDE)
- THE SYMBIONT CARRIES A VIRUS THAT'S TOXIC TO THE WASP'S YOUNG

- OTHER SYMBIONTS (NOT *BUCHNERA*, BUT COEXISTING WITH *BUCHNERA*) IMPROVE THE APHID'S TOLERANCE FOR HIGH TEMPERATURES, ENABLING IT TO THRIVE IN NEW ENVIRONMENTS

WHAT'S IN IT FOR THE APHID

- THE SYMBIONT SYNTHESIZES SEVERAL AMINO ACIDS THAT ARE REQUIRED FOR THE APHIDS' METABOLISM
- APHIDS IN WHICH THE SYMBIONT HAS BEEN ARTIFICIALLY REMOVED HAVE EXTREMELY LOW SURVIVAL AND FECUNDITY
- PROTECTION IN THE FORM OF RESISTANCE TO PARASITES AND ENVIRONMENTAL STRESS

WHAT'S IN IT FOR THE SYMBIONT

- WITH VERTICAL TRANSMISSION TO THE APHID'S PROGENY, SURVIVAL IS ESSENTIALLY GUARANTEED
- COST → MAJOR GENOME DEGRADATION → NO POSSIBILITY OF RETURNING TO AN INDEPENDENT LIFESTYLE
- THE BACTERIA CANNOT UNDERGO TRANSFORMATION AND LACK PLASMIDS, THEREFORE, THEY CANNOT RECOMBINE DNA AND REPLACE BAD GENES
- ONLY A FEW OF THE BACTERIA TRAPPED INSIDE AN APHID PASS ALONG TO THE NEXT GENERATION (REMEMBER POPULATION BOTTLENECKS) → FURTHER REDUCTION IN RECOMBINATION CAPABILITIES
- BUILDUP OF MUTATIONS STEADILY ERODES THE NUMBER OF WORKING GENES
- *BUCHNERA* HAS JUST 600 GENES (COMPARED WITH THE CA. 5,000 IN *ESCHERICHIA COLI*)
- HOWEVER THEY CAN COUNTERACT DECAY BY HORIZONTAL GENE TRANSFER → ESSENTIAL GENES FROM THE MICROBE MIGRATE TO THE HOST GENOME (LIKE GENES FROM MITOCHONDRIA DID). THAT WAY THEY CAN BENEFIT FROM THE HOST'S SEXUAL REPRODUCTION, WHICH ENABLES RECOMBINATION AND INTACT COPIES TO REPLACE MUTATED ONES.

HOW SMALL IS SMALL FOR A SYMBIONT'S GENOME? WHEN IS AN ENDOSYMBIONT AN ORGANELLE?

- SMALLEST SO FAR: *CARSONELLA RUDDII*, SYMBIONT OF JUMPING PLANT LICE
- RELATED TO APHIDS, ALSO DINES ON PLANT SAP
- IT SUPPLIES THE HOST WITH SOME ESSENTIAL AMINO ACIDS
- GENOME: CA. 160,000 BASE PAIRS (FOR COMPARISON, *E. COLI* IS CA. 4,500,000)
- NUMBER OF PREDICTED GENES: 182 (LOWEST ON RECORD). FOR COMPARISON: *MYCOPLASMA GENITALIUM* (SEXUALLY TRANSMITTED, SMALL PATHOGENIC BACTERIUM THAT LIVES ON THE SKIN CELLS OF THE URINARY AND GENITAL TRACTS IN HUMANS) HAS THE SMALLEST GENOME OF ANY SELF-REPLICATING ORGANISM, AT 521 GENES
- REDUCTION OF THE SYMBIONT'S GENOME TO ONLY ESSENTIAL GENES FOR THE HOST AND SYMBIONT **COLLECTIVE GENOME**

Jumping Plant Louse

A SYMBIONT'S "THREE FACES OF EVE": *HODGKINIA* IN 17-YEAR CICADAS

- BECAUSE THE CICADA LIFE CYCLE TAKES YEARS UNDERGROUND, NATURAL SELECTION ON ENDOSYMBIONT POPULATIONS IS RELAXED FOR MANY BACTERIAL GENERATIONS
- THIS ALLOWS THE SYMBIONT GENOMES TO DIVERSIFY WITHIN THE HOST FOR YEARS WITH ONLY PUNCTUATED PERIODS OF SELECTION WHEN THE CICADAS REPRODUCE
- AS A RESULT, THE ANCESTRAL *HODGKINIA* HAS SPLIT INTO THREE GROUPS OF PRIMARY ENDOSYMBIONTS, EACH ENCODING A SUBSET OF THE ESSENTIAL GENES FOR THE SYMBIOSIS
- THE HOST NOW REQUIRES ALL THREE SUB-GROUPS OF SYMBIONT
- IN A FURTHER TWIST, EACH SUB-SYMBIONT HAS SEEN ITS GENOME SPLIT INTO DOZENS OF CIRCLES

WHEN GENOME DEGRADATION GOES TOO FAR, CHANGE YOUR SYMBIONT

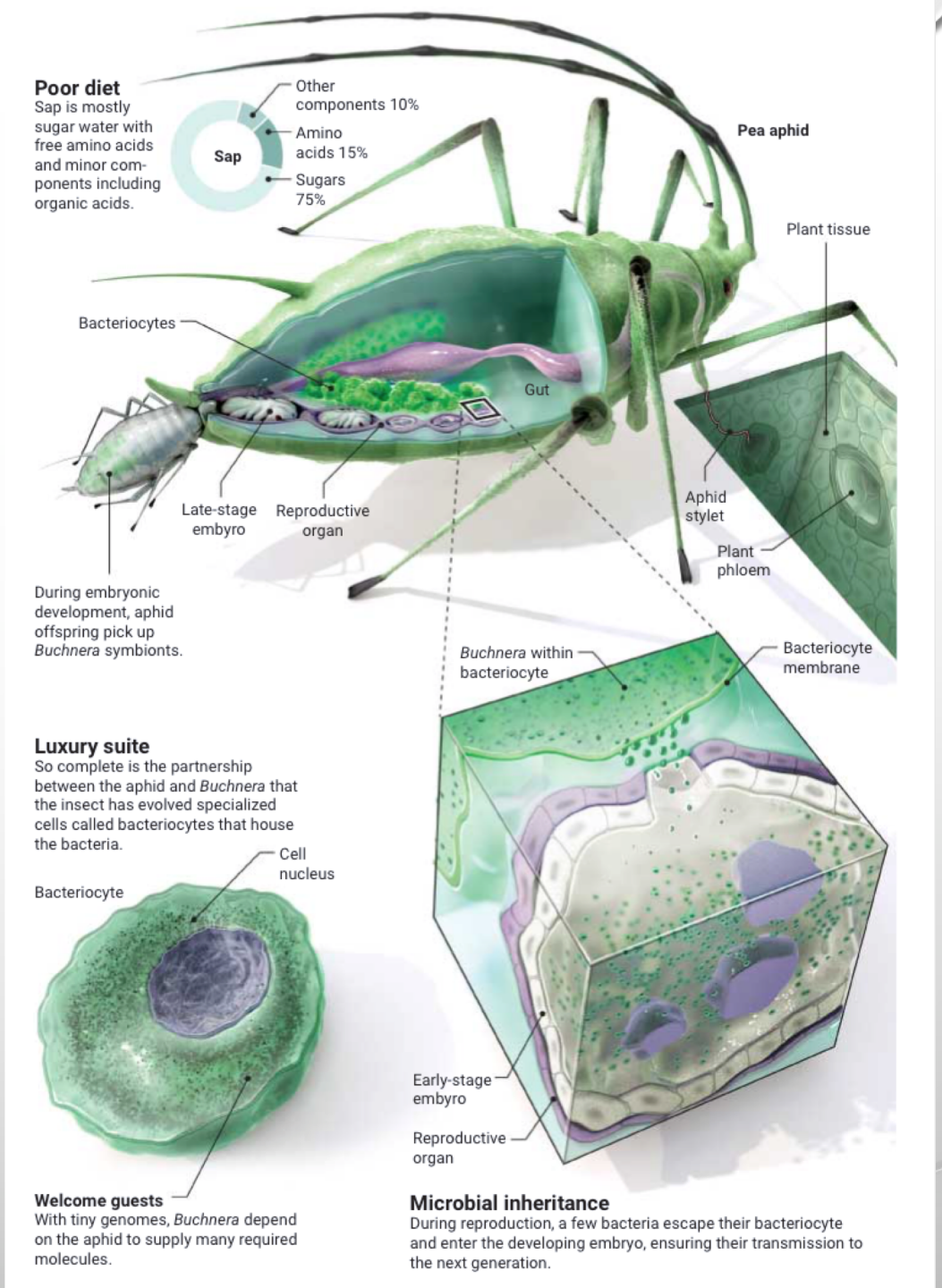
- OVER EVOLUTIONARY TIME, THE VERY FEATURES THAT HAVE STRENGTHENED OBLIGATE SYMBIOTIC RELATIONSHIPS (LIKE INTEGRATED METABOLISM) MAY ALSO PRESENT AN EVOLUTIONARY HAZARD
- THE SMALL EFFECTIVE POPULATION SIZE OF VERTICALLY TRANSMITTED OBLIGATE SYMBIONTS PROMOTES THE ACCUMULATION OF DELETERIOUS MUTATIONS RESULTING IN REDUCED GENE FUNCTION AND SIGNIFICANT GENE LOSS
- AS ENDOSYMBIONT GENOMES CONTINUE TO DETERIORATE, FUNCTIONS MAY BECOME SIGNIFICANTLY COMPROMISED
- ONE SOLUTION TO THE EVOLUTIONARY DEGRADATION OF OBLIGATE SYMBIONT FUNCTIONS IS THE ACQUISITION OF NOVEL SYMBIONTS
- THIS HAPPENED IN SOME APHIDS → THEY DITCHED *BUCHNERA*, AND GAINED ANOTHER BACTERIUM WITH THE SAME METABOLIC CAPABILITIES → **IT'S THE SONG, NOT THE SINGER**

FROM PARENT TO PROGENY: PASSING ON YOUR ENDOSYMBIONT

- MATERNALLY TRANSMITTED TO THE EGG → APHIDS
- VIA MOTHER'S MILK → TSETSE FLIES
- VIA INGESTION: MOUTH-TO-MOUTH OR ANUS-TO-MOUTH → TERMITES

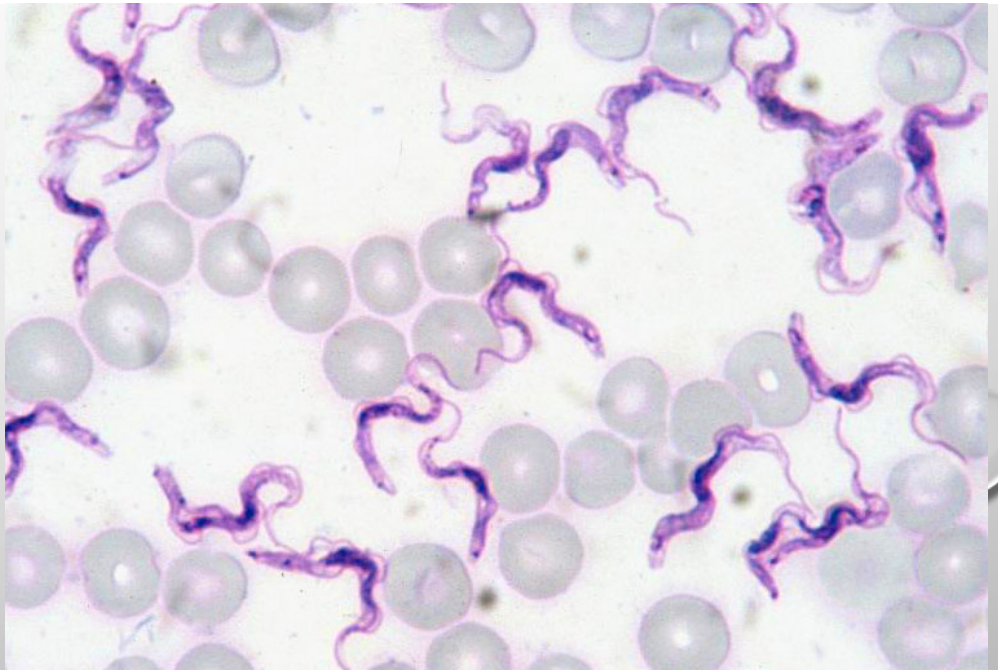
APHIDS

Symbionts are transmitted from maternal bacteriocytes to adjacent blastulae (early embryonic stage) at the ovariole tips. This transmission is active and specific; other microbes that are present are **not** transmitted (in lab experiments)



TSETSE FLIES AND *WIGGLESWORTHIA*

- LARGE BITING FLIES THAT INHABIT MUCH OF TROPICAL AFRICA
- VECTORS OF TRYPANOSOMES: HUMAN SLEEPING SICKNESS
- ENDOSYMBIONT: *WIGGLESWORTHIA*: SYNTHESIZES KEY B-COMPLEX VITAMINS WHICH THE TSETSE FLY DOES NOT GET FROM ITS DIET OF BLOOD
- REMOVING THE SYMBIONT MAKES THE FLIES MORE SUSCEPTIBLE TO TRYPANOSOME INFECTION
- WITHOUT THE SYMBIONT, FEMALES CANNOT SUPPORT THE DEVELOPMENT OF THE LARVA; THEY BECOME STERILE
- THE MOTHER TRANSFERS THE SYMBIONT TO THE LARVA VIA ITS "MILK"



A TSETSE FLY IS BORN

Tsetse females are viviparous (intrauterine embryogenesis and larvigenesis) and they provide for all larval nutrition in the form of milk secretions by milk glands

A TERMITE'S GUT

- TERMITES CONSUME DEAD PLANTS AT ANY LEVEL OF DECOMPOSITION
- TERMITES ARE A MAJOR SOURCE (11%) OF ATMOSPHERIC CH_4 PRODUCED FROM THE BREAKDOWN OF CELLULOSE
- SYMBIOTIC PROTOZOA IN THE GUT DIGEST CELLULOSE (*TRICHONYMPHA)*
- THEY ALSO HAVE BACTERIAL SYMBIONTS OF THEIR OWN (A KIND OF MATRYOSHKA DOLL THAT ENSURES FULL DIGESTION OF CELLULOSE)
- WHEN TERMITES MOLT, THEY DISCARD THE LINING OF THE DIGESTIVE SYSTEM → OFF GO THE SYMBIONTS
- THUS THEY HAVE TO REPLENISH THEIR GUT MICROBIOTA AFTER EVERY MOLT
- THIS IS ACCOMPLISHED BY PROCTODEAL TROPHALLAXIS, WHERE NESTMATES EAT EACH OTHER'S HINDGUT FLUID TO ACQUIRE ENDOSYMBIONTS