INTIMATE STRANGERS:
MICROBIAL PARTNERS IN THE NATURAL WORLD

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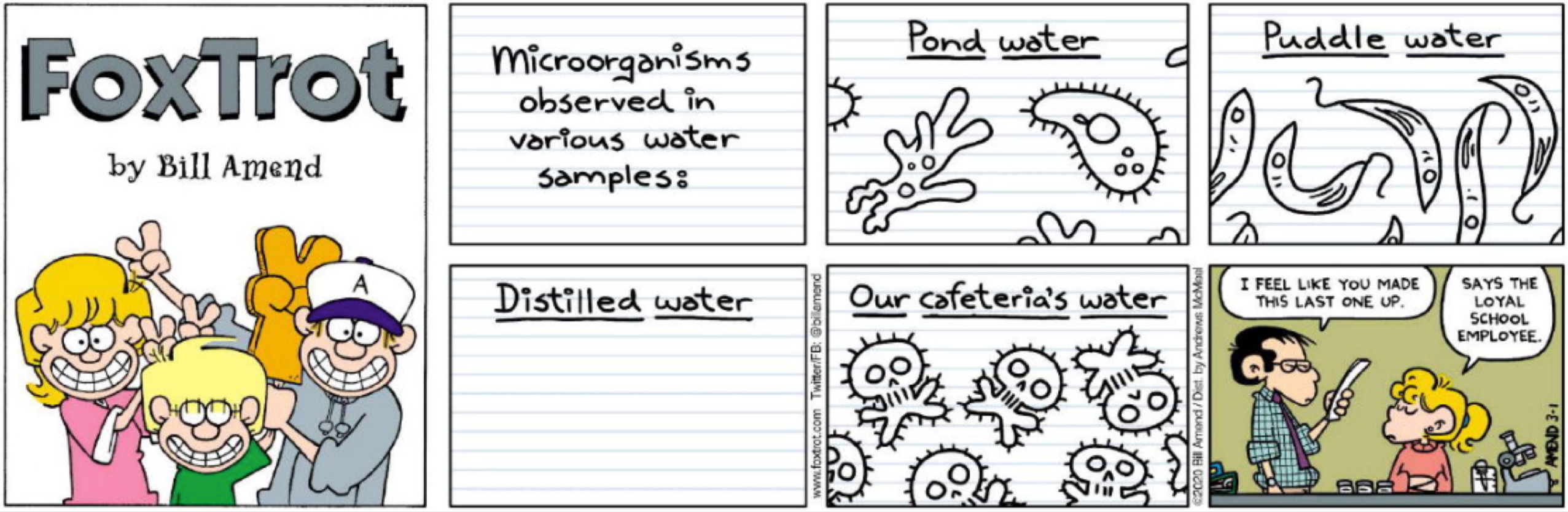
SPRING 2020


FoxTrot
by Bill Amend
Microorganisms observed in various water samples:
Pond water
Puddle water
Distilled water
Our cafeteria's water
www.foxtrot.com Twitter/FB: @billamend
©2020 Bill Amend / Dist. by Andrews McMeel
I FEEL LIKE YOU MADE THIS LAST ONE UP.
SAYS THE LOYAL SCHOOL EMPLOYEE.
AMEND 3-1

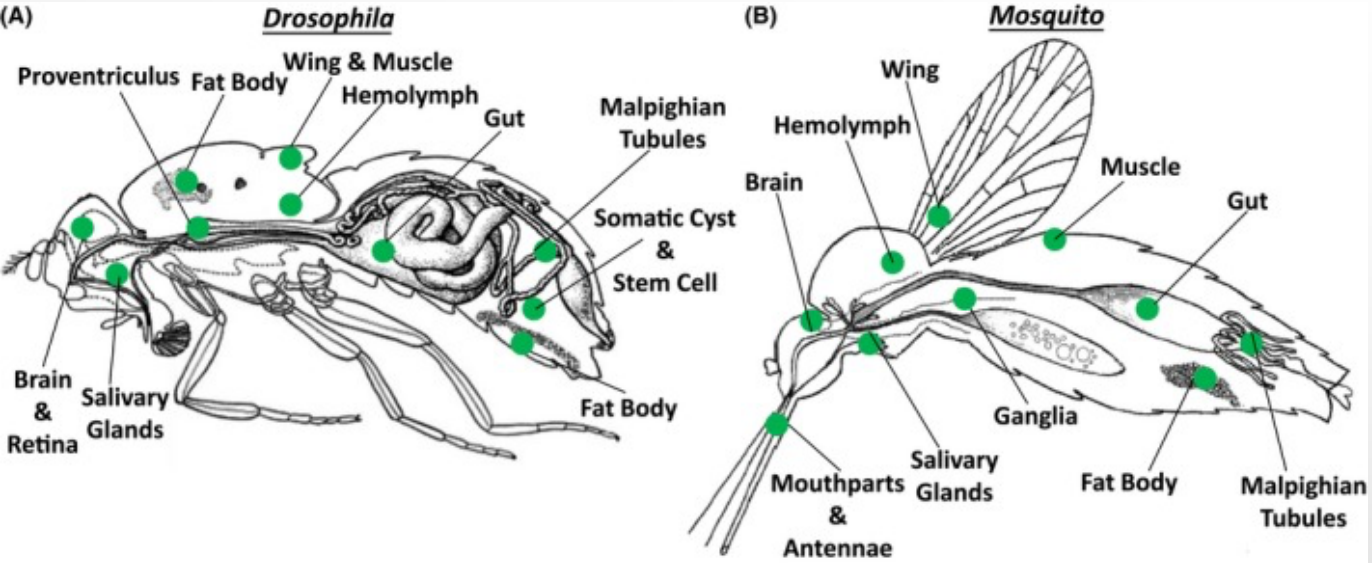
LECTURE 6:
EXPLORING MICROBIAL INTERACTIONS – III

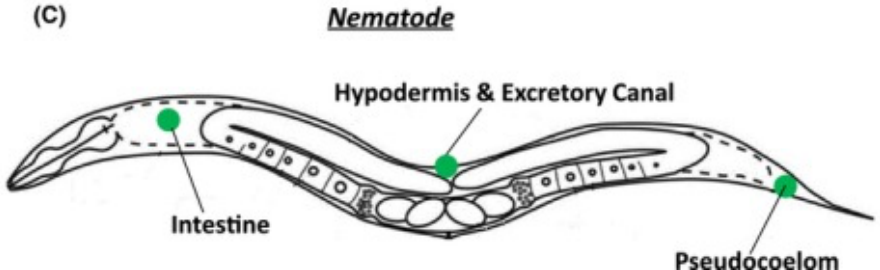
SEX AND THE SYMBIONT – THE TALE OF *WOLBACHIA*

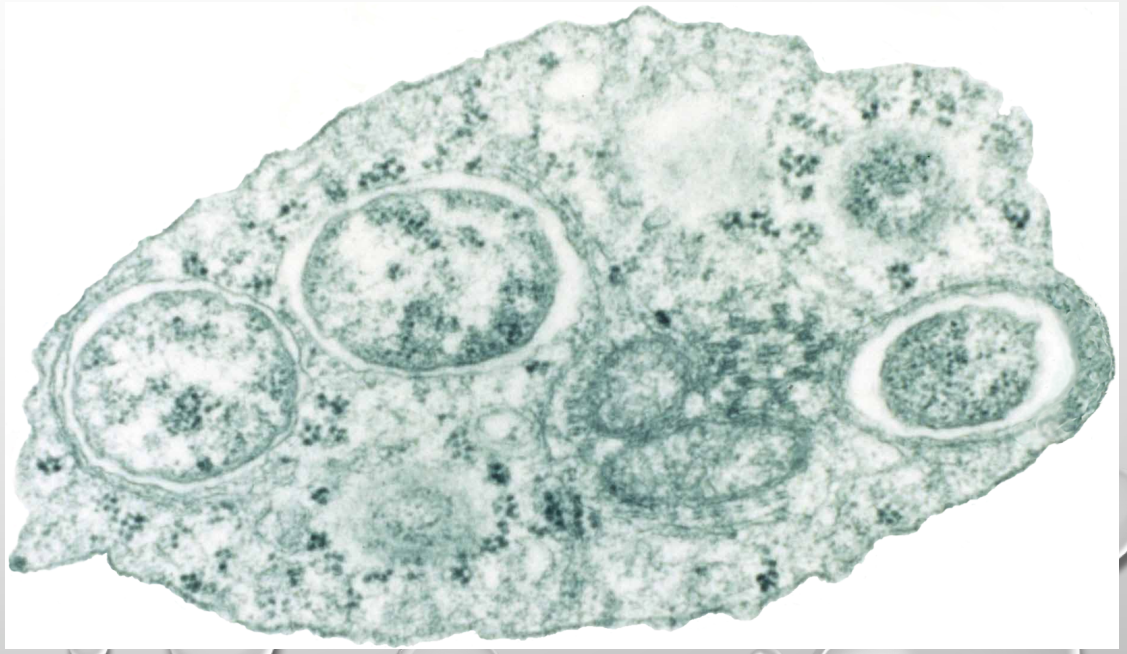
VERTEBRATES AS MICROBIAL PARTNERS

SEX AND THE SYMBIONT: THE TALE OF *WOLBACHIA*

- MOST WIDESPREAD HERITABLE MICROORGANISM (SYMBIONT) KNOWN
- THEY ARE PRESENT IN MORE THAN 65% OF ALL INSECT SPECIES; ALSO WIDESPREAD IN ARACHNIDS, CRUSTACEANS, AND NEMATODES

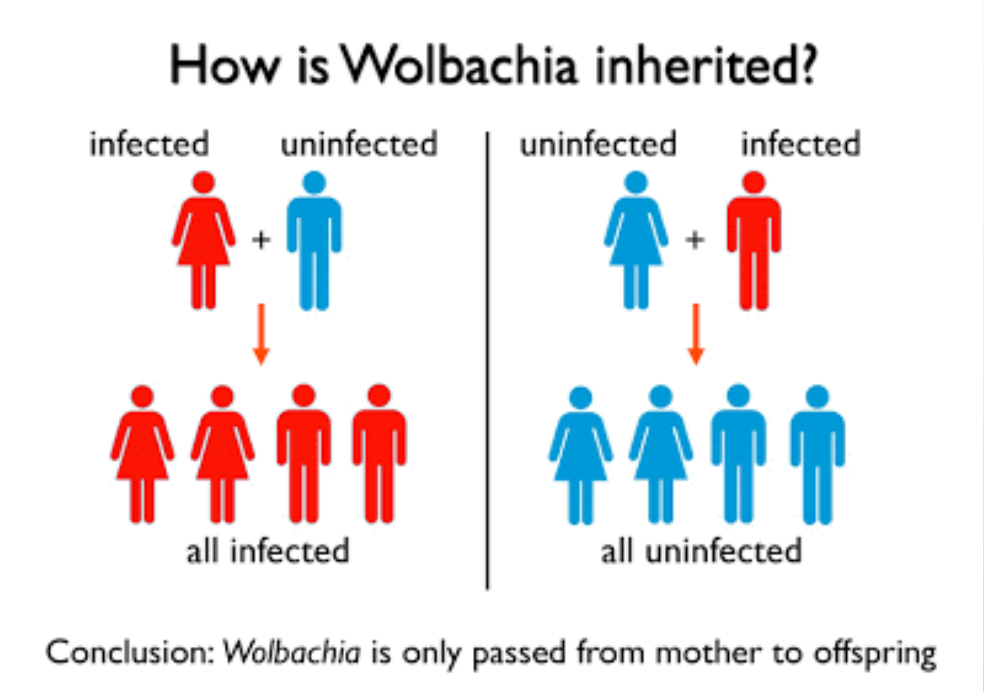






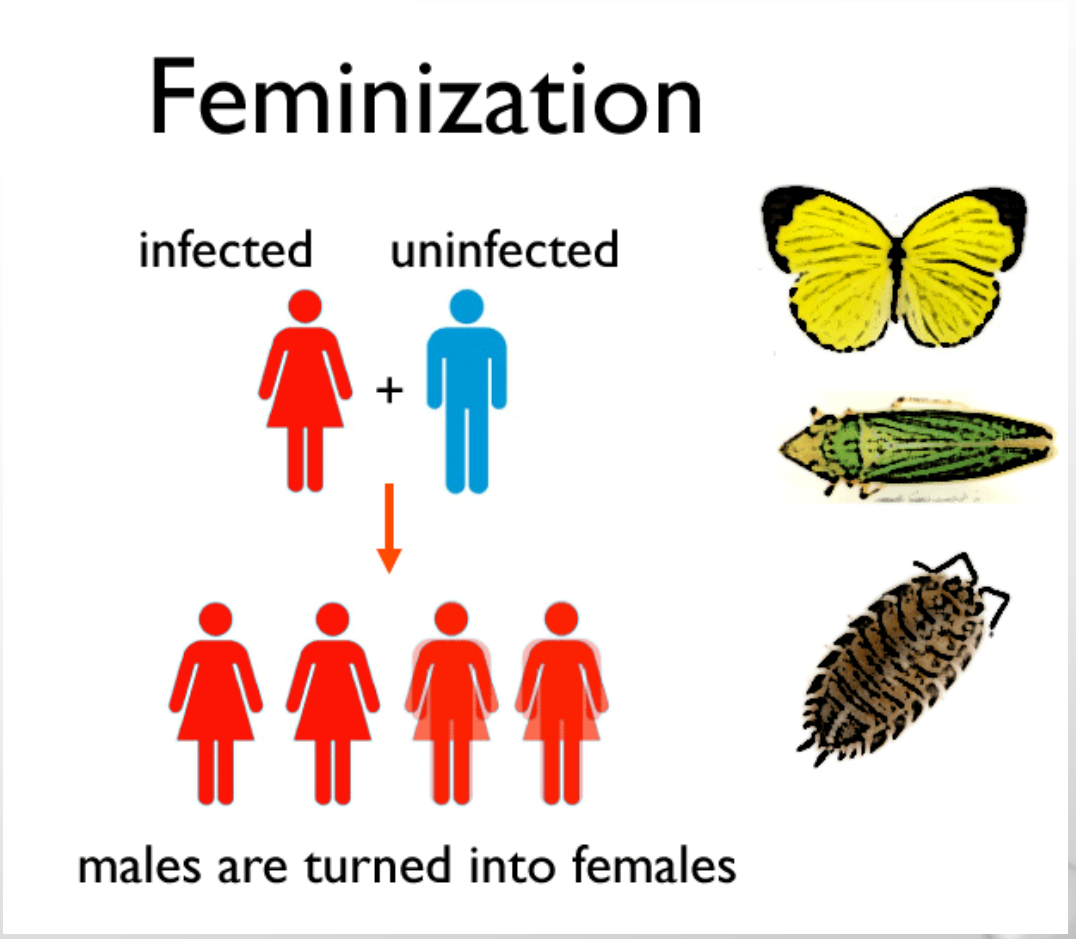
SEX AND THE SYMBIONT: THE TALE OF *WOLBACHIA*

- INTRACELLULAR, MATERNALLY INHERITED BACTERIAL ENDOSYMBIONT (TRANSMITTED VERTICALLY TO EGGS)
- MALES CAN BE INFECTED BUT THEY DO NOT TRANSMIT *WOLBACHIA* TO OFFSPRING
- *WOLBACHIA* MANIPULATES THE REPRODUCTIVE BIOLOGY OF HOSTS TO INCREASE ITS OWN TRANSMISSION



STRATEGY NO. 1 – FEMINIZATION

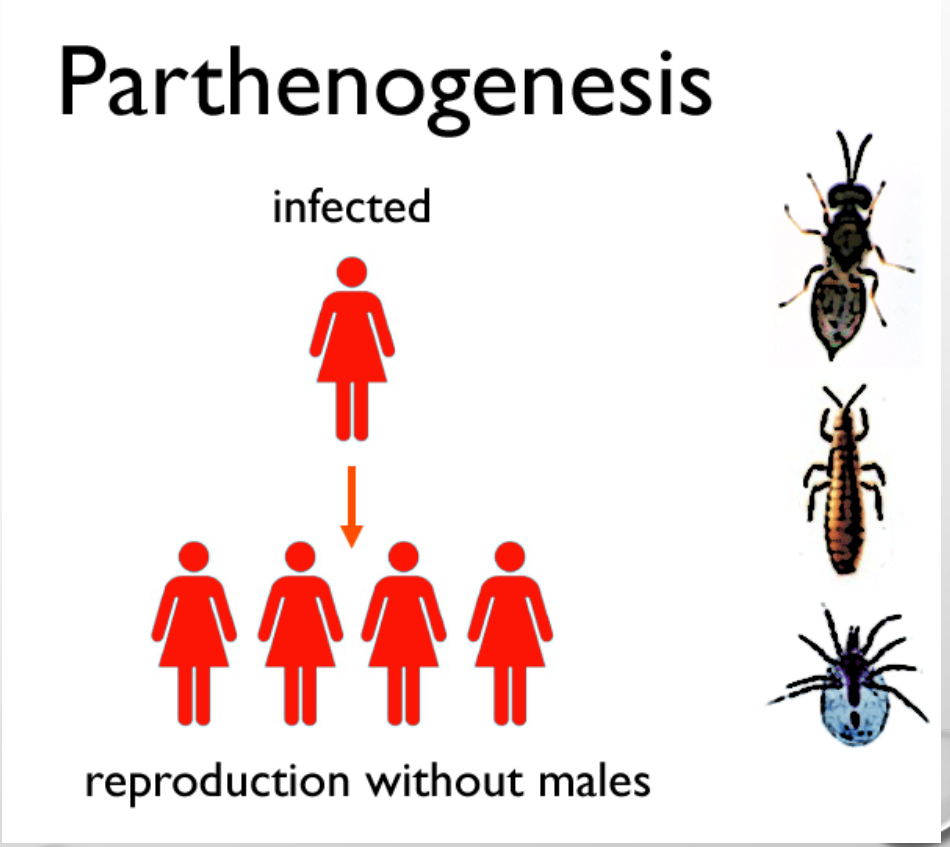
- MOST OBVIOUSLY BENEFICIAL STRATEGY FOR A MATERNALLY INHERITED BACTERIUM
- CONVERSION OF INFECTED GENETIC MALE OFFSPRING INTO FEMALES → DOUBLES THE POTENTIAL FOR TRANSMISSION TO THE NEXT GENERATION
- INFREQUENT



STRATEGY NO. 2 – PARTHENOGENESIS

- WITH MALES BEING AN EVOLUTIONARY DEAD END FOR *WOLBACHIA* INHERITANCE, ANOTHER OBVIOUS STRATEGY OF HOST MANIPULATION BY A MATERNALLY INHERITED ENDOSYMBIONT IS TO INDUCE PARTHENOGENESIS, THE PRODUCTION OF FEMALE OFFSPRING WITHOUT FERTILIZATION BY SPERM. AS WITH *WOLBACHIA*-INDUCED FEMINIZATION, PARTHENOGENESIS INDUCTION DOUBLES THE POTENTIAL TRANSMISSION OF WOLBACHIA TO THE NEXT GENERATION, BECAUSE ALL THE PROGENY ARE FEMALE

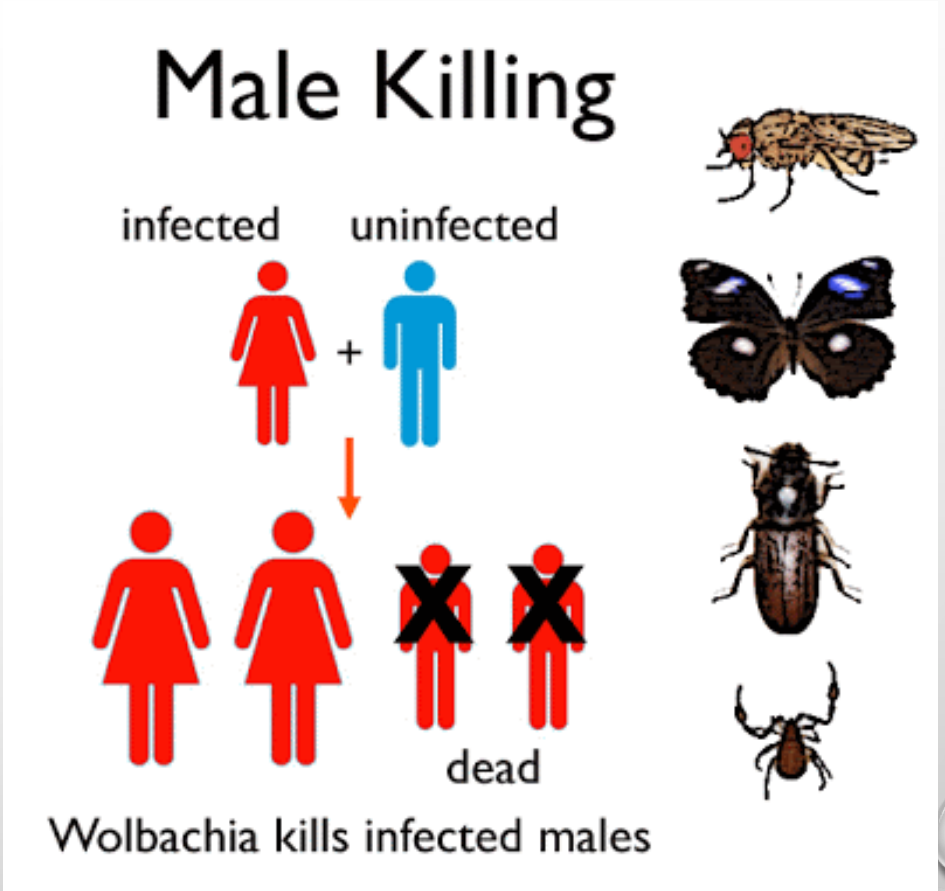
- INDUCES GAMETE DUPLICATION: X → XX



STRATEGY NO. 3 – MALE KILLING

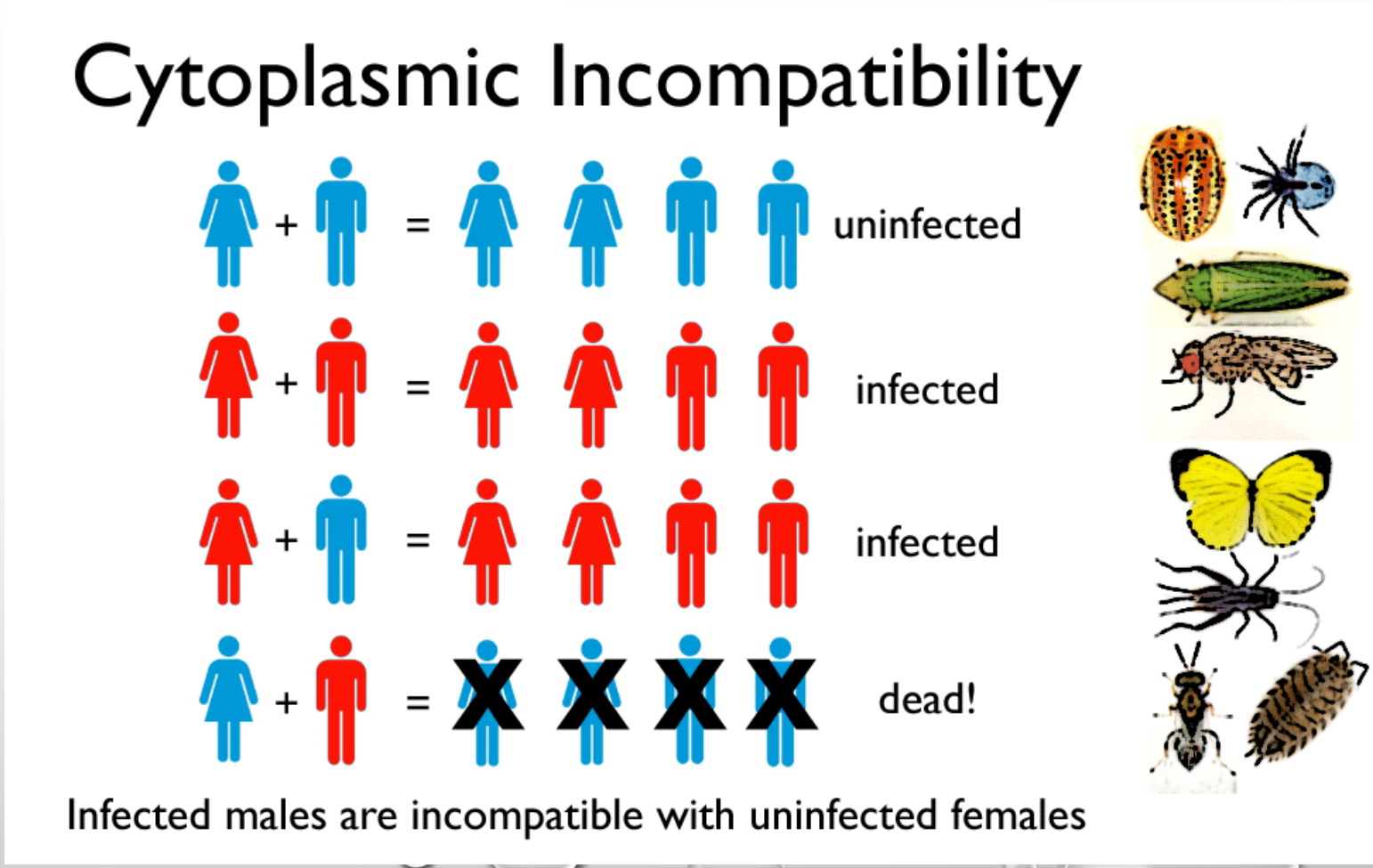
- KILLING OF GENETIC MALES
- ONLY EVOLVED WHEN THE KILLING OF INFECTED MALES BENEFITS THE SURVIVING INFECTED FEMALE SIBLING
- CONSEQUENTLY, ONLY HOSTS WITH HIGH SIBLING COMPETITION FOR RESOURCES ARE THOSE IN WHICH MALE-KILLING SHOULD PERSIST

- IN (INFECTED) MALE EMBRYOS, A GENE THAT ENCODES A PROTEIN REQUIRED FOR MASCULINIZATION IS TURNED OFF → ABNORMAL APOPTOSIS (PROGRAMMED CELL DEATH) → DEATH OF MALE EMBRYOS



STRATEGY N0. 4 – CYTOPLASMIC INCOMPATIBILITY

- MOST COMMON
- WHEN AN INFECTED MALE MATES WITH A NON-INFECTED FEMALE ALL OFFSPRING DIE (INCOMPATIBLE CROSS)
- ALL OTHER COMBINATIONS OF CROSSES ARE COMPATIBLE
- THE ENDOSYMBIONT CAN RAPIDLY SPREAD THROUGH THE POPULATION
- IN A MIXED POPULATION (WITH BOTH INFECTED AND UNINFECTED INDIVIDUALS), THE PRESENCE OF WOLBACHIA-INFECTED MALES INCREASES THE RELATIVE FITNESS OF INFECTED FEMALES BY REDUCING THE FITNESS OF UNINFECTED FEMALES

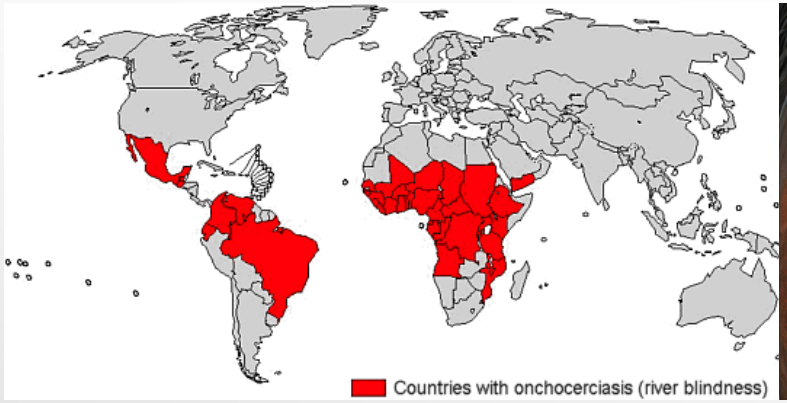


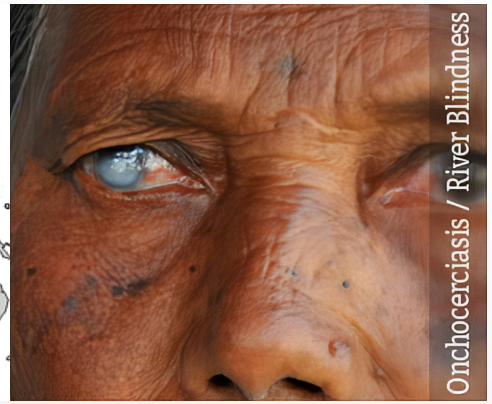
ADVANTAGES FOR THE HOST

- INCREASED RESISTANCE TO VIRUSES
- INCREASED RESISTANCE TO INSECTICIDES
- CAN ALSO PROVIDE B VITAMINS
- INCREASED FECUNDITY: INFECTED FRUIT FLIES PRODUCE MORE OFFSPRING THAN NON-INFECTED
- IN NEMATODES THAT CAUSE HUMAN DISEASE, IT PROVIDES THE HOST WITH CHEMICALS NECESSARY TO ITS REPRODUCTION AND SURVIVAL

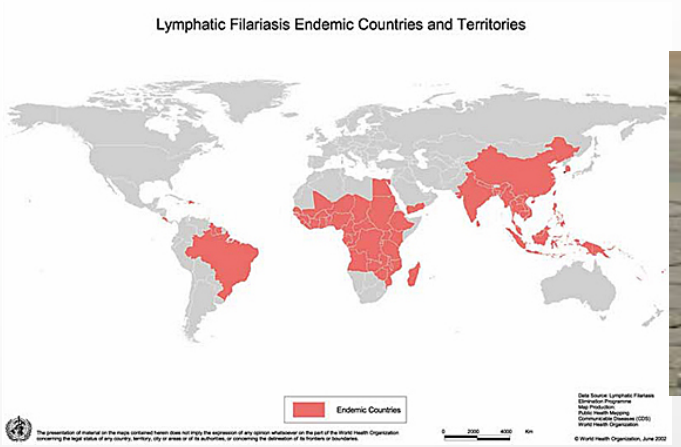
WOLBACHIA AND HUMAN DISEASES – FILARIAL NEMATODES

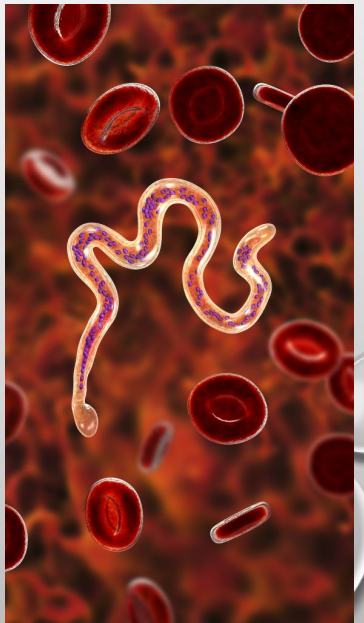
- RIVER BLINDNESS





- ELEPHANTIASIS





WOLBACHIA AND HUMAN DISEASES – FILARIAL NEMATODES

- *WOLBACHIA* INFECTS NEMATODES THAT CAUSE RIVER BLINDNESS AND ELEPHANTIASIS
- IN FILARIAL NEMATODES THEY ARE ESSENTIAL. ANTIBIOTIC ELIMINATION OF *WOLBACHIA* RESULTS IN INFERTILITY, INHIBITION OF EMBRYOGENESIS, ARRESTED ADULT GROWTH, AND DEATH OF THE WORM
- A LARGE PART OF THE PATHOGENICITY OF FILARIAL NEMATODES IS DUE TO HOST IMMUNE RESPONSE TOWARD THEIR *WOLBACHIA* SYMBIONT
- THEREFORE, TO TARGET THE WORM, GET RID OF *WOLBACHIA*
- CURRENT STRATEGIES FOR CONTROL OF FILARIAL NEMATODE DISEASES INCLUDE ELIMINATION OF THEIR SYMBIOTIC *WOLBACHIA*
- USE AN ANTIBIOTIC AND KILL THE SYMBIONT, RATHER THAN DIRECTLY KILLING THE NEMATODE WITH FAR MORE TOXIC ANTINEMATODE MEDICATIONS

MOSQUITO-TRANSMITTED HUMAN DISEASES

- *AEDES*: DENGUE, CHIKUNGUNYA, ZIKA, YELLOW FEVER
- THE BURDEN OF *AEDES*-TRANSMITTED DISEASE HAS INCREASED CONSIDERABLY OVER THE PAST 50 YEARS
- THE INCIDENCE OF DENGUE, NOW THE WORLD'S MOST COMMON MOSQUITO-BORNE VIRAL DISEASE, GREW MORE THAN 30-FOLD DURING THIS PERIOD
- DENGUE IS ESTIMATED TO INFECT AROUND 400 MILLION PEOPLE PER YEAR, AND OVER HALF OF THE WORLD'S POPULATION IS AT RISK OF THE DISEASE
- CHIKUNGUNYA VIRUS EMERGED FROM AFRICA IN THE MID-2000S, SPREADING FIRST ACROSS INDIA AND ASIA AND THEN INTO THE AMERICAS IN 2013
- ZIKA VIRUS OUTBREAKS OCCURRED IN THE SOUTH PACIFIC IN 2013 AND IN THE AMERICAS IN 2015
- YELLOW FEVER, FOR WHICH AN EFFECTIVE VACCINE EXISTS, IS RE-EMERGING. RECENT OUTBREAKS STARTED IN ANGOLA IN LATE 2015, AND THE VIRUS QUICKLY SPREAD TO THE DEMOCRATIC REPUBLIC OF CONGO, KENYA AND CHINA. IN LATE 2016, HUNDREDS OF CASES OF YELLOW FEVER WERE REPORTED IN BRAZIL

DENGUE IN THE WORLD

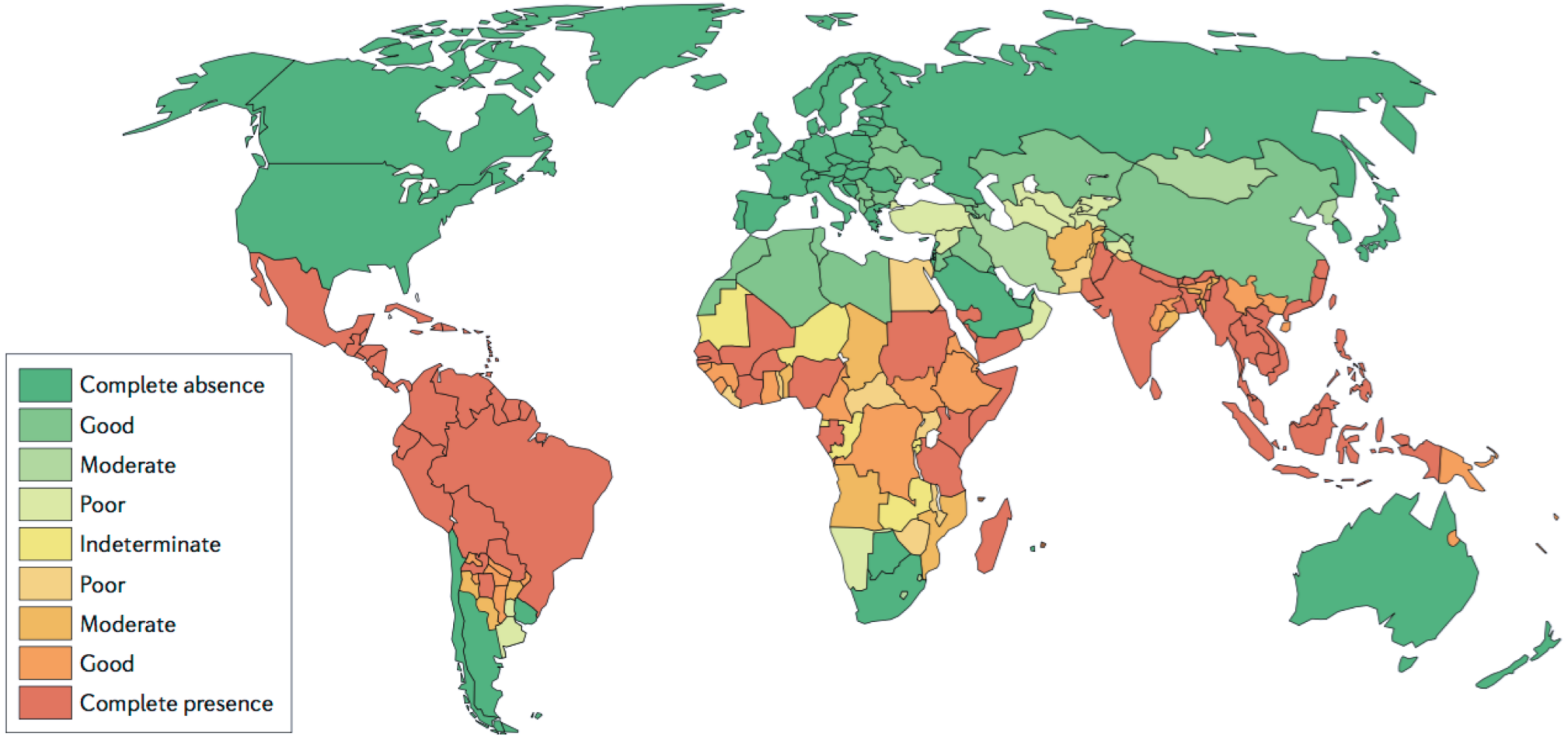


Fig. 1 | **The global distribution and burden of dengue.** Evidence consensus map showing the complete absence to complete presence of dengue. Green colours indicate evidence consensus towards absence of dengue, and orange and red colours indicate consensus towards presence of dengue. Darker colouring indicates more data supporting a conclusion about the presence or absence of dengue in a country. Figure adapted from REF.[4], Macmillan Publishers Limited.

STRATEGIES FOR CONTAINING *AEDES* MOSQUITOES

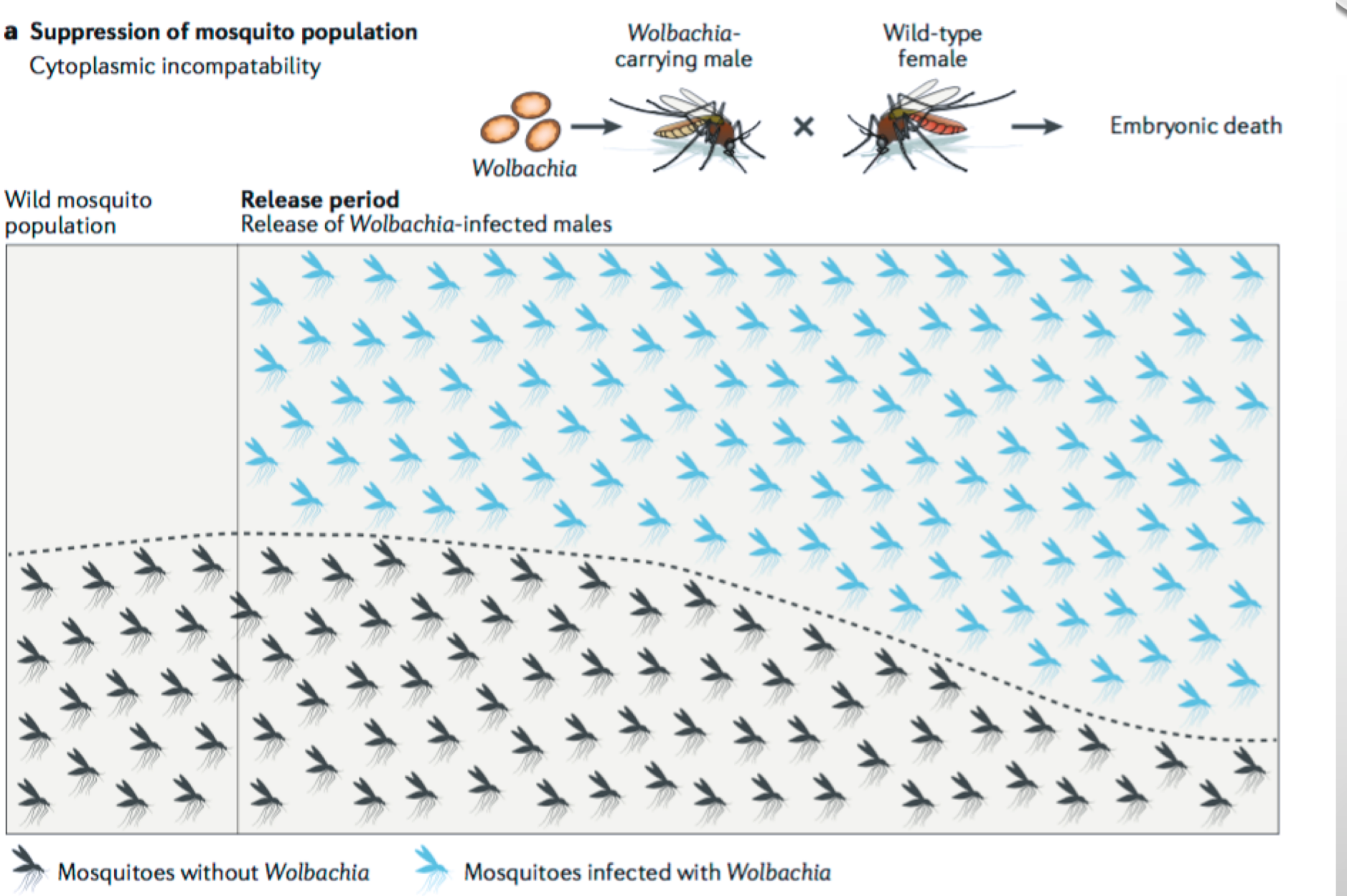
- TRADITIONAL METHODS (REMOVAL OF BREEDING SITES OR THE APPLICATION OF INSECTICIDES TARGETING EITHER LARVAE OR ADULTS) ARE UNABLE TO COPE IN THIS NEW GLOBAL CONTEXT

- NOVEL APPROACHES INVOLVING RELEASE OF MOSQUITOES AIM TO:

- 1) REDUCE THE VECTOR POPULATION
- 2) MODIFY THE VECTOR TO MAKE IT REFRACTORY TO PATHOGEN TRANSMISSION

I – REDUCING VECTOR POPULATION

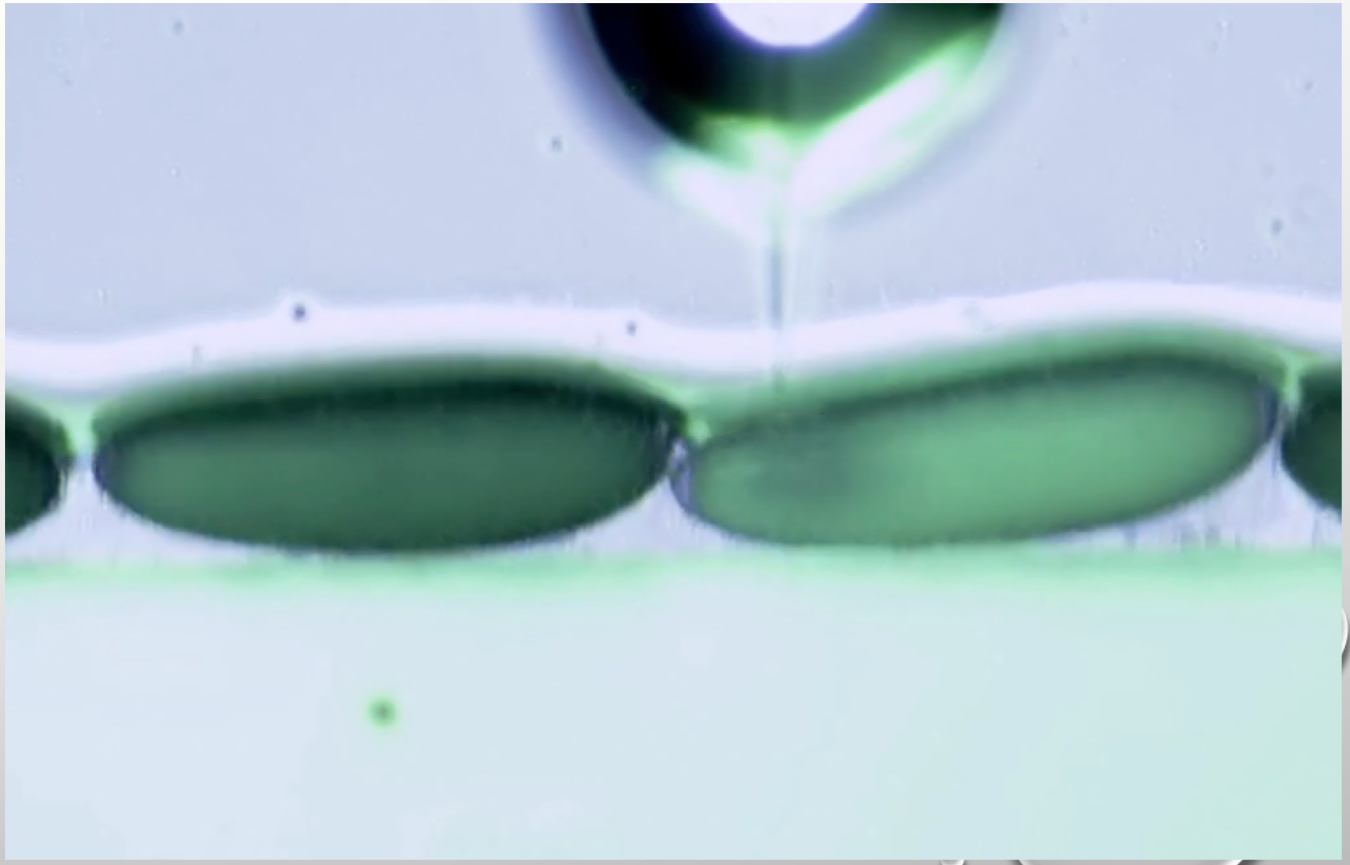
- REARING AND RELEASING LARGE NUMBERS OF MALE MOSQUITOES THAT CANNOT PRODUCE VIABLE OFFSPRING WHEN THEY MATE WITH WILD FEMALES
- ADVANTAGE: FEMALES WILL NOT BE RELEASED → COMMUNITIES SHOULD EXPERIENCE NO INCREASE IN BITING RATE
- REQUIRES LARGE NUMBERS OF MALES TO BE RELEASED OVER A LONG TIME PERIOD AND UNLESS THE POPULATION IS COMPLETELY ELIMINATED, IT IS EXPECTED TO RECOVER QUICKLY
- USING *WOLBACHIA*: *WOLBACHIA* STRAIN IS STABLY INTRODUCED INTO A COLONY OF A MOSQUITO SPECIES. ONLY MALE MOSQUITOES CARRYING *WOLBACHIA* ARE RELEASED INTO A WILD POPULATION TO MATE WITH WILD-TYPE FEMALES
- OWING TO CYTOPLASMIC INCOMPATIBILITY INDUCED BY *WOLBACHIA*, NO OFFSPRING CAN BE PRODUCED. IF MALES ARE RELEASED IN HIGH ENOUGH NUMBERS, MORE INCOMPATIBLE MATINGS WILL OCCUR, AND ULTIMATELY, THE MOSQUITO POPULATION COLLAPSES



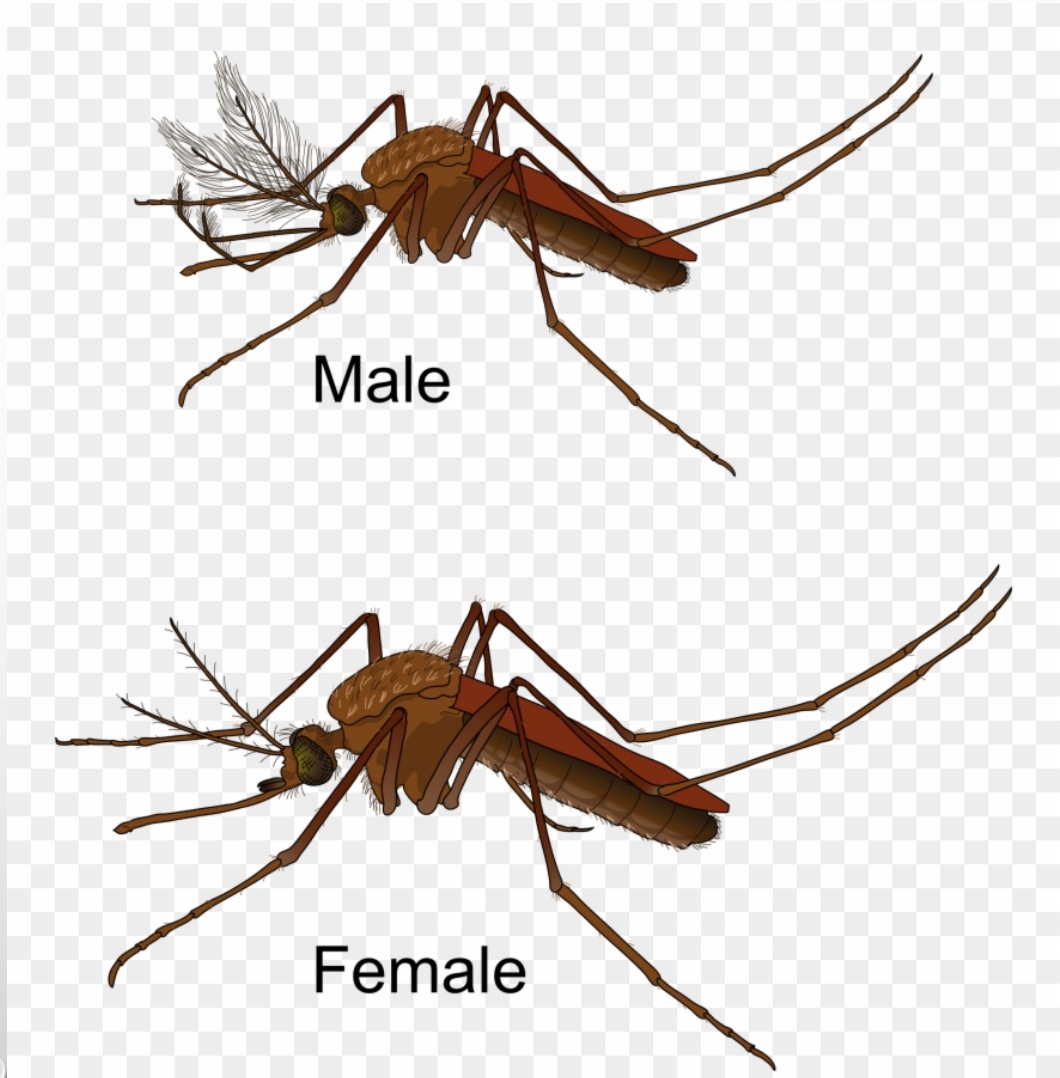
From Nature Reviews – Microbiology (2018)

ENGINEERING *WOLBACHIA* INTO MOSQUITOES

In contrast to many insects, including many mosquito species, *A. aegypti* is not a natural host for *Wolbachia*, and therefore to use *Wolbachia* to modify a mosquito population, the bacteria must be introduced into the mosquito through microinjection and a stable colony needs to be established



BREEDING ENGINEERED MOSQUITOES

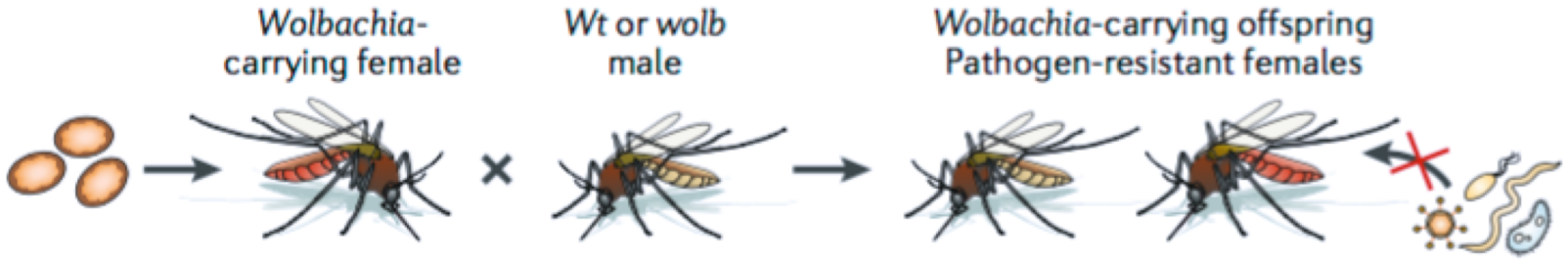


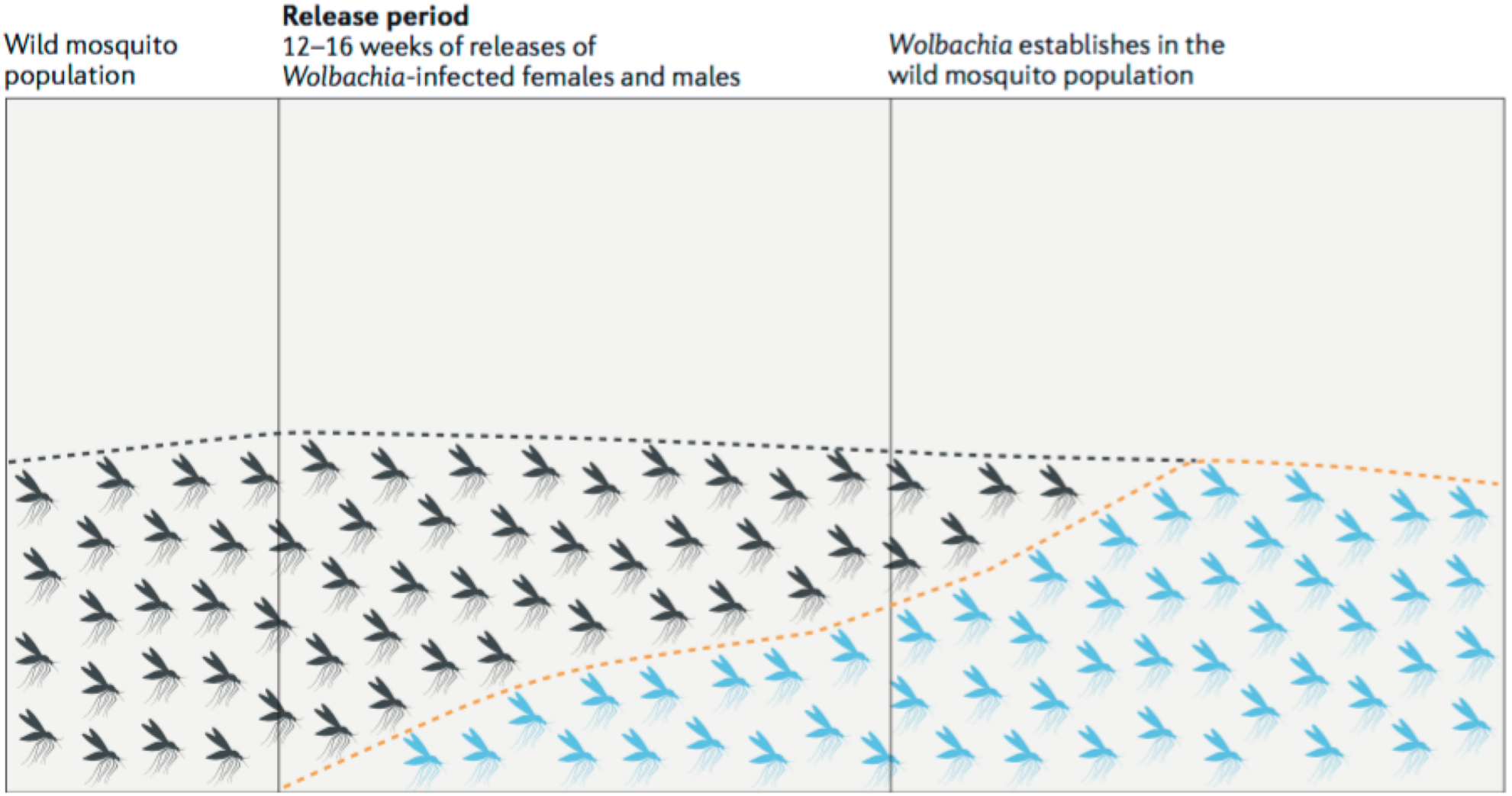
II – MOSQUITO POPULATION MODIFICATION APPROACHES

- RELEASE OF BOTH MALE AND FEMALE MOSQUITOES THAT CARRY A HERITABLE FACTOR THAT REDUCES OR BLOCKS THEIR ABILITY TO TRANSMIT VIRUSES, SUCH AS DENGUE OR ZIKA
- A NUMBER OF DIFFERENT *WOLBACHIA* STRAINS WERE SHOWN TO PREVENT THE TRANSMISSION OF A RANGE OF VIRUSES AND PARASITES IN LABORATORY STUDIES BY PREVENTING PATHOGEN REPLICATION WITHIN THE INSECT
- BY RELEASING BOTH MALE AND FEMALE MOSQUITOES THAT ARE INFECTED WITH *WOLBACHIA* INTO A WILD POPULATION, IT SHOULD BE POSSIBLE FOR *WOLBACHIA* TO INVADE THAT POPULATION
- THE *WOLBACHIA*-INFECTED FEMALES WOULD THEN HAVE GREATLY REDUCED ABILITY TO TRANSMIT A VIRUS TO HUMANS, AND DISEASE SHOULD DECLINE

b Modification of mosquito population

Pathogen blocking





ENGINEERING *WOLBACHIA*-INFECTED MOSQUITOES

- AS OF NOVEMBER 2019, EIGHT DIFFERENT *WOLBACHIA* STRAINS HAVE BEEN TRANSINFECTED INTO *A. AEGYPTI*

- IT WAS SHOWN THAT *WOLBACHIA* CAN LIMIT THE TRANSMISSION OF A RANGE OF HUMAN PATHOGENS BY *A. AEGYPTI*, INCLUDING DENGUE, ZIKA AND CHIKUNGUNYA VIRUSES, WHICH SUGGESTS THAT THIS INTERVENTION SIMULTANEOUSLY TARGETS MULTIPLE DISEASES

FIELD TRIALS

- AS OF NOVEMBER 2019:
  - WOLBACHIA-INFECTED MOSQUITOES HAVE BEEN DEPLOYED IN 10 COUNTRIES, IN AREAS WHERE 4.4 MILLION PEOPLE LIVE
- BETWEEN AUGUST 2016 AND MARCH 2017, WORLD MOSQUITO PROGRAM RESEARCHERS RELEASED LAB-GROWN MOSQUITOS CARRYING *WOLBACHIA* IN INDONESIA, AMONG A POPULATION OF ABOUT 65,000 PEOPLE AND IN TWO AREAS NEAR RIO DE JANEIRO, BRAZIL, POPULATION 100,000 PEOPLE
- RESULTS: 76% REDUCTION IN DENGUE TRANSMISSION AMONG THE INDONESIAN STUDY POPULATION, AND A MORE THAN 70% REDUCTION AMONG THE BRAZILIAN POPULATION IN 2018 TO 2019. IN BRAZIL, THE RESEARCHERS ALSO NOTED A MORE THAN 70% REDUCTION IN CHIKUNGUNYA
- BETWEEN 2011 AND 2017, WORLD MOSQUITO PROGRAM RESEARCHERS RELEASED *WOLBACHIA*-INFECTED MOSQUITOES IN NORTH QUEENSLAND, AUSTRALIA. ACCORDING TO THE RESEARCHERS, THE EFFECT WAS "SUSTAINED AT A HIGH LEVEL 8 YEARS POST RELEASE." IN SEPTEMBER 2019, THE REDUCTION IN DENGUE CASES WAS 96%

IN THE US

- IN 2017, THE EPA APPROVED THE USE OF *WOLBACHIA* FOR MOSQUITO CONTROL
- ZIKA MADE ITS WAY TO THE UNITED STATES IN 2016, WITH THE FIRST CASES OF LOCALLY ACQUIRED ZIKA TRANSMISSION REPORTED IN MIAMI, FLORIDA
- STARTING IN FEBRUARY 2018, THE CITY OF SOUTH MIAMI, MIAMI DADE COUNTY, WITH TWO BIOTECH COMPANIES, BEGAN A PROJECT IN WHICH *WOLBACHIA*-INFECTED *AE. AEGYPTI* MALES WERE RELEASED INTO AN APPROXIMATELY 150-ACRE AREA IN SOUTH MIAMI
- MALES CAUSE CYTOPLASMIC INCOMPATIBILITY (CI) WHEN THEY MATE WITH WILD TYPE *AE. AEGYPTI* FEMALES, WHICH LEADS TO EGG-HATCH FAILURE → THE REPEATED, INUNDATIVE RELEASE OF MALES CAN ACT AS A SPECIES-SPECIFIC, SELF-DELIVERING PESTICIDE
- PEAK WEEKLY RELEASE OF APPROXIMATELY 375,000 MALES
- RESULTS REPORTED IN MAY 2019 COMPARING THE TREATED AND UNTREATED AREAS SHOW A REDUCTION OF *AE. AEGYPTI* FEMALES OF MORE THAN 75 PERCENT

THE CASE OF MALARIA

- CAUSE: *PLASMODIUM* (UNICELLULAR EUKARYOTE)
- SPREAD BY AN INFECTED FEMALE *ANOPHELES* MOSQUITO
- TRYING TO ENGINEER *WOLBACHIA* INTO MOSQUITO EGGS HAS NOT BEEN SUCCESSFUL: THE MICROBE TAKES HOLD BUT IT DOES NOT GET TO THE OVARIES → CANNOT TRANSMIT IT TO PROGENY
- RECENTLY (2018) WILD POPULATIONS OF THE MALARIA MOSQUITO INFECTED WITH *WOLBACHIA* HAVE BEEN FOUND IN TANZANIA, BURKINA-FASO AND MALI
- WOLBACHIA-INFECTED MOSQUITOES ARE AT LEAST TWO TIMES LESS LIKELY TO HARBOR THE MALARIA PARASITE, SUGGESTING A PROTECTIVE EFFECT OF THE ENDOSYMBIONT AGAINST THIS PATHOGEN IN THE MOSQUITO

LESSONS IN EVOLUTION: *WOLBACHIA* AND HORIZONTAL GENE TRANSFER (HGT)

- 20-50 % OF INSECT SPECIES HAVE EVIDENCE OF *WOLBACHIA* GENES **IN** THEIR GENOMES
- MANY ARE SMALL FRAGMENTS OF THE MICROBIAL GENOME
- IN ONE SPECIES OF *DROSOPHILA* THE **ENTIRE** *WOLBACHIA* GENOME WAS TRANSFERRED (THAT'S ABOUT 1,000,000 BASE PAIRS OF DNA)
- THIS IS THE MOST DRAMATIC EXAMPLE OF HGT

ARE THESE MICROBIAL GENOME 'INVADERS' SERVING A PURPOSE? – THE ROOT-NODE NEMATODE

- MICROSCOPIC WORMS THAT ARE PLANT PARASITES
- THEY RUIN CA. 5% OF ALL THE WORLD'S CROPS
- THEY LATCH ONTO THE CELLS OF THE ROOTS AND SUCK OUT THE INNARDS
- BUT PLANT CELLS ARE SURROUNDED BY A TOUGH CELL WALL OF CELLULOSE
- THE WORM HAS CA. 60 GENES THAT JOINTLY DEGRADE CELLULOSE
- BUT NO ANIMAL CODES FOR THOSE GENES (THEY ONLY OCCUR IN IN FUNGI AND BACTERIA)
- THE WORM HAS ACQUIRED THE GENES FROM BACTERIA
- THEY TURN ON THE GENES IN THEIR THROAT GLANDS

MORE EXAMPLES: THE COFFEE BORER BEETLE

- MOST HARMFUL COFFEE PESTS
- MICROBES IN ITS GUT DETOXIFY CAFFEINE
- A BACTERIAL GENE IN THE BEETLE'S GENOME ACQUIRED BY HGT ALLOWS THE LARVA TO DIGEST THE CARBOHYDRATES IN THE COFFEE BEAN (NO OTHER INSECT CAN DO THAT)

THE CITRUS MEALYBUG

- NATIVE TO ASIA
- PEST OF CITRUS TREES, AND ALSO CROP PLANTS, ORNAMENTALS AND WILD FLORA
- CONTAINS AN ENDOSYMBIONT, *TREMBLAYA*, THAT IN TURN CONTAINS ANOTHER ENDOSYMBIONT, *MORANELLA*
- ALL PARTNERS ARE REQUIRED TO SYNTHESIZE SOME AMINO ACIDS
- WITHIN THE MEALYBUG'S GENOME THERE ARE 22 BACTERIAL GENES THAT ARE NEEDED FOR AMINO ACID SYNTHESIS THAT CAN BE TRACED BACK TO THREE OTHER LINEAGES OF BACTERIA THAT ARE NOT THERE ANY LONGER
- THE MEALYBUG CONTAINS (ALL OR PART) OF FIVE BACTERIA: TWO THAT STILL EXIST AS ENDOSYMBIONTS AND THREE MORE THAT IT ONCE HAD, AND HAS NOW VANISHED AFTER TRANSFERING SOME GENES INTO THE MEALYBUG GENOME

THE HOLOBIONT

- RECASTING AN ORGANISM NOT AS A DISTINCT SEPARATE ENTITY BUT AS A COMMUNITY → THE SUM OF THE HOST ORGANISM'S PLUS IT'S SYMBIOTIC MICROBES
- THE HOLOBIONT FUNCTIONS AS A WHOLE: ANATOMICALLY, METABOLICALLY, IMMUNOLOGICALLY, DEVELOPMENTALLY, AND DURING EVOLUTION
- THE HOST GENOME IS HIGHLY CONSERVED, AND GENETIC CHANGES WITHIN IT OCCUR SLOWLY, WHEREAS THE MICROBIOME GENOME IS DYNAMIC AND CAN CHANGE RAPIDLY IN RESPONSE TO THE ENVIRONMENT BY INCREASING OR REDUCING PARTICULAR MICROBES, BY ACQUISITION OF NOVEL MICROBES, BY HORIZONTAL GENE TRANSFER, AND BY MUTATION