Lecture 1

Parasitism

Parasites are organisms which live in or on other organisms (like animals, plants, fungi, bacteria, etc.), drawing sustenance from the host causing it harm. It has been estimated that more than 50% of eukaryotic organisms are parasitic or have at least one parasitic phase during their life cycle.

Parasites are categorised by their common parasitic lifestyle; this may also include those molecules whose function has not been yet defined (like prions).

Parasitology is concerned mostly on protozoa, worms and arthropods behaving as parasites.

Types of interactions:

- **Mutualistic Relationship**, in which two partners benefit from coexistence without losing the ability to live independently (mutualism). Sea anemone and clownfish are an example.
- **Commensalism** describes a feeding relationship, in which one partner benefits without providing any reciprocal benefits nor imposing any cost to the other.
- **Antagonistic Relationship** occurs when a guest organism extracts nutrients from its host (which incurs a cost from the relationship). In this category we find parasites, which can also cause damaging effects such as injury, inflammation, or toxic metabolites production, reducing the evolutionary fitness of the host.
  There are different forms of parasitism:
  - Obligate parasites, having no alternative other than their way of life. It is possible that sex define parasitism in some organisms (like mosquitoes)
  - Facultative parasites are not necessarily dependent by parasitism (Triatoma infestans is an example)

  There are also various kinds of hosts, like Definitive Hosts (either harbour adult parasites or becomes the place where they reproduce), Intermediate hosts (hold larval form) and paratenic host.

Many are the effects that this interaction can result in, depending on the Virulence of the parasite, the susceptibility of the host species and the condition of the individual in the moment of the inoculum. What is also pivotal is the Time factor, defined as how much time passes between the inoculum and the effects on the hosts.

Coevolution

It is a term that describes the evolution of distinct species of organisms which evolve in close association and influence one another, so these organisms evolve together by interacting in their shared ecosystem. Those species that are closely coupled (host/parasite relationship) strongly affect the other individual, and vice versa. However, since the parasite tends to negatively affect the host, the latter tends to develop ways to resist the parasite, and so the more resilient individuals of the same species become to a given parasite, the more they will affect positively their fitness. But this is also true for the parasite, since trying to resist against new defence pathways of the host can lead them to new strategies to increase their survivability, for example by developing new evasion mechanisms. We should also consider that both host and parasite are subjected to evolutionary pressure through intraspecific competitors competing for the same ecological niche and sexual partners.

Lecture 2/3

Vector-Borne Diseases

VBD are human illnesses caused by parasites, viruses and bacteria that are transmitted by mosquitoes, sandflies, triatomine bugs and many other insects. The major vector-borne diseases, together, account for 17% of all infectious diseases. The burden of these diseases is highest in tropical and subtropical areas and they disproportionately affect the poorest populations, since the lack of healthcare, hygiene and food boosts the spreading of these pathologies.

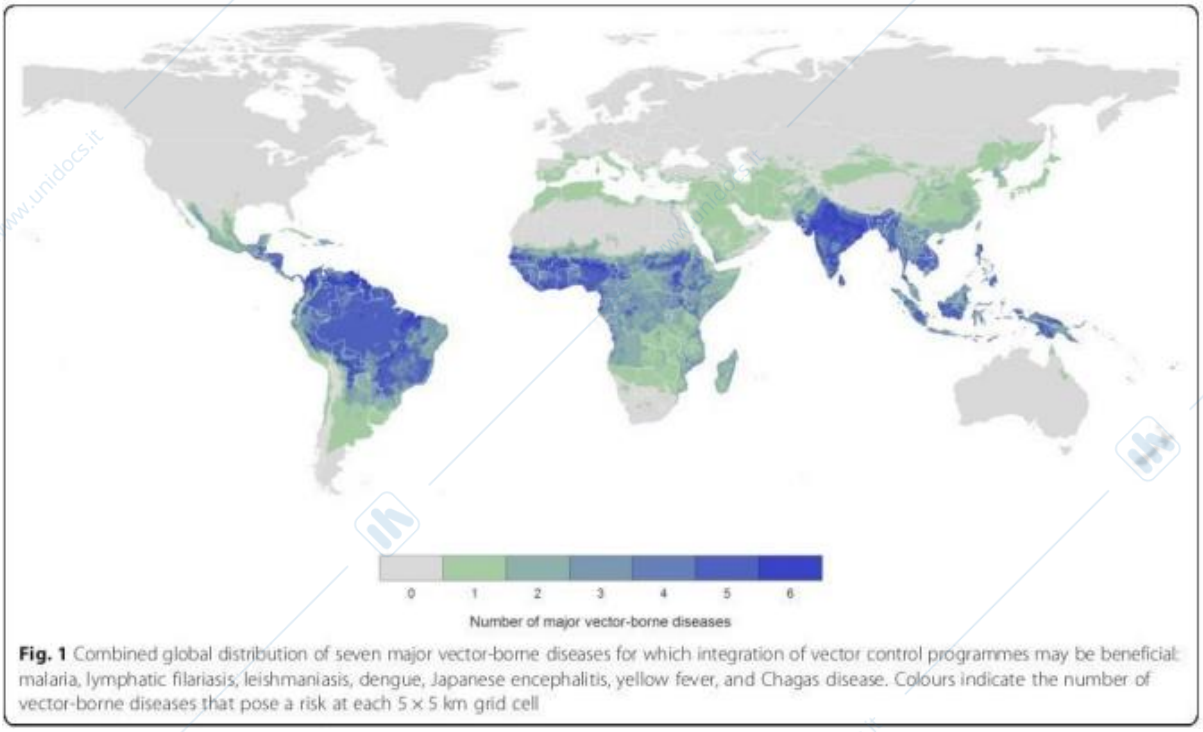
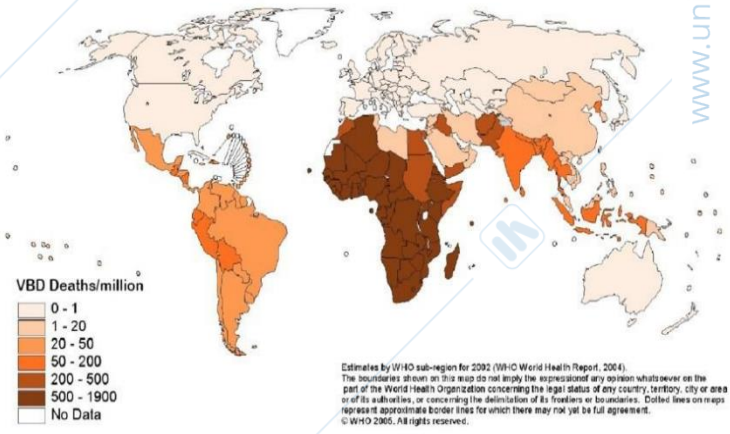


Fig. 1 Combined global distribution of seven major vector-borne diseases for which integration of vector control programmes may be beneficial: malaria, lymphatic filariasis, leishmaniasis, dengue, Japanese encephalitis, yellow fever, and Chagas disease. Colours indicate the number of vector-borne diseases that pose a risk at each 5 × 5 km grid cell

Distribution of vector-borne diseases is determined by complex demographic, environmental and social factors. These diseases profoundly restrict socioeconomic status and development in countries with the highest rates of infection, many of which are located in the tropics and subtropics.

In addition, global travel and trade, unplanned urbanization, and environmental challenges such as climate change can impact on pathogen transmission, making transmission season longer or more intense or causing diseases to emerge in countries where they were previously unknown.



The rapid expansion of global trade and transportation since 1700 has been associated with the spread of mosquito-borne diseases such as yellow fever and dengue. Today's integrated global

economy has accelerated the introduction of pathogens and their vectors to new hosts and geographic ranges.

Asian tiger mosquitoes were first recorded in Europe in 1979 on the coast of northern Albania in huge piles of used tires and subsequent investigations demonstrated that the species had been also successfully established in many other parts of the country

Ae. albopictus was subsequently detected in Italy (initially in the area of Genoa) in September 1990, due to the international trade of used tires. Now is regarded as one of the most heavily infested European country by Ae. Albopictus. At the end of 19th Century, malaria cases amounted to 2 million with 15,000–20,000 deaths per year. Malignant tertian malaria was present in Central-Southern areas and on the islands. Early in the 20th Century, the most important act of the Italian Parliament was the approval of laws regulating the production and free distribution of quinine and the promotion of measures aiming at the reduction of the larval breeding places of Anopheline vectors. The eradication was done in 4 steps:

1. Larval and adults' management with larvicide and insecticides. Paris green is an inorganic compound. It is a highly toxic emerald-green crystalline powder that has been used as a rodenticide and insecticide, and also as a pigment, despite its toxicity. Paris green was heavily sprayed by airplane in Italy, Sardinia, and Corsica during 1944 and in Italy in 1945 to control malaria.
2. Improved housing and education. Mosquito nets are still considered an essential garrison for the control of malaria in endemic localities.
3. Drainage of the marshes. It was part of a detailed engineering effort to ditch, dike and pump surface waters out of the marshland and into the sea.
4. An improved medical treatment. Free distribution of quinine to workers and settlers in malaria endemic areas to treat fever attacks were launched.

Italy was the first country to develop special legislation for the fight against malaria, representing an example of integration between scientific, political, social, and economic knowledge. → INTEGRATED CONTROL STRATEGIES

Climatic conditions are also significant drivers of VBD as some of the vectors are cold-blooded; thus, climate change can shift the geographical ranges of VBD transmission. Consider that socially and economically disadvantaged groups suffer disproportionally from infectious diseases in Europe, as well from the rest of the world.

Now, Vectors are living organisms that can transmit infectious diseases between humans or from animals to humans. Many of these vectors are bloodsucking insects, which ingest disease-producing microorganisms during a blood meal from an infected host (human or animal) and later inject it into a new host during their subsequent blood meal. **Mosquitoes are the best known disease vectors**, but also ticks and other bugs are able to carry diseases.

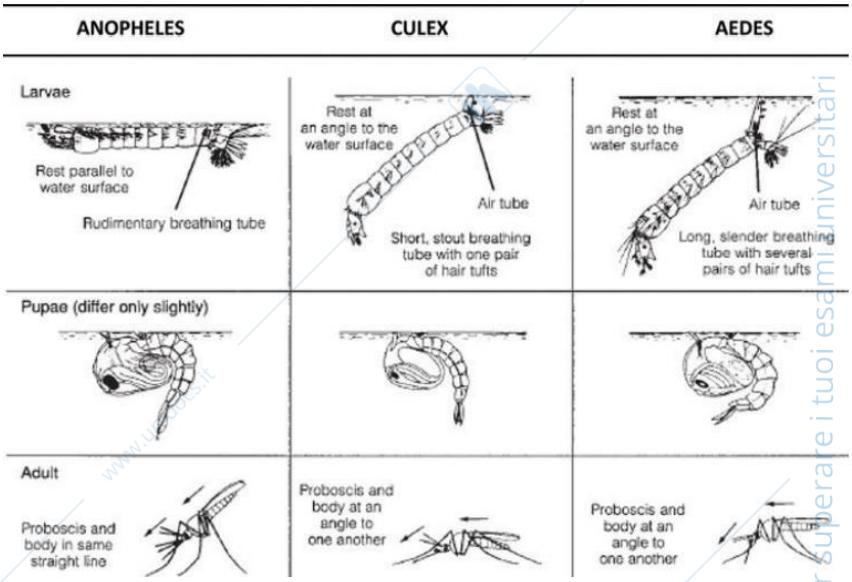
Main protagonists are:

- Aedes mosquitoes (Dengue fever, Zika, Rift Valley fever , Yellow fever, Chikungunya)
- Anopheles mosquitoes (Malaria, Virus)
- Culex mosquitoes (Japanese encephalitis, Lymphatic filariasis, West Nile fever)
- Tsetse flies (Sleeping sickness or African trypanosomiasis)
- Fleas (Plague, transmitted by fleas from rats to humans, Rickettsiosis)

Mosquitoes

In countries with a temperate climate they are more important as nuisance pests than as vectors. Control measures are mostly directed against only one or a few of the most important species and can be aimed at the adults or the larvae. Some species bite in the morning or evening and at night; others feed during the day. Mosquitoes may bite indoors or outdoors.

Mosquitoes have four distinct stages in their life cycle: egg, larva, pupa, and adult. Larvae's habitat could be Running water, transient water, permanent water, or containers.



One of the most important characteristics of **mosquitoes is their ability to carry malaria**, a diseased caused by protozoan parasite. Malaria is widespread in the tropics and also occurs in subtropical and temperate regions and is among the most important causes of death in Africa, especially among children. Malaria parasites enter the human body via the bite of a malaria-carrying mosquito of the genus Anopheles.

Malaria etiological agents

- Plasmodium falciparum: most common and deadly type of malaria infection. Can lead to cerebral malaria
- Plasmodium vivax: most common, it causes relapse if treatment was not completed.
- P. Ovale: occurs mainly in tropical West Africa and rarely in the Western Pacific.
- P. malaria: is found worldwide but has a very patchy distribution

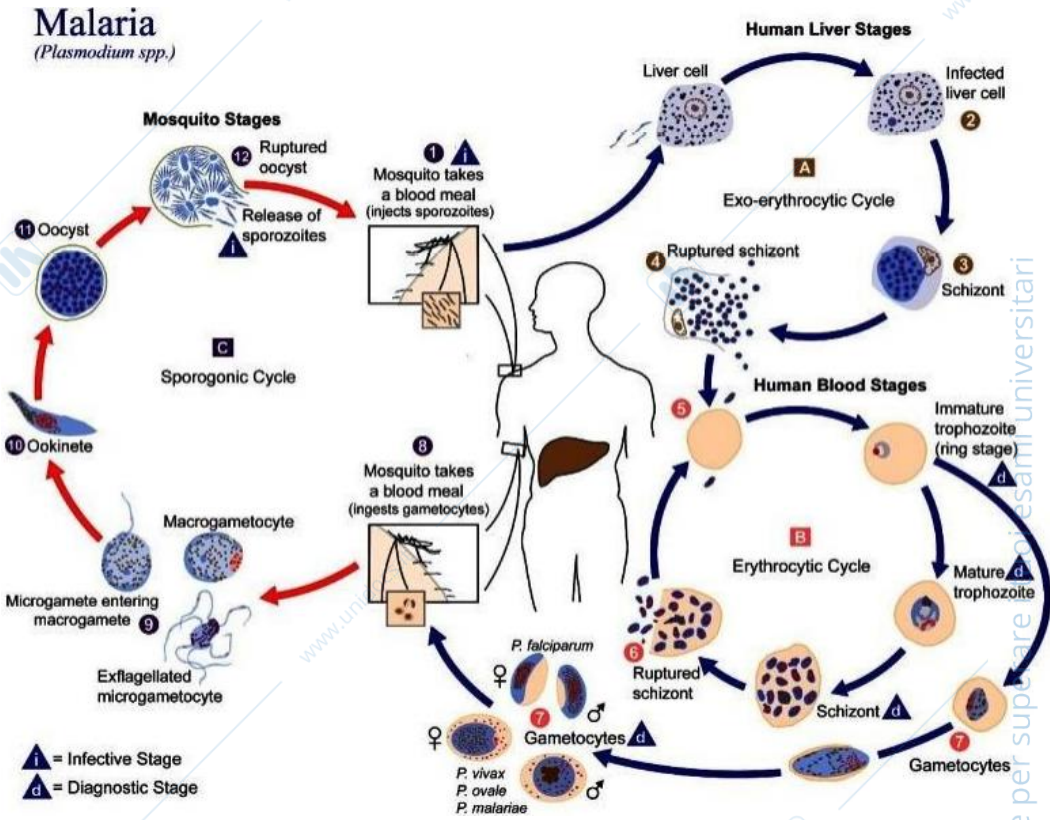
Life Cycle

Plasmodium

The parasites invade the liver via the bloodstream and multiply. During this period, the victim does not feel ill. After about 9 days or longer, depending on the species, the parasites (called merozoites) enter the bloodstream, invade the red blood cells, and again multiply. The blood-stage parasites within a host usually undergo a synchronous schizogony.

A few days after the appearance of the first symptoms some merozoites develop into gametocytes, the sexual stage in the life cycle. Anopheles mosquitoes that feed on a person with gametocytes in the blood become infected and the parasites undergo another phase of reproduction in the insects.

At the end of this process a new generation of malaria parasites, called sporozoites, migrates to the salivary glands of the mosquito where they remain until the insect bites a person and injects the sporozoites together with its saliva into a new human host. The sporozoites then invade the liver and the cycle is repeated**. The pathology and clinical manifestations** associated with malaria **are almost exclusively due to the asexual erythrocytic stage of the parasites.**



Plasmodium infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occurring at either 48 or 72 hour intervals. The severity of the attack depends on the Plasmodium species as well as other circumstances.

In contrast to the other three species, P. falciparum can produce serious disease with mortal consequences. These potentially high parasitaemia are due in part to the substantial number of merozoites produced and the ability of P. falciparum to invade all erythrocytes.

Antimalarial Quinine

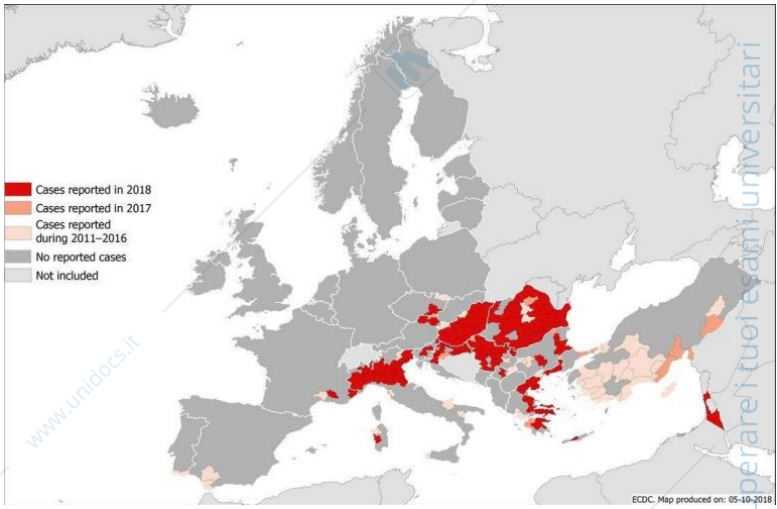
Quinine was first extracted from the bark of the South American cinchona tree and isolated. In 1944, the total synthesis of quinine was achieved by Woodward and Doering. [124 Quinine] exhibits specific toxicity against Plasmodium and has antipyretic (fever reducing) activity. Therefore, it has long been used as an antimalarial drug. Although many other antimalarial drugs such as chloroquine have been developed based on the structure of quinine, it is still widely used since it is the sole compound to which Plasmodium has no resistance.

Nowadays, vaccination proved to be extremely effective against malaria.

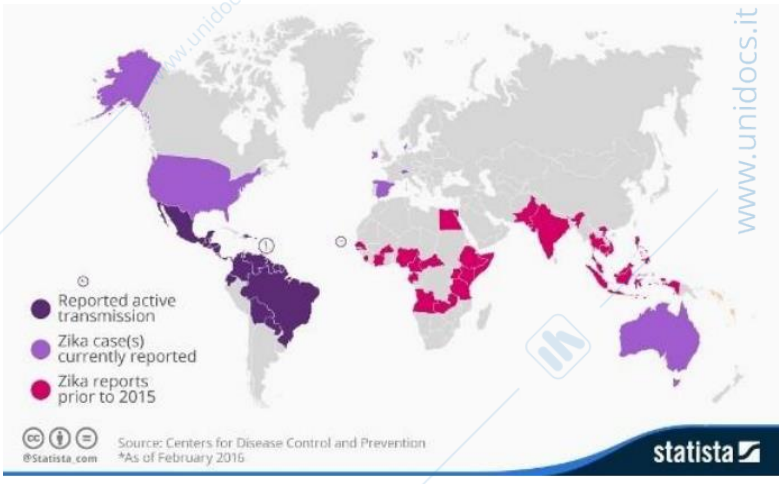
Lecture 5/6

Along with malaria, also West Nile Virus, Zika and Dengue Virus are dangerous diseases spread by mosquitoes:

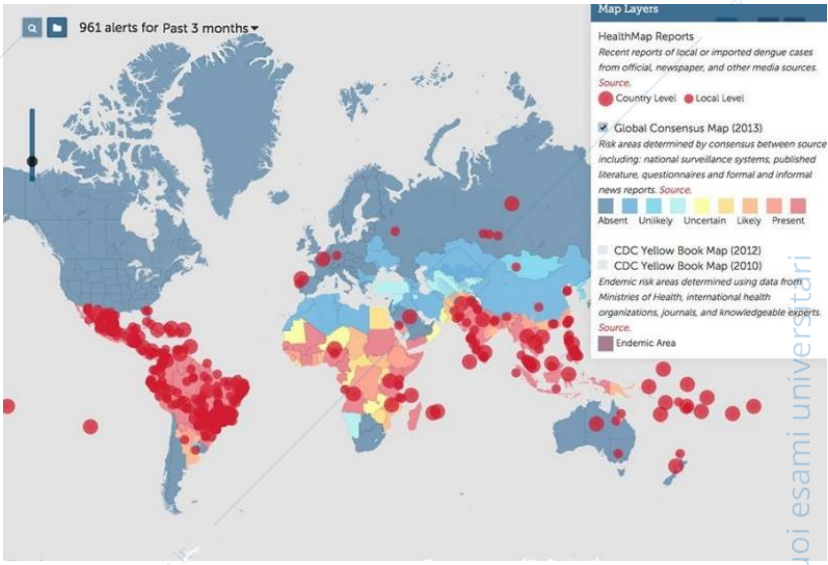
- **WNV** is a virus that can cause encephalitis or meningitis and can be carried by a mosquito after a blood meal on a bird affected by the virus. Mosquitoes of the genus Culex play a key role in WNV circulation in Europe, although WNV has also been detected in mosquitoes belonging to the genera Aedes, Anopheles and Culiseta. Common symptoms include fever, headache, mild rash, and swollen lymph nodes; it is also possible, although in a small percentage, that WNV can cause Permanent neurological damage Encephalitis, possibly leading to coma. Yet, mostly population above 45/50 y.o. are more susceptible to the virus, and a safe behaviour in those regions with a high risk of contagion is the best solution to avoid WNV.



- **Zika Virus** has a very low percentage to arouse severe symptoms as for that patients usually recover by their own. However, Zika may cause birth defects, since infected subjects are likely to pass the virus either by blood or by non-protected sexual contacts: Microcephaly is one birth defect linked to Zika Causes a smaller than normal head compared to infants of the same age It is associated with developmental and functional delays. Other problems have been detected among foetuses and infants infected with Zika virus before birth, such as eye defects, hearing loss, and impaired growth. Its vectors are the Aedes Aegypti, an aggressive mosquito that bites mostly during the day and it's more likely to be found in tropical areas; the Aedes Albopictus is another common vector. While travelling it is important to use repellents containing DEET or picaridin, protect as much as possible the skin to avoid bites and be careful of standing water areas.



- **Dengue Virus** is caused by several closely related viruses, called **dengue types 1, 2, 3 and 4**. The disease is transmitted from person to person mainly by Aedes aegypti, but Aedes albopictus can also act as a vector. Severe dengue (Dengue Haemorrhagic Fever) was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. The



incidence of dengue has grown dramatically around the world in recent decades. The actual numbers of dengue cases are underreported and many cases are misclassified. In August 2020, during the coronavirus disease (COVID-19) pandemic, five locally acquired cases of dengue virus type 1 were detected in a family cluster in Vicenza Province, North-East Italy, areas in which Ae. Albopictus mosquitoes are endemic. Dengue should be suspected when a high fever (40°C/104°F) is accompanied by 2 of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito. Severe dengue is a potentially deadly complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs occur 3–7 days after the first symptoms in conjunction with a decrease in temperature (below 38°C/100°F) and include: severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness, and blood in vomit. The next 24–48 hours of the critical stage can be lethal; proper medical care is needed to avoid complications and risk of death and proper maintenance of the patient's body fluid volume is critical to severe dengue care. The main method to control or prevent the transmission of the virus transmitted by mosquitoes is to combat vector mosquitoes, one technique being the Vector Control approach with Wolbachia Bacteria.

Wolbachia

Wolbachia are found in up to 70% of insect species and in many terrestrial arthropods and are vertically transmitted with the egg from an infected female to her progeny. In order to enhance their spreading success throughout host populations, Wolbachia can manipulate host reproductive biology. With the discovery of this mechanism, the Symbiotic Control approach seems to be particularly promising. It can be divided in three main methods:

1. The disruption of microbial symbionts required by insect pests
2. The manipulation of symbionts that can express anti-pathogen molecules within the host
3. The introduction of endogenous microbes that affect life-span and vector capacity of the new hosts in insect populations.

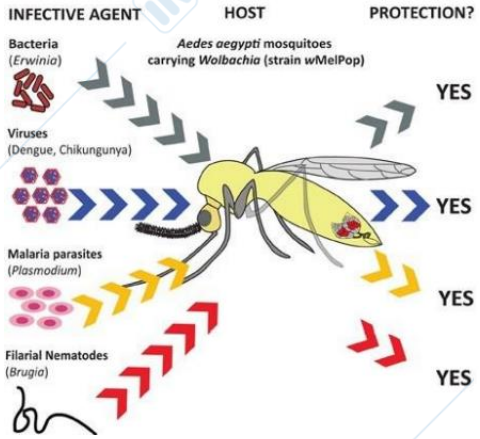
As for Wolbachia, it causes a particular phenomenon called Cytoplasmic Incompatibility, which results in sperm and eggs being unable to form viable offspring. The effect arises from changes in the gamete cells caused by intracellular parasites like Wolbachia, which infect a wide range of insect species. If we consider an insect population in which a percentage of individuals is infected with Wolbachia that induces CI:

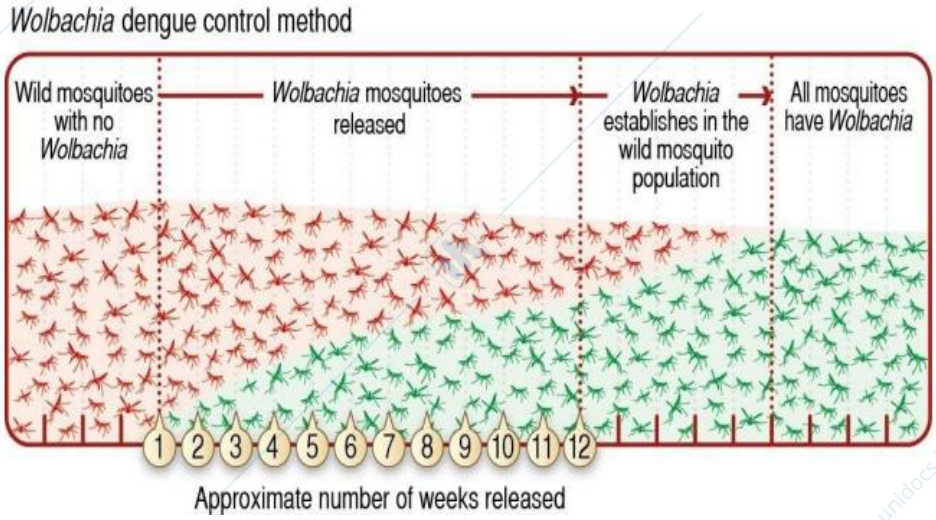
- Infected females can successfully mate with both uninfected males and infected males
- Uninfected females can successfully mate only with uninfected males.

Thus, Wolbachia gives a reproductive advantage to infected females! (or if we prefer, a disadvantage for uninfected females!) Since infected females have a higher physical form, and since Wolbachia is transmitted by them, Wolbachia is more easily spread in the host population.

The popcorn strain of Wolbachia (wMelPop), discovered in Drosophila Melanogaster, does not cause big problems to the insects during development, but in mature insects it reproduces massively in different tissues, shortening host's lifespan and inducing cytoplasmic incompatibility.

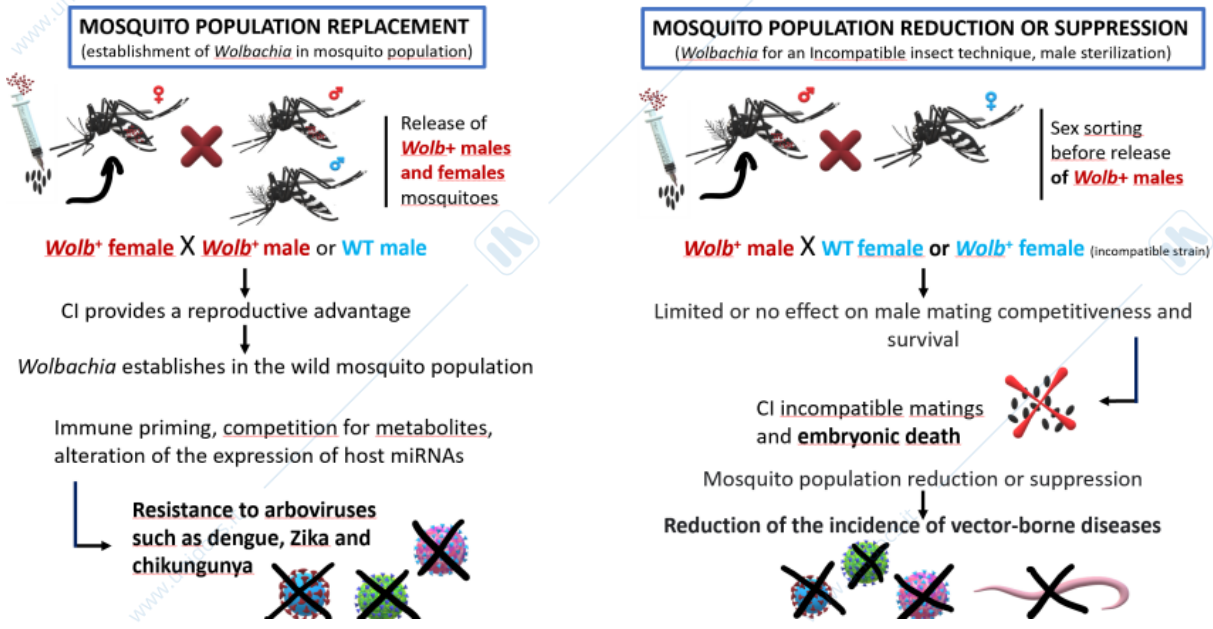
An important issue of Ae. Aegypti is the fact that it doesn't host Wolbachia: this is due to the fact that Wolbachia wMelPop is able to activate the mosquito's immune system. So, even if the population won't lower in numbers, this approach can be used to reduce or remove the bacteria and viruses that hosted by the mosquito. The Influence of Wolbachia symbionts on the inhibition / reduction of transmission capacity, as well as the protection of mosquitoes against infections is a good method to keep under control those diseases affect by wMelPop.





More generally, Wolbachia is not pathogenic and it is not transmitted to man, spreads quickly through CI, the Wolbachia+ mosquito IS NOT CONSIDERED A MOSQUITO GENETICALLY MODIFIED (TRANSGENIC), the Wolbachia+ strain is protected from pathogens.

Aedes aegypti MOSQUITOES



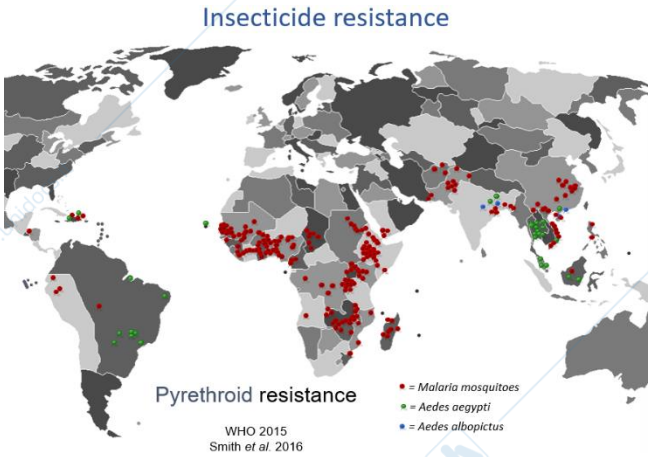
Lecture 7/8

Insect control with Genes and Transgenes

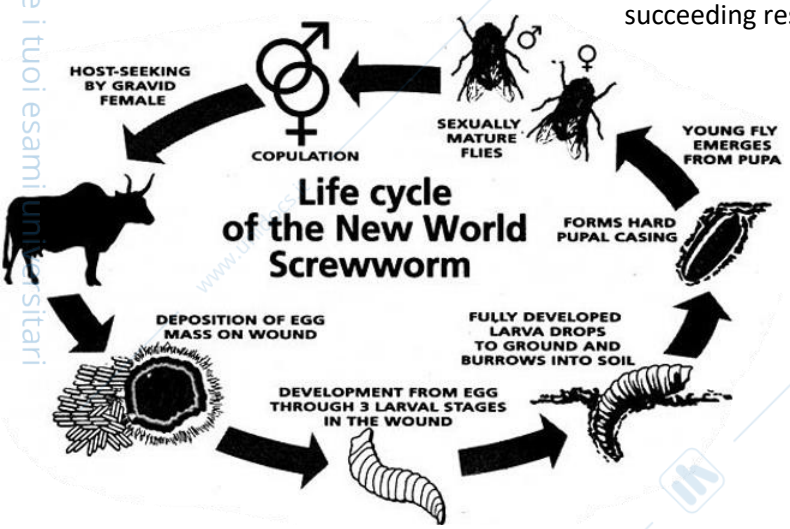
Importance in studying insects resides in the necessity to find bio-acceptable solutions to those issues caused by insects on crops, without decimating the population of those insects which are fundamental for the overall biosystem (like bees).

As the 'Fastest' solution could be pointed towards bio-compatible insecticides, it is also extremely economically demanding to produce such molecules in mass (considering the amount of work and research required to even find the mentioned molecule).

Moreover, without appropriate studies, insecticides will surely have negative effects on nature, as we have already seen (DDT to Neonicotinoids had dire drawbacks on the population of bees, risking their extinction), and the targets of these products have a high probability to adapt and resist to insecticides, which make their use questionable on many levels →→



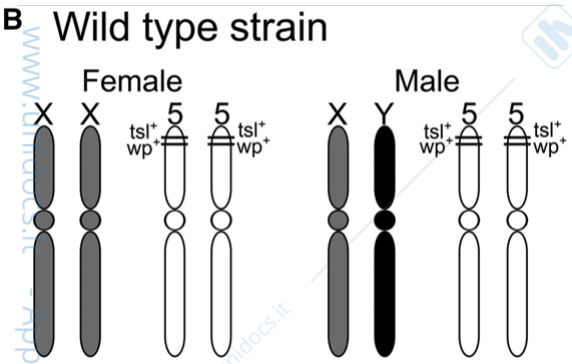
An approach that seemed to be developmentally sustainable is the Sterile Insect Technique (SIT). By understanding which sex is tactically the most favourable to sterilisation (in terms of efforts/results ratio). Une of the first application of SIT is the screwworm fly in USA: the technique procedure was done in Curacao Island (islands are a closed system, so the percentage of a dangerous outbreak on the ecosystem are contained), producing succeeding results in 1953.



Another species examined is the Ceratitis Capitata (or Medfly), which exploits fruits to deposit eggs, which become larvae that grow in the soil, later becoming pupae and adults. In particular, Ceratitis have been a very big issue for fruits production in the Mediterranean, and so SITs studies have been conducted.

Now, when dealing with these insects, we will encounter several issues.

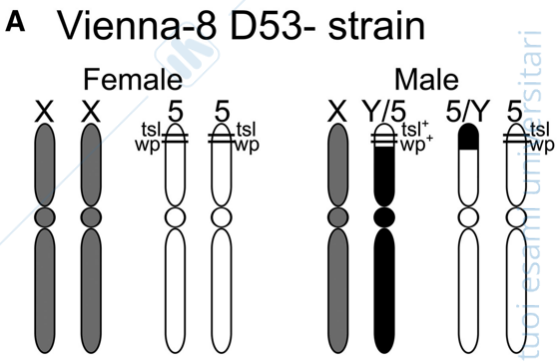
First issue is given by the need to release males only in the SIT experiment. This is done by genetic sexing strains, modifying somehow species genes in order to differentiate between sexes. More in details, by exploiting genetic mutations of those genes sensitive to temperature.



"Normal" flies are not temperature sensitive and have brown pupal case

By translocating a portion of the male Y chromosome with chrm 5, males becomes insesitive to T variations, while females,

upon reaching 34°, change their pupae color into white. Thus is possible to separate the sexes.



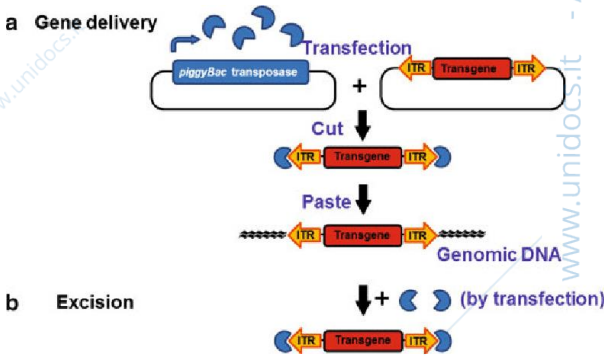
Mutant female flies are temperature sensitive (34°C) and have white pupal case, while males do not feel the change in temperature

As sexes have been differentiated, it is possible to kill the unwanted sex (females)

It is preferred to kill subjects during embryo phase in order to reduce the costs of maintainance of the not desired population.

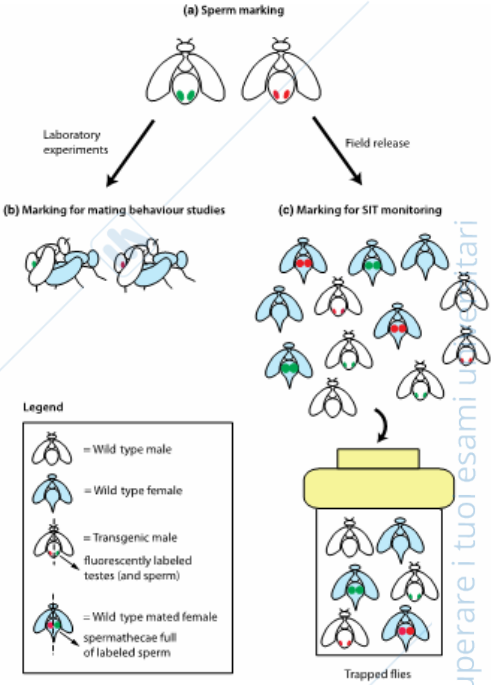
The Second problem is given by recapture step: how do we recognize which males are the ones being transgenetically modified? One solution could be the usage of a fluorescent dust, which unfortunatly doesn't cover for every impeding situation (like rain or males fighting). In general, marking the target with a reliable marker should be sought.

The transgenic activity is controlled by a series of techniques picked based on the target organism: general feature is the use of Transposons, DNA material that is able to move around the genome with a series of processes conduced by Transposase enzyme. It is composed by the Transposon DNA sequence and two sides short sequences that are recognized by the Transposase. After excision, the transposon is moved to another site and is inserterd by the enzyme.



In particular, the **PiggyBac (PB)** transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition**, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the original sites and integrates them into TTAA chromosomal sites**. The powerful activity of the PiggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes. The TTAA-specific transposon piggyBac is rapidly becoming a highly useful transposon for genetic engineering of a wide variety of species, particularly insects.

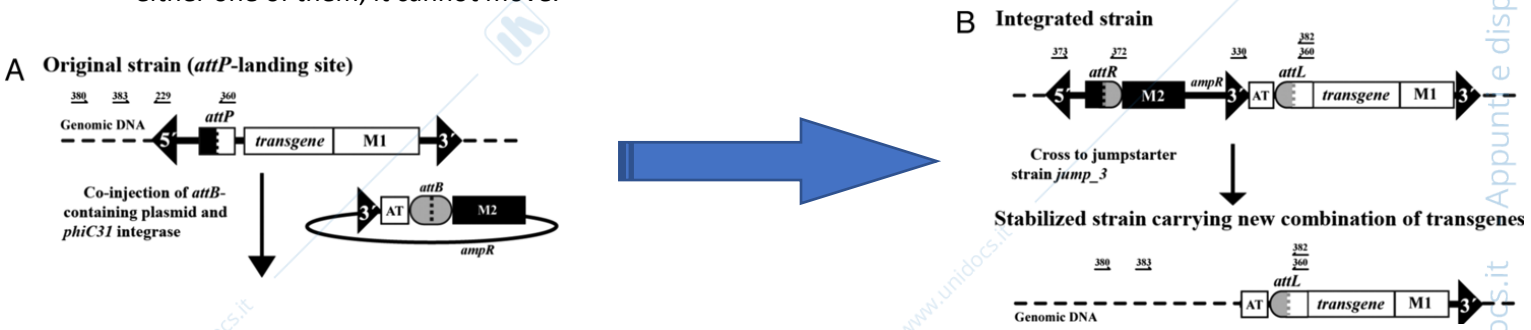
We also need to recognize which individuals have been successfully modified, so it it widely used the GFP cassette, a series of gene markers that produce fluorescent proteins. When transgenic males produce sperm during coupling, colored sperm get into the egg of the female, producing color and making possible to visualise which female has to be checked during parenting.



Another important factor to keep in mind is that transposons are not static, enev if they had already moved once. In fact, they are continuously moving around in the genome, making very difficult to pinpoint their exact location, and to keep them in place. Latter being a crucial component of the SIT procedure, since the movement of novel transposons can have dire consequences along the genome if not carefully positioned.

So how to immobilise a transposon?

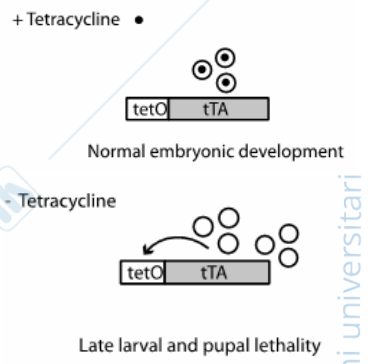
There are few strategies, one of which is commonly used on flies: The insertion of a plasmid carring the complementary flank sequence **Upstream** the transposable DNA seq. In this way, after the first jump, the plasmid is in jumping cassette and acts as the transposon in order to be excised in its place. Keep in mind that a transposon requires BOTH flanking sequences to be recognized as such, and so to jump: without either one of them, it cannot move.



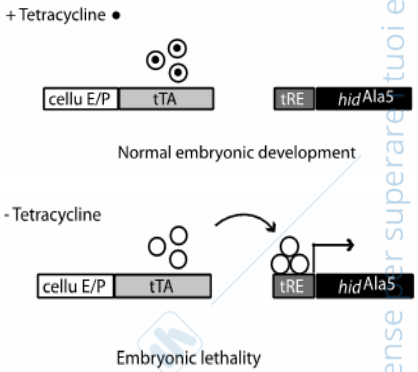
Another important quest is the strategy to use when we want to kill a certain generation of at least part of it. Take into example the situation of mentioned above, the necessity to kill all the females of a filial generation in order to have only males to free. As we said, we may want to kill these individual in their embryonic state, in order to save money, time and resources. But how do we kill embryos?

It is possible to exploit the Lethality system, which has two main mechanisms:

1. One component system. With the presence of Tetracyline, tTA proteins produced during the embryonic development are correctly functioning. With the absence of tetracycline, tTA proteins binds to tetO promoter, repressing it and inducing Late larval/ Pupal lethality



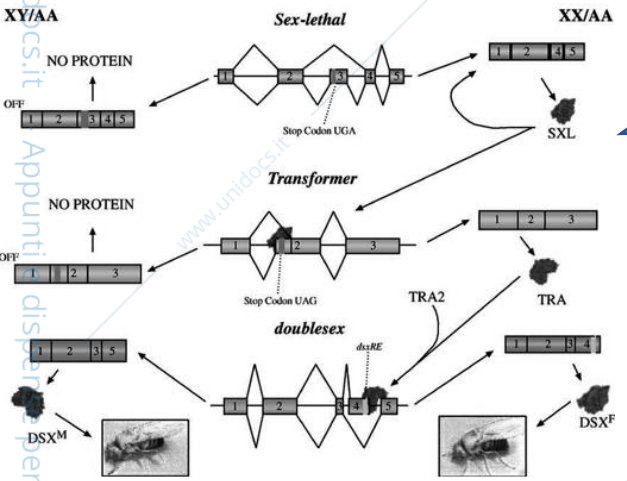
2. Two component system is very similar to One component system, with the difference of the presence of another cassette, tRE-hidAla5, being targeted by tTA proteins and inducing death. This cassette is just a checkpoint to maintain under control death rate of the embryos

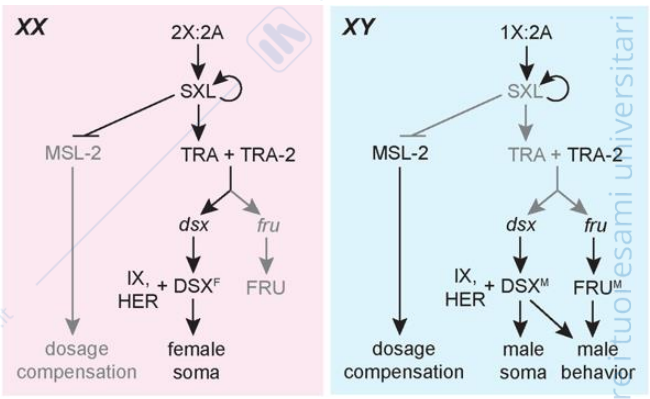


Transgenic mosquitoes are actually used to reduce dangerous populations around the world: **Friendl Mosquitoes** are male *Aedes aegypti* mosquitoes that carry a "self-limiting" gene. When this Mosquitoes mate with wild females, their offspring inherit a copy of this gene, that prevents them surviving to adulthood, and a fluorescent marker gene that produces a protein throughout the body of the insects, which glows when you shine a special light on it. Since the offspring do not mature to reproduce, there is a reduction in the wild pest population. They **are being used to effectively fight *Aedes aegypti*** in projects in Brazil, Panama and the Cayman Islands, suppressing wild populations by **more than 80% relative to an untreated area** – a level of control greater than that typically achieved with insecticides.

Sex determination and transgenic strategies

Drosophila sex determination pattern is shown: it works by alternative splicing of splicing factors and transcription factors





Now, the sex is established molecularly during the syncytial stage of the embryos, before cellularization, thus ee can use this knowledge to develop new transgenic tools to kill prematurely the desired portion of the population, or just to produce mutant strains lacking main characteristics of species.

One is the usage of Tetracycline system to differentiate between sexes, by inserting into the abovementioned determination patterns a signal to activate (inactivate) the tetracycline system.

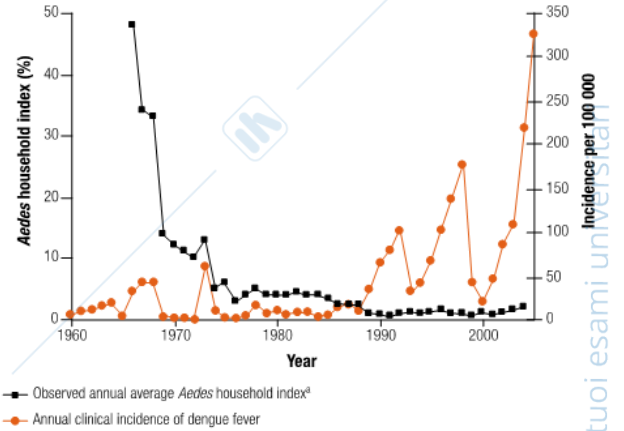
The other mechanism is a little different, by exploiting some alternative splicing patterns, instead of killing embryos, can force the undevelopment of those key traits of the species, like for example the grow of healthy wings: while males, which do not require blood meals, develop fully functional wings, the transgenic females produce proteins inside veins, nervs and muscles controlling the movement of wings, thus making them incapable of flight (which is pivotal for the fitness). Thus, females are used fro mating with males, reducing the number of individuals capable to reproduce (no flight → no blood meal → no larvae).

These are not the only pathways exploitable for the job, but in order to proceed it is important to review the ability of the Zinc Fingers nucleases, TALEN and in general homing nucleases: these protein complexes are able to induce double stranded cuts on the DNA, able to select with a certain degree of specificity which sequence to cut.

This is at the base of what is called X-Shredding, a genetic sex ratio distorter (systems aimed at effecting a bias in the reproductive sex ratio of a population) that interferes with the transmission of the X-chromosome by inducing multiple DNA double-strand breaks during male meiosis. As powerful as it looks, it wasn't as accurate as with the discovery of the CRISPR-CAS9 complex: it is more accurate compared to the other techinques, exploiting a peculiar DNA molecule, called gDNA or guide DNA, that is the complementar strand of the target sequence to cut. Thus, it is less prone to error-induced mutations. This led to the implementation of the X-shredding strategy to a newer, more accurate version of it, succeding the aims set.

And yet, even if the numbers of Aedes individuals is greatly reduced (ranging around 80% less of the total household populations of the targeted regions), it has been observed that an increase in dengue fever cases in exact coincidence with the lowest number of Aedes individuals.

Fig. 1. **Observed annual average *Aedes* household index and annual clinical incidence of dengue fever**



This peculiar event hasn't been justified yet, as the possible reasons related to it can be multiple and interconnected. In my considerations, it is possible that the Aedes species, which carry malaria virus, due to the reduced numbers of individuals, is not injecting one of the most dangerous viruses, which with a high degree of probability competes with others viruses for their establishment inside the host. No more main competitor in the body could result in an higher degree of freedom for those diseases that were less strong in respect to it.

Lecture 9

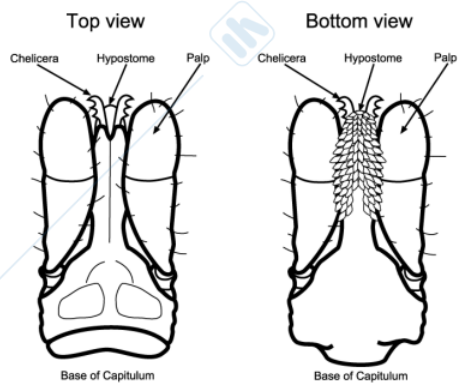
Ticks

These parasites are very dangerous and resilient if placed in the correct environment, as their ability to feed undetected is incredibly useful for parasites of this range.

There are two main categories of ticks:

1. Ixodidae Hard Ticks, characterised by a hard dorsal plate and a sectorized life cycle (Eggs, larvae, nymph, and adults). Can range between 1 to 3 hosts per life cycle (mostly mammals), and females require a very large blood meal depositing 5ks eggs in only one time in their lifetime of 1-2 years. This species follow seasonal activity patterns.
   Humid and dry environments, preferred cities and small towns in general.
2. Argasidae Soft Ticks have a leathery folded cuticle, can have more than 2 nymphal stages, have multi-host life cycle (at least 3, mainly bats and birds), female require repeated small blood meals and can produces several small batches of 500 ca eggs. Do not follow seasons. Their environment is predominantly dry, but can also occur in caves (consider bats as feeding hosts)

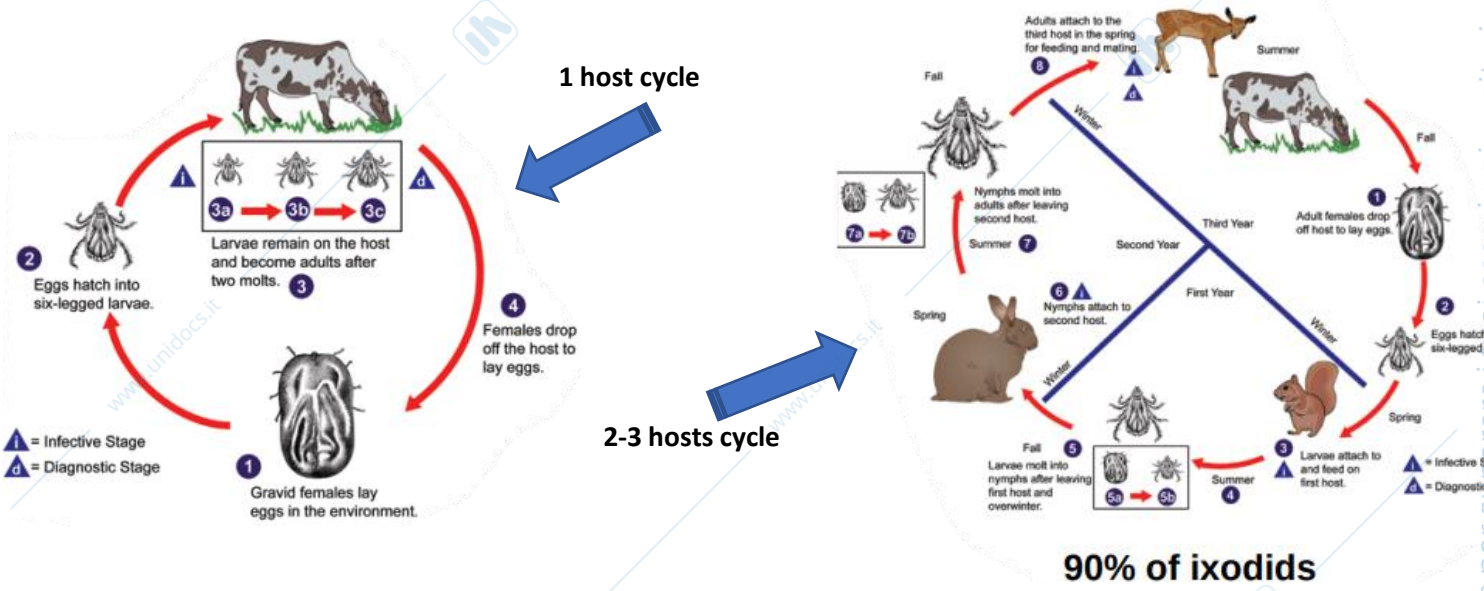
Their feeding process requires blood during some or all life stages, defining them as obligate ectoparasites. It starts with the contact with the host, which is followed by its recognition and the opening of the skin in order to access the inner part of the epithelial tissue. Then it inserts hypostome, producing cement like substance in order to attach strongly to the host (for ixodidis), later starting the Salivate/Feeding cycle. When satisfied, it detaches and drops.



The saliva contains anticoagulants, as well as antihemostatics and vasodilators. It can also block host local immune system response (like inflammation etc).

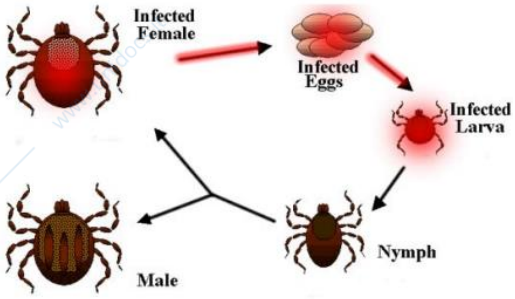
Hard ticks (ixodids) feed gradually (2 days larvae, 13 day females) and can create new cuticle to expand abdomen, while Soft ticks (argasidis) have small meals lasting around 35 to 70 minutes.

The ixodid life cycles with 1, 2, 3 hosts

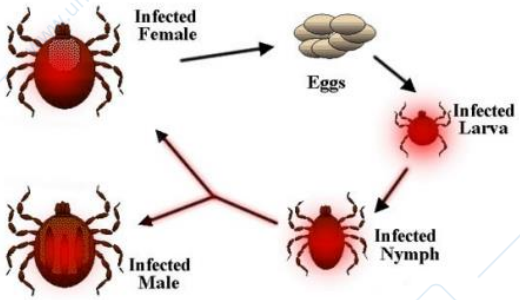


Now, a good portion of Ixodidae and Argasidae is very strict regarding host specificity, "hunting" the host through odours, like CO_2 production, ammonia, and other wastes (butyric and lactic acids), sometimes visual images and vibrations.

These abilities, as well as their feeding processes, the high reproductive potential, the sclerotised nature of their bodies (which protects them from environmental stress), and the low numbers of natural enemies, make ticks impressively good vectors for diseases



Transovarial transmission



Transstadial transmission

A good example is given by Ixodes Ricinus, belonging to the hard ticks, that has a wide geographical distribution, from EU to Portugal to Russia and so on, entailing the high resilient capabilities of the species. Ricinus is sensitive to climatic conditions, requiring a relative humidity of at least 80% and are restricted to areas of moderate to high rainfall with good vegetation. Ixodes Ricinus is therefore primarily observed across Europe in deciduous woodland and mixed forest but can be found in a range of habitats that support its blood hosts and a moist microclimate.

There are many ways to control and prevent ticks' attacks:

- Use of chemical repellents, like DEET (N,N-diethyl-3-methylbenzamide) which has high efficacy, broad target spectrum (it is active not only against ticks). Applications on the skin or clothes for repellent action (Effectiveness depends on the concentration of use)
  Moreover, tools have been developed for the targeted administration of the repellent on deer, against adult ticks, and on rodents against immature ticks (Four-poster feeder, Bait box etc.)
- Biopesticides that involves the use of species that feed on harmful organisms while being non-toxic to humans or non-target species. It can also exploit the natural enemies of ticks: birds, parasitoid wasps, Bacillus thuringensis and fungi (Beauveria bassiana).
  E.g. Ixodiphagus hookeri, a parasitoid wasp specialized in parasitizing ixodid ticks. After laying, the wasp eggs hatch and the larvae begin to feed on the ticks in the form of nymphs, leading them to death before they pass into the adult form.
  So, Biopesticides are among the biological control strategies.
  There are 20 species of fungi associated with ticks.
  Entomopathogenic fungi are good candidates for biological control of ticks as they are capable of perforating the integument of ticks: in particular, the Beauveria bassiana and Metarhizium anisopliae are promising for the control of Rhipicephalus microplus and other ticks

Figure 3 Mycelium and conidiophores of *Beauveria bassiana* on an engorged larva of *Rhipicephalus sanguineus* s.l. at 20 days post infection.

- Hosts' control

Lecture 10

Integrated control of VBD

An approach based on actions to control vector populations, to reduce prevalence/abundance of a pathogen into the host and to reduce vectorial capabilities of the arthropod.

Symbiotic control implies the use of symbiotic organisms to provide anti-disease strategies and includes the use of symbionts to control pest populations. It involves a treatments targeted on mutualistic endosymbionts by exploiting symbionts that manipulates the reproduction of the host.

Paratransgenesis is the use of symbionts engineered to produced anti-pathogen molecules as a strategy to re-colonize vector populations. It exploits competition between microorganisms for survivability of the needed one. Paratransgenesis is a "population replacement" strategy for interfering with pathogen development via genetic modification of symbiotic microbes to produce antipathogen effector molecules in the host. There are several basic requirements for the implementation of the paratransgenesis strategy:

- The symbiotic bacterium should preferably originate from the disease-transmitting vector and have a stable symbiotic relationship with the vector
- The symbiotic bacterium can be cultured
- The symbiotic bacterium can be genetically manipulated

- The effector gene product should not impair symbiont and vector fitness
- The effector gene product should be secreted to assure interaction with the target.

It is an approach to interfere with pathogen development and differs from the genetic modification of the insect itself. The principle of the approach is to engineer an insect symbiont to secrete antipathogen molecules and introduce the engineered symbiont into the vector: it has a number of attractive features and could help overcome some of the limitations of transgenesis. For example, considering that mosquitoes carry a diverse microbiota in their midgut, the site where the parasite development occurs, making this compartment a prime target for intervention. Plus, the gut bacterial number increases dramatically, by hundreds of fold, after ingestion of a blood meal, correspondingly increasing the output of effector molecules produced by recombinant bacteria: thus transgenes are much easier to spread into mosquito populations via engineered bacteria than via transgenic mosquitoes. Finally, genetic manipulation of bacteria is much simpler and faster than genetic manipulation of mosquitoes.

Effector Molecules

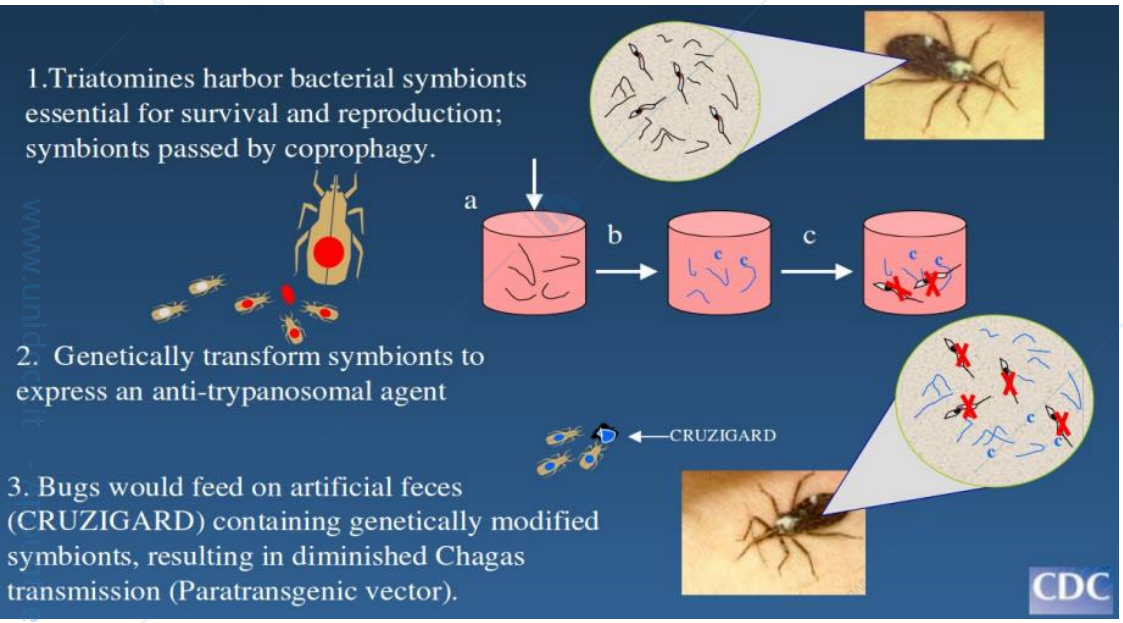
These molecules can be grouped into three classes based on their modes of action against parasites:

1. Parasite killing including host antibacterial peptides such as defensins, gambicin and cecropins, and peptides from other sources that lyse parasites, such as scorpine
2. Interaction with parasites, like EPIP, a Plasmodium Enolase–Plasminogen Interaction Peptide, is a peptide that inhibits mosquito midgut invasion by preventing plasminogen binding to the ookinete surface
3. Manipulation of mosquito immune system. The mosquito's innate immune system plays an important role in inhibiting the Plasmodium parasite development in the mosquito. Thus, boosting mosquito immune-related genes can lead to reduced mosquito vectorial competence

Example: the parasitic protozoan Trypanosoma cruzi, the causative agent of Chagas disease, is transmitted by the triatomid bug Rhodnius prolixus. The Rhodnius obligate Gram-positive bacterium Rhodococcus rhodnii was genetically engineered to produce the antimicrobial peptide cecropin A and fed to naïve R. prolixus nymphs. The expression of the anti-parasite peptide by the genetically modified symbionts significantly reduces T. cruzi's ability to survive in the bug.

If untreated, infection is lifelong and can be life threatening. The impact of Chagas disease is not limited to the rural areas in Latin America: large-scale population movements from rural to urban areas of Latin America and to other regions of the world have increased the geographic distribution and changed the epidemiology of Chagas disease.

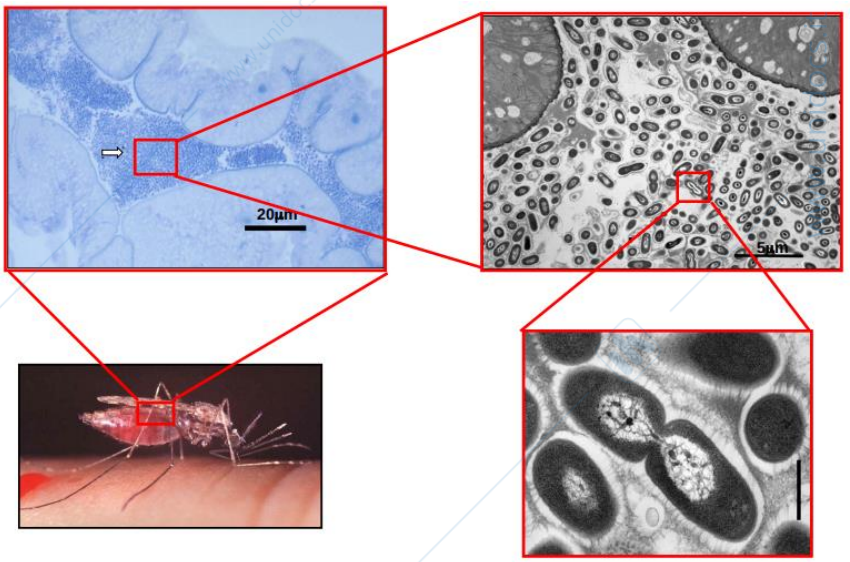
Vector-Symbiont intervention



CRUZIGARD: consisting of an inert guar gum matrix dyed with India ink. The CRUZIGARD preparation was mixed with genetically modified R. rhodnii and used to impregnate cages constructed of thatch and adobe building materials from Chagas-endemic regions of Guatemala

Field caught adult R. prolixus were placed in the cages and removed after eggs were laid. Nymphs were allowed to mature in the CRUZIGARD-treated cages. Nine months later, genetically altered R. rhodnii were detected in approximately 50% of F1 adults and comprised nearly 95% of total CFUs in these bugs, demonstrating that CRUZIGARD may be useful as a gene dispersal strategy even in environments where competing microbes are present. To increase the volume and duration of CRUZIGARD ingestion, and consequently increase rates of vector inoculation with transformed symbiont, on-going collaborations to develop triatomine attractants and semiochemicals to supplement the current CRUZIGARD formulation are underway.

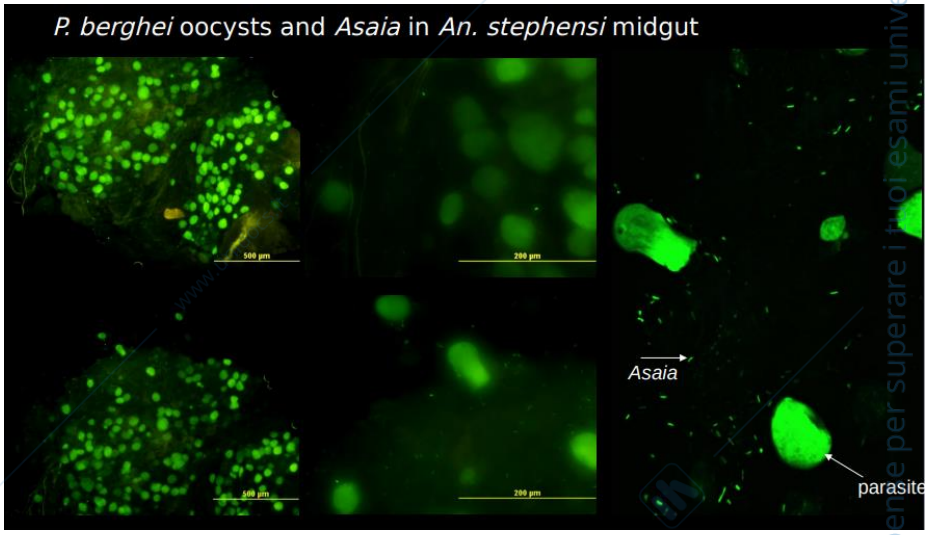
Example: Paratransgenesis for the control of malaria transmitted by Anopheles stephensi mosquitoes



Asaia is an acetic acid α-proteobacterium, rod shaped and proteobacterium, rod shaped and peritrichously flagellated bacteria. Does not (or weakly) oxidize ethanol to acetic acid while produces acid from D-proteobacterium, rod shaped and glucose, D-proteobacterium, rod shaped and fructose, L-proteobacterium, rod shaped and sorbose, dulcitol and glycerol; it is well adapted to live in different kind of sugar-rich environments. Moreover, it has been originally isolated in phytotelmata from tropical flowers. ϖLarvae of Diptera are ubiquitous and conspicuous in phytotelmata; the most common phytotelm dipterans are mosquitoes.

Asaia is a culturable bacterium and it was engineered for the expression of GFP, And it can be inserted into subjects through cages with cotton pad soaked in sugar solution plus transformed bacteria, resulting into fluorescent tissues under the right chemicals. It is possible to see the amount of transgenic Asaia inside mosquitoes' bodies, and in particular the regions more colonized by it. Then, through oral route, it is assessed the horizontal transmission of this bacterium and through mating the vertical transmission, by checking whether larvae are fluorescent or not. It was very important to define the location of proliferation of Asaia in mosquitoes since it had to be competitive with Plasmodium. By recombinant strains of both Asaia and Plasmodium expressing fluorescent protein, they studied the relative localization of the two organisms.

Inside Asaia were inserted plasmids containing genes for lethality competition. In particular SCORPINE (a peptide component of scorpion venom) which kills by lysing parasites Anti-Pbs21 (immunotoxin): a single chain antibody (scFv) against P. berghei ookinete surface protein 21-Shiva 1 fusion protein which lyses ookinetes after binding to them. The combination of these and many other effectors, plus the positioning of Asaia proliferation, defined this strategy as promising.



Plasmid	Effector	Full Size	Effector + PhoA
pNB95	none	110kDa	47kDa
pNB96	Pro-EPIP	113kDa	50kDa
pNB97	scorpine	119kda	55kDa
pNB98	EPIP	114kDa	51kDa
pNB99	PLA2	128kDa	64kDa
pNB101	Prochitinase	112kDa	48kDa
pNB102	Pbs21-Shiva1	142kDa	79kDa

In a similar fashion, another application as Symbiont-based control strategy involved the cooperation of Asaia with Wolbachia.

Consider that in mosquitoes, Wolbachia induces the production of reactive oxygen species (ROS); primes the innate immune system of the mosquito, especially the Toll signalling pathway; induces the production of various antimicrobial effectors. Taking into account that Wolbachia surface protein (WSP) was shown to elicit innate immune responses via TLR2 and TLR4 activation, in both humans and mice, it also stimulates the Innate Immune system in mosquitoes.

Wolbachia Surface Protein (WSP) is an Outer Membrane Protein (OMP) with eight beta-barrel transmembrane structure which participates in host immune response, cell proliferation, pathogenicity and alteration of apoptosis. But how is it related to Asaia?

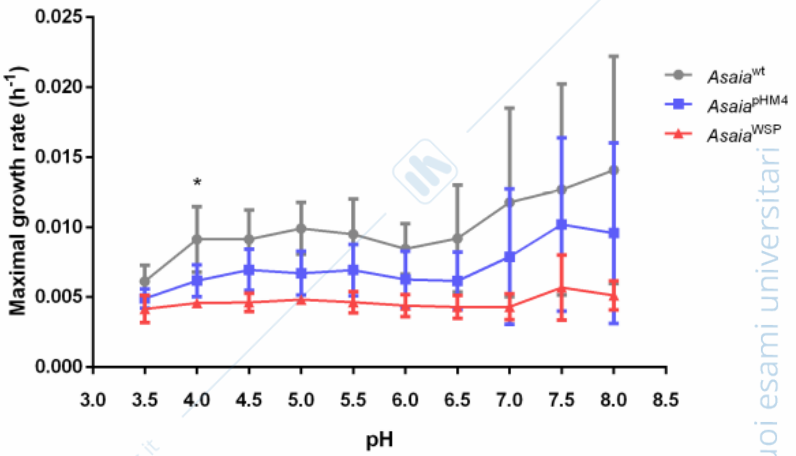
It has been engineered an extracellular symbiotic bacterium of mosquito vectors (Asaia) for the expression of an effector molecule of an intracellular symbiotic bacterium (Wolbachia). Asaia has been transformed for expression of WSP from Wolbachia of Dirofilaria immitis, wDim

Why Asaia? It is an alpha-Proteobacterium, associated with arthropod vectors, cultivable and transformable with exogenous DNA, a non-pathogenic bacterium

The gene coding for the surface protein of Wolbachia has been cloned for D. immitis in two bacteria: E. coli and Asaia.

Particular attention have been given to Asaia, for which it had been made a FITNESS MEASUREMENTS of Asaia^{WSP} and Asaia^{pHM4}, the fitness parameters have been defined for the different strains in culture conditions for different pH.

MGRs were estimated as the slope of the best regression line which fitted to the 24 h growth curves calculated for either of the strains by measuring OD620 at ten different pH values in GLY medium.



Experimental results of Semi-proteobacterium, rod shaped and quantitative analysis of the antimicrobial peptides (AMPs) defensin and cecropin and the nitric oxide synthase (NOS) expression in haemocytes show that Asaia^{WSP} Induces II response in haemocytes primary cultures.

Plus, from Phagocytosis tests, phagocytic activity was evaluated in vitro using cultured Ae. aegypti and An. stephensi haemocytes exposed to bacterial cells from Asaia^{WSP} and Asaia^{pHM4} strains incubated with FITC-proteobacterium, rod shaped and fluorescent beads suspension. Asaia^{WSP} induces phagocytosis in mosquito cells.