mental selection, we were able to select a YFV well adapted to a transmission by *Ae. albopictus* (Amraoui et al. *Sci Rep* 2018). This result should alert about the potential of YFV to initiate an urban cycle in Brazil, like in the past.

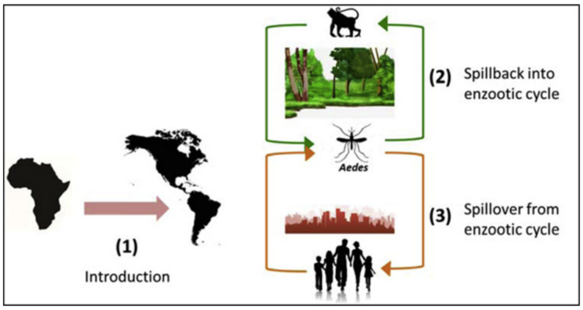

Fig. 1 The three main steps in the emergence of an arbovirus.

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Benefits and limitations of emerging techniques for mosquito vector control

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In response to a broad governmental referral, the French High Council for Biotechnology has published an opinion on the use of genetically modified (GM) mosquitoes for vector control [1]. Emerging techniques of vector control were developed to overcome (i) the lack of therapies, preventive treatments and vaccines for most mosquito-borne diseases, and (ii) the limitations of existing vector control techniques (the situation is particularly critical regarding insecticides: in France, essentially only one insecticide is used against adult mosquitoes (deltamethrin), and its efficacy decreases due to resistance evolution in mosquito populations).

To date, only one GM mosquito-based technique has been developed to an operational level, Oxitec's RIDL technique, which seeks to reduce a mosquito population by repeated mass releases of sterilising transgenic males [2]. Two other techniques under development rely on CRISPR-based gene drive, seeking to spread a genetic trait in a wild population, either to eliminate the population by spreading sterility [3] or to make the target mosquitoes incapable of transmitting pathogens [4].

To identify the specific benefits and limitations of the different GM mosquito-based techniques, a cross-analysis of different vector control techniques was conducted with respect to possible objectives, efficacy and sustainability, technical constraints and risks to health and the environment. Consideration was given to both existing techniques (chemical, biological, physical, and environmental) and emerging techniques based on release of mosquitoes, whether GM (RIDL and the different gene drive techniques) or non-GM–irradiated (standard sterile insect technique (SIT)) or carrying *Wolbachia*[1] (incompatible insect technique (IIT) and spread of pathogen interference (PI) technique).

As specified by the referral, we considered the mosquito-borne diseases and vector species present across France, including overseas territories. The French territories being dispersed across the world, the most notable mosquito-borne diseases worldwide were considered, namely dengue, chikungunya, Zika, yellow fever, West Nile fever, for the viral diseases, and malaria and lymphatic filariasis for the parasitic diseases. We focused on the corresponding local vector species: mainly *Aedes aegypti* and *Aedes albopictus*, and species of Anopheles and Culex.

These vector species have very distinct features, not only in distribution and vector competence, but also in bio-ecology (reproduction modes, potential for survival, host preferences, peaks and sites of aggressiveness, invasive potential...). The vector systems themselves (the triad mosquito/pathogen/vertebrate host), as well as the diversity of situations encountered across the territories add another layer of complexity. This overall complexity must be understood and taken into account in order to design the most appropriate vector control strategy.

Cross-analysis of the different vector control techniques has been conducted in great detail and has made it possible to identify specific features and relative benefits and limitations of each of these techniques. Detailed results are developed in HCB's opinion (HCB, 2017).

At a more general level, we found:

– no divide between GM and non-GM techniques or between emerging and existing techniques (Fig. 1);

– shared characteristics within different sets of techniques, i.e. (i) techniques based on release of mosquitoes, (ii) population reduction techniques[2] vs. population modification techniques[3], (iii) self-limiting techniques[4] vs self-sustaining techniques[5];

– complementarity of the techniques.

Lastly, we found that the benefits and limitations of these vector control techniques cannot be treated in a generic manner, but will depend on the target vector species, the intended objective, and the broader context (epidemiological, environmental and socio-economic context, including available human and financial resources).

Key highlights for each of these broad conclusion points are developed below.

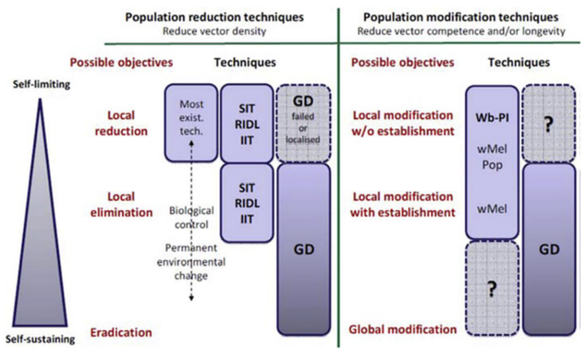

Fig. 1 Possible objectives and sustainability potential of existing and emerging vector control techniques (Insects Grand Conference Talk, C. Golstein and P. Boireau, 14 March 2019). Most exist. tech.: Most existing vector control techniques, including use of chemical insecticides; SIT: standard Sterile Insect Technique (mostly based on irradiated mosquitoes); RIDL: Release of Insects carrying a Dominant Lethal (GM-mosquito based technique); IIT: *Wolbachia*-mediated Incompatible Insect Technique; GD: Gene Drive techniques, for population elimination or modification (GM-mosquito based techniques in this report); Wb-PI: *Wolbachia*-mediated spread of pathogen interference; *w*Mel Pop and *w*Mel: two different strains of *Wolbachia*.

Because they operate through mating between released mosquitoes and field mosquitoes, a key feature of all techniques based on mosquito release is an unprecedented specificity of action, confined to the released mosquito species and any interfertile (sub)species. This has the major benefit of minimizing the direct impact of vector control on health and the environment. It does, however, entail as many individual interventions as there are species of non-interfertile vector mosquitoes to be targeted on a given site.
Population reduction techniques, whether or not they use mosquito release, and whether or not the released mosquitoes are GM, have in common:
– an environmental impact associated with the reduction of target mosquito population density and depending on the target species' role in the ecosystem. This impact varies according to, amongst other factors, whether the relevant species is autochthonous or invasive, whether its habitat is urban or natural, whether specialist predators exist, the extent to which the population is reduced (simple reduction, local elimination, or eradication of the species[6]), the duration of the effects of a technique (depending, amongst other things, on how isolated the treated area is), and the specificity of the technique (techniques involving mosquito release being the most specific);
– the potential for unintended replacement of the target population by the population of another vector species, which increases the more the target population is reduced and the more this reduction persists over time.
Population modification techniques, whether or not they use GM mosquitoes, have in common:
– less of an impact, in principle, with regard to environmental and health risks, since they should not affect the density of mosquito populations. An assessment of the risks associated with the induced modification is still necessary;
– persistence and varying invasiveness of the modifications induced, with the need to consider the evolution and long-term effects of the factors responsible for these modifications (*Wolbachia*, transgenes), including their potential for transfer to other species.
Self-limiting techniques, whether or not they make use of mosquito release, and whether or not the released mosquitoes are GM, have in common:
– the advantage of being controllable and adjustable in the light of monitoring data;
– the drawback of calling for demanding maintenance in the long-term.
Self-sustaining techniques, whether or not they use GM mosquitoes, have in common:
– the advantage of not calling for maintenance or large-scale infrastructure;
– the drawback of being fairly inflexible, or even without the possibility of control (e.g., of intended spread affecting a whole species).
Complementarity of existing and emerging vector control techniques is well illustrated in Fig. 2, which represents the efficiency of the techniques depending on target population density.
Fig. 2 illustrates that:
– the efficacy of conventional methods of vector control is independent of density beyond a certain density threshold of the target mosquito population. Below this threshold, it declines with density until it is nullified before it can lead to elimination;
– conversely, reduction techniques such as the sterile insect technique SIT and the derived techniques such as RIDL and IIT can only be effective below a certain density threshold of the target mosquito population (depending on the ratio of released males to wild males and on the competitiveness of the males released in comparison with wild males). Beneath this threshold, they are all the more effective when the density is lower, thus leading to local elimination (referred to as "Eradication" on the figure) of the population.
These different context-dependent efficacy profiles for the various vector control techniques mean that compatible, complementary techniques ought to be combined in an integrated vector control approach.

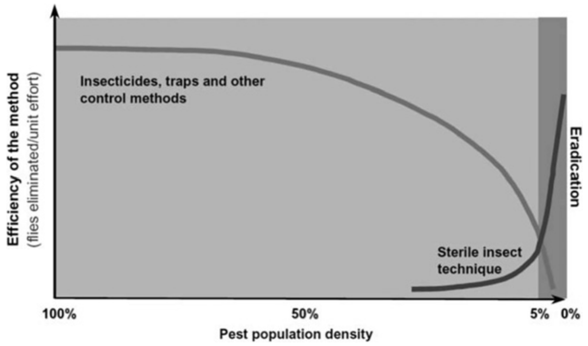

Fig. 2 Vector control efficiency optimization by combining the sterile insect technique with conventional control methods (Feldmann and Hendrichs, 2001, in PAAT Technical and Scientific Series, Number 3, FAO, Rome, Italy).

As of now, gene drive techniques are still under development. Additional research is required before considering any field application, including reducing development of resistance, developing knowledge and procedures for assessing the long-term effects of gene drive on ecosystems, and strategies for controlling the spread of gene drive.
Self-limiting sterile insect techniques (SIT, RIDL, IIT) could be tested step by step on a precautionary basis for the purpose of contributing to vector control in French territories, depending on the vectors concerned, in combination with the conventional techniques currently used for integrated vector management. If successful, employing IIT, SIT or the RIDL technique would in particular help reduce insecticide use. In addition to a lesser risk of exposure for humans and ecosystems, lower insecticide use owing to the use of techniques based on mosquito release would preserve insecticide efficacy by lessening pressure for selection of resistance. This would thus enable insecticide use to be reserved specifically for epidemics and public health emergencies.
More generally, the choice between different existing and emerging vector control techniques or combinations of techniques should be informed by the intended objective, by vector biology and behavior, and by the epidemiological, environmental, and socio-economic context.

Disclosure of interest The authors declare that they have no competing interest.

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[1] All original research is referenced in HCB's [Haut Conseil des biotechnologies, France] scientific opinion: HCB Scientific Committee (2017). Scientific Opinion of the High Council for Biotechnology concerning use of genetically modified mosquitoes for vector control in response to the referral of 12 October 2015 (Ref. HCB-2017.06.07). (Paris, HCB), 142 p. Available online: http://www.hautconseildesbiotechnologies.fr.
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[1] Intracellular bacteria naturally common in arthropods and endowed with specific properties that can be used for vector control.
[2] Population reduction techniques refer to techniques aiming at reducing vector density under the threshold required for pathogen transmission.

They include techniques based on mosquito release (SIT, IIT, RIDL, gene drive for elimination) or existing techniques (chemical, biological, physical and environmental control techniques), only RIDL and gene drive techniques using GM mosquitoes.

[3] Population modification techniques refer to techniques aiming at reducing vector competence and/or longevity. They include only techniques based on mosquito release, namely gene drive for population modification, using GM mosquitoes, and *Wolbachia*-mediated spread of PI.

[4] Self-limiting techniques refer to vector control techniques with effects that are limited in space and time unless application of the technique is maintained. They include most existing vector control techniques (e.g., chemical control) as well as the sterile insect technique SIT and the derived techniques RIDL and IIT.

[5] Self-sustaining techniques refer to vector control techniques whose effects spread across space and last over time without calling for any maintenance. They include some existing techniques such as biological control (to an extent), gene drive techniques and *Wolbachia*-mediated spread of PI. More rigorously, there is a continuum of techniques between these two extremes of self-limiting and self-sustaining.

[6] The objective of eradicating a species, which would be a specific feature of gene drive techniques for elimination, is theoretical at this stage.

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A transdisciplinary consideration of sand flies & leishmaniasis

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It has been hundred years since Phlebotomine sand flies were first identified as transmitters of the medically important parasites called *Leishmania*. The key players, investigated by scientists during the first 60 years, were the insect, the parasite, and the mammalian host. Forty years ago, plants were included as potentially influential players in the transmission process. During the past ten years, we have witnessed a further expansion to include bacteria and viruses as influencers of transmission and the realisation that there is a fascinating network of microbes interacting with surprising consequences for the control of the leishmaniases.

This presentation focussed on the recent inclusion of the bacterial players in the sand fly–*Leishmania* drama. It was also a personal reflection on the urgent need for entomologists and other biologists to harness their creative endeavours to engage with policy makers and the public about what insects can teach us and the huge importance of insects and their microbes in a human centred world.

Influence of gut microbiota on Leishmania interactions

I am very fond of the writing of US-based insect pathologist, Ed Steinhaus, who wrote the following apt statement in 1960 "A comprehensive understanding of the biology of insects requires that they be studied in an ecological context with microorganisms as an important component of the system". There is certainly ample opportunity for Phlebotomine sand flies to interact with elements of the microbial world. Adult sand flies are plant feeders; the male only feeds on plants and females require plants as well as blood for egg development. The *Leishmania* parasite develops entirely inside the female gut of the fly and is therefore exposed to the fly gut microbiota. Hence microbes may be acquired during feeding on plants or animals as well as being vertically transmitted via the larvae and pupal stage. During the 1980's and early 1990's, the idea that bacteria may have an important influence on *Leishmania* was often met with some incredulity; experts even stated their belief that the sand fly gut was 'sterile'. The recognition of the importance of bacterial interactions with *Leishmania* and the sand fly vector finally started to gain some attention post 2010 after a few published studies on the sand fly gut microbiota. Our work on the gut microbiota of sand flies was the first to ask the important question: "Does the gut microbiota influence *Leishmania* development in sand flies?" We examined the effect of yeast and bacterial colonisation of the gut on the subsequent development of *Leishmania mexicana* population in the gut of the South American vector *Lutzomyia longipalpis*. We showed that certain species of bacteria and yeast, previously isolated from wild caught sand flies, significantly suppressed the *Leishmania* [1]. This demonstrated the potential for other microbes already present in the sand fly to interfere with *Leishmania* transmission. Kelly et al. [2] questioned whether some gut microbes might be beneficial to *Leishmania* survival in the sand fly. They showed that, although bacterial diversity decreased in their model of *Lu. longipalpis*, there was a dominance of Acetobacteraceae associated with an increase of the *Leishmania* population. If we think of the sand fly gut as an ecological niche, the initial occupation of that niche will therefore determine the potential for *Leishmania* transmission and the potential of the sand flies to act as successful vectors.

There are potential costs for the sand fly to harbour *Leishmania* and we posed the question: "Does *Leishmania* infection of the sand fly gut confer any benefit on the sand fly?" Are there circumstances under which *Leishmania* might protect the sand fly during its occupation of the gut? We found that *Leishmania* infected sand flies were able to survive for longer after being fed with a bacterial insect pathogen compared to control flies [1].

So, we finally understand that the gut microbiota are important players in the activity of the sand flies as hosts of *Leishmania*. A more recent study extended the influence of the fly microbiota to include a role in the development of *Leishmania* in the mammalian host. When the female sand fly acquires a blood meal through a bite, there is a transfer of *Leishmania* gut bacteria into the wound [3]. The co-transfer of bacteria seemed to be significant as the bacterial antigens primed the host immune system by triggering the inflammasome, leading to an increase in *Leishmania* dissemination through the body of the mammalian host [3]. These recent studies served to underline the importance of the microbial ecology of insect vectors and the need to consider the unseen players in predictive models for determining transmission of medically important parasites and pathogens by insects.

In the second part of my presentation I focussed on examples of creative projects featuring my work with microbes and insects. The primary role of the bioscience researcher is to discover more about life around us and to communicate our discoveries firstly to our science peers but also to the public and decision makers. However, I suggest that it is now more important than ever for scientists including entomologists to engage in public debate on issues that affect the health of the planet and all its inhabitants, including insects. There are many ways to engage with the public; clear factual presentations are useful, but the audience may often be selective and narrow. I am interested in engaging with audiences using artistic means, less directly, through emotional engagement, often with playful elements. Engagement may be through unexpected, novel interventions or by modifying a 'known' commonplace activity. My presentation gave three examples of projects developed through my experience of working with insect vectors of disease.

My first example was the production of a children's audio story called *Tropical Tales*, done in collaboration with a group of artists led by Bisakha Sarker, an Indian creative dance performer and choreographer. We developed a folk tale–very loosely based on the story of Jonah and the Whale. In the tale, a young Indian boy dreams that he was swallowed by a sand fly where he fought with *Leishmania* inside a cavernous insect gut. He then woke up and created a cure for his sisters leishmaniasis after dreaming about a plant that kills *Leishmania*. This story was presented as a series of cartoons (Fig. 1) and the user had a 'talking pen'(with which audio can be given in five different languages) activated by touching each picture.

The second example took place in an art gallery in Liverpool. Here we took part in a 'Bed-In'–in celebration of the John Lennon and Yoko Ono peace protest Bed-In. The intervention was called 'Bednets Not Bombs,' which was meant to be a statement about the funding available for research into tropical diseases and insect control in comparison to the vast funding