Q&A

MALARIA

Evolution in vector control

Yannis Michalakis and François Renaud

Each week some 20,000 people die from malaria. There will be no magic ways of reducing this dreadful toll, not least because the mosquito vector and the parasite itself have formidable abilities to resist control measures. Angles of attack that rest on evolutionary principles are being explored.

Why hasn't malaria been eradicated?

The reason for the failure is threefold. First, there are no drugs that can cure people on a routine basis and on a large scale. Second, there are as yet no vaccines that can provide protection against the unicellular *Plasmodium* parasites that cause the disease. And finally, no large-scale, effective yet environmentally respectful way of controlling the vectors — the mosquitoes that transmit the parasites — has yet been applied in areas of intense malaria transmission. Political, social and economic factors also contribute to the burden imposed by malaria and to the failure to eradicate it.

What is the basic biology involved?

To complete its complex life cycle, *Plasmodium* needs to infect a vertebrate and then a mosquito host (Fig. 1). For human malaria, the vertebrate host is man, but other mammals, birds and reptiles can be infected by their own malaria-causing parasite. Only female mosquitoes transmit the disease. They become infected when they take a blood meal at night, and pass the parasite on during a meal roughly 10–14 days later; the disease symptoms of chills and fever are caused by *Plasmodium* proliferation in red blood cells. More than 20 species of the mosquito genus *Anopheles* can transmit human malaria, although with variable efficiency. A notable feature is that even in areas where most, if not all, humans are infected, only a small proportion of mosquitoes are.

What are the ways of trying to combat malaria?

There are two broad approaches: direct, involving vaccination to prevent infection or drugs to cure it; and indirect, involving attempts to reduce or stop transmission by the mosquito vector. Vector control is the subject of this article.

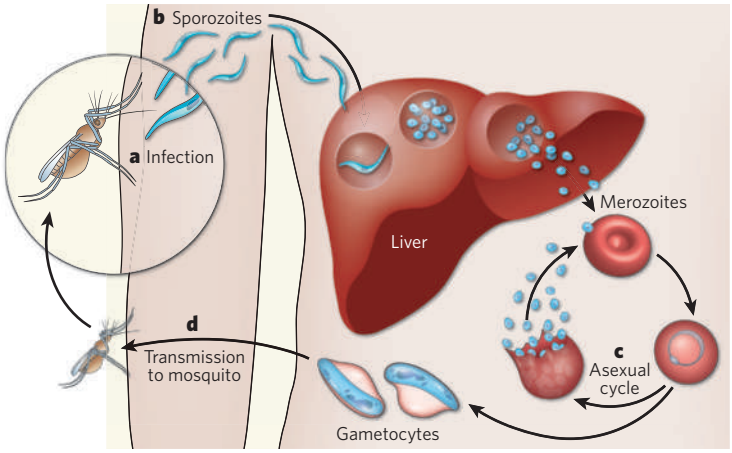

Figure 1 | Basic features of the *Plasmodium* life cycle. **a**, Egg development in female *Anopheles* mosquitoes requires a blood meal. In the process, infected females inject the sporozoite form of the parasite into a human host. **b**, Sporozoites are carried in the bloodstream to liver cells, where they proliferate asexually, and then, as merozoites, invade red blood cells. **c**, An asexual cycle within red blood cells, at which disease is clinically manifest as fever and chills, is followed by the production of male and female gametocytes. **d**, These are transmitted back to a mosquito during a blood meal, where they fuse to form oocysts that duly divide to create sporozoites. These migrate to the salivary glands, where the cycle of infection starts again. Details of the life cycle differ for different species of *Plasmodium*, with *P. falciparum* causing the most virulent form of human malaria. Development within the mosquito is temperature-sensitive, and takes 10–14 days or longer.

Why emphasize vector control?

The development of drug resistance within populations of *Plasmodium* and the inability, so far, to formulate effective vaccines have limited the direct ways of tackling malaria. Another reason for revisiting vector control is that many people carry and transmit the parasite, but don't show the symptoms of malaria and are not treated. Finally, vector control is historically the cheapest and most successful approach, and evolutionary biology is now offering fresh perspectives on it.

What are the approaches to vector control?

They aim either at lowering vector density or at avoiding infectious bites. The first large-scale programmes were attempts to destroy the habitat of mosquito larvae by draining wetlands. But this approach is costly, and mosquitoes can breed in small bodies of water that cannot be eliminated. Killing larvae or adults with chemical insecticides is another approach, and there have also been studies with biological-control agents such as nematodes and fungi. With the development of molecular biology came the hope that the release of genetically manipulated mosquitoes would prove effective.

How are insecticides used?

Spraying with chemical insecticides has been the commonest form of mosquito control because it has proved effective, at least locally. Application of DDT, for example, causes significant decreases in mosquito densities. Other products, both artificial and natural (such as bacterial toxins), directed towards larval or adult mosquitoes, are widely used today. Insecticide-impregnated bednets have a double advantage: they both protect people from infectious bites and reduce vector populations.

What are the problems with insecticides?

Insecticides are typically toxic not only to vectors but to many other organisms, in some cases including humans. Moreover, sooner or later mosquitoes develop resistance to insecticides, either by becoming able to metabolize them, or by modifying the insecticide's target molecule. Typically, resistance comes at a fitness cost to mosquitoes, but the vectors have been able to counteract pretty much any insecticide used against them. In evolutionary terms, the benefit from resisting insecticides

at lethal doses is always much larger than the accompanying cost. Surprisingly, we do not know whether insecticide-resistant mosquitoes are more or less effective malaria vectors.

How does genetic modification enter the picture?

The rise of insecticide resistance prompted research into the release of genetically manipulated mosquitoes (GMMs), with the aims of limiting *Plasmodium* transmission or reducing mosquito population densities. One strategy is best known from attempts to release male mosquitoes that can mate but cannot reproduce successfully, although other approaches, such as the use of site-specific 'selfish genes', have been proposed (Box 1). Another involves producing mosquitoes in which *Plasmodium* development is halted at any stage.

What are the problems with GMMs?

Apart from the political controversy associated with any use of genetically modified organisms, there are practical drawbacks. The success rate of genetic-transformation techniques is low, leading to possible inbreeding-related decreases in GMM population viability. The genetic manipulations often involve laboratory strains, whose ability to survive in the wild, let alone invade wild mosquito populations, is questionable. It is not clear that GMMs designed to resist *Plasmodium* infection would necessarily have a fitness advantage, or, given the low prevalence of mosquito infection even in areas where many humans are affected, that such resistance would spread rapidly. For most, though not all, proposed GMMs, their spread and maintenance in the wild have to be ensured by the repeated release of modified mosquitoes; strategies based on naturally invasive driving mechanisms, such as homing endonuclease genes or transposable elements, present an advantage in this respect (Box 1). There is also the complication that malaria is transmitted by many mosquito species, and GMMs might have to be designed for each of them or restricted to the main culprits. Moreover, if genotype–genotype (mosquito–*Plasmodium*) interactions exist at the within-species level, modification of a given gene might affect *Plasmodium* only on a local scale. Finally, release of *Plasmodium*-resistant mosquitoes might increase transmission of other *Anopheles*-borne diseases, such as filariasis.

How about enlisting the natural enemies of mosquitoes?

Such organisms include nematodes that kill mosquito larvae, microsporidia that infect larvae, and fungi that attack adults (Box 2, overleaf). The fungal parasites can induce more than 80% mosquito mortality within 14 days of infection (a critical period, as we will see later), and are especially effective against *Plasmodium*-infected mosquitoes. They can be sprayed indoors, on places where mosquitoes rest before or after blood meals, and can be combined with other control or prevention strategies, such as bednets.

Box 1 | Approaches involving genetically modified mosquitoes (GMMs)

The production of sterile male mosquitoes can be achieved non-genetically through radiation treatment, or by introducing dominant lethal genes into them. This works, for example, by introducing genes that are lethal during larval development, unless the mosquitoes are fed a specific substance such as an antibiotic. The GMMs survive in the lab because they are fed the antibiotic. But when they are released and then mate with wild females, their offspring die because of the absence of the antibiotic in the environment.

An approach that involves 'selfish genes' entails, for example, the use of homing endonuclease genes (HEGs) that encode an enzyme that recognizes a 20–30-base-pair sequence on chromosomes not containing the HEG, and cleaves it. The cleaved copy uses the HEG-carrying copy as a repair template, and thus the HEG spreads in the population. Because the HEG lies in the middle of the recognition sequence, the chromosome carrying it is protected from future attack.

For use in vector control, HEGs are required that recognize and insert into a specific sequence of an essential mosquito gene. When these GMMs are heterozygotes, with one of a gene pair disrupted and the other not, they will survive and reproduce. Such constructs will initially spread because they are driven by the bias in HEG transmission. Once their frequency is sufficiently high in the mosquito populations, substantial numbers of inviable homozygotes, in which both copies of the gene are disrupted, will result, possibly causing the vector population to crash.

GMMs are also used to spread *Plasmodium* resistance among mosquitoes. The first stage consists of identifying genes that stop *Plasmodium* development in the mosquito, or prevent transmission, and then engineering mosquitoes that express this characteristic. This is the relatively easy part. The problem then consists of associating this construct with a naturally invasive driving mechanism, which could involve transposable elements, meiotic drive, HEGs or bacteria called *Wolbachia*.

In principle, this approach does not harm the vector, only *Plasmodium*, and the driving mechanisms may help to overcome the other disadvantages related to the release of GMMs. But the association between the resistance gene and its driver may be broken by genetic recombination, and some of the drivers — such as transposable elements — may generate insects with undesirable characteristics. Also, it is difficult to assess the risk, and the consequences, of the driving mechanisms and transgenes spreading to other species. **Y.M. & F.R.**

... and the drawbacks are?

Like GMMs, mosquito predators and parasites must spread and be maintained in wild populations, while not eliciting resistance in the target. The spread has to be achieved by human agency, and so requires the production of large quantities of these agents; the existence of industrial formulations for some of them, for example fungi or nematodes, is a definite plus. If rearing these natural allies is relatively easy, it can also satisfy the demand for maintenance, although any ally able to meet that demand itself would obviously be a favourable option. The development of resistance is much more of a problem. Widespread use of natural allies would drastically shift selection in favour of mosquitoes able to resist them, as has happened with chemical insecticides. The consequences, including the ensuing selective pressure imposed on *Plasmodium*, can neither be generalized nor ignored. A further consideration is the specificity, or lack of it, of natural allies. Perhaps killing any insect present in a human habitation is a good thing, but this too is an issue that merits debate. Alternatively, mosquito-specific isolates could be selected and used.

How does the timing of intervention come into things?

Like some other vector-borne agents, such as the virus that causes dengue fever, *Plasmodium* has an intriguing feature: after it has entered a mosquito, it takes quite a long time, 10–14 days or more, to produce the stages transmissible to humans (Fig. 1). The timing of the effect of an insecticide, whatever its nature, may thus be crucial. An agent that acts once transmission has taken place will not be very helpful; and one that imposes a large burden on mosquito fitness will favour any mechanism that makes mosquitoes resistant. But get the timing of intervention right, and it might be possible to dissociate the effects on *Plasmodium* transmission from the effects on mosquito fitness.

How might this influence anti-vector strategies?

Until recently, all such strategies worked on the assumption that negative effects on vector fitness are desirable. But what we really want to do is limit pathogen transmission. An ideal approach would cause as little harm as possible to the vector, to avoid eliciting resistance, but as much harm as possible to the pathogen. This is where the evolutionary theory of senescence comes in.

What is the evolutionary theory of senescence?

The strength of natural selection declines with age. In consequence, selection against mutations with negative effects late in life is much weaker than selection against mutations with negative effects early in life. From an evolutionary standpoint, senescence can thus be explained either

by the accumulation of mutations having deleterious effects late in life (the 'mutation accumulation' theory; Fig. 2), or by the fixation of mutations that are advantageous to their bearer when it is young and detrimental when it is old (the 'antagonistic pleiotropy' theory).

How can this theory be applied in vector control?

A consequence of the fact that *Plasmodium* requires a long developmental period within mosquitoes is that malaria is transmitted only by relatively old mosquitoes. Females undergo cycles during which a blood meal is necessary to produce eggs, which are laid on water, with each cycle lasting 2–4 days. Because their daily survival rate is 80–90%, most females will go through few such cycles before they die (fewer than 20–40% would go through more than four cycles). Thus, a strategy killing mosquitoes later in their life, but before they transmit malaria, would mimic senescence and disrupt transmission. Such approaches would generate little, if any, selection for resistance in the mosquito population, and would require 'late-life-acting insecticides'.

Can this theory be put into practice?

Pathogenic fungi seem to have the desirable properties: they induce high mortality relatively late in a mosquito's life but before the insect transmits malaria (Fig. 2). These fungi are even more virulent in malaria-infected mosquitoes, which is a bonus: this should further slow the advent of resistance to the fungi while at the same time favouring resistance to *Plasmodium* among mosquitoes. The same principles are behind a project to combat dengue fever, a viral disease transmitted by *Aedes* mosquitoes, by introducing *Wolbachia* bacteria into the vector through genetic manipulations that have little effect on vector fitness but that can disrupt dengue transmission. The manipulation of malaria-transmitting mosquitoes in like fashion is under way. *Wolbachia* could in principle spread in mosquito populations because there is a driving mechanism: infected males effectively sterilize females uninfected by the same bacteria as the males. But although this strategy is based on the same evolutionary principles as that involving the fungi, there are two drawbacks. First, large-scale application in the field will encounter the same issues as other approaches involving GMMs. Second, there are examples from *Wolbachia* interactions with fruitflies, butterflies and even mosquitoes that show that bacterial virulence and/or within-host density evolves rapidly. So frequent release of mosquitoes carrying 'unevolved' bacteria could be required.

How might *Plasmodium* respond to fungal attack on its vector?

One way would be for the parasite to shorten its development time within the mosquito. Here we enter some fascinating and largely uncharted territory. We can assume that the present long development time is advantageous, but if late-life-acting insecticides were to be widely applied, the selection 'landscape' for *Plasmodium* would change dramatically. Genes that determine slower development would become a liability, and this prompts various questions. Is there a link between *Plasmodium* development time and virulence in mosquitoes? If faster development leads to lower virulence, there would be selection against any naturally occurring resistance mechanisms in the mosquitoes, and parasite transmission would be enhanced. And is there a link between development time in mosquitoes and infectiousness and/or virulence in humans? Selection experiments on varying *Plasmodium* development times should allow us to explore these questions.

What's next?

By dissociating fitness effects on the vector from effects on parasite transmission, late-life-acting insecticides offer a new angle of attack on malaria by limiting the potential evolution of insecticide resistance. The approach will require more testing of both theory and practice, and proven methods, such as insecticide-treated bednets or insecticide spraying, will remain the mainstays for malaria control. Although much research is devoted to the separate development of control strategies, that is perhaps at the expense of assessing the combinations of approaches that could produce the best results in designing evolution-proof inhibition of *Plasmodium* transmission. Papers published in the past couple of months show that a combination of conventional insecticides and insect-killing fungi can work synergistically in directly lowering malaria transmission and decreasing insecticide resistance. Evolutionary thinking can greatly contribute to disease control, particularly in devising ways to limit disease transmission, to avoid the development of resistance and to predict the potential evolutionary responses of parasites and vectors. ■

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Box 2 | Fungal allies in vector control

The fungi concerned are types of Sordariomycetes, and the species of principal interest are *Beauveria bassiana* and *Metarhizium anisopliae*. The fungal spores attach to the insect cuticle when, for instance, a mosquito rests on a wall that has been sprayed with an appropriate preparation. The spores then germinate and penetrate the cuticle, after which they develop in the insect's haemocoel (the equivalent of the circulatory system). The fungi breach the cuticle using enzymes, and then overcome the insect's immune system either by producing cryptic forms that the immune system does not 'see', or by secreting substances that suppress immunity. Ultimately, the fungus kills its host and produces spores.

It is unlikely that the fungi will be able to self-sustain and provide long-term infectivity; repeated applications will probably be necessary. Spore viability, a critical component for the success of this approach, depends on ambient temperature and humidity, and variation among fungal strains for spore viability is poorly understood. The effects of these fungi on mosquitoes have been investigated in the lab and in a pilot study in Tanzania, and formulations are being developed for use in the field. Such work may profit from previous experience, because the same fungi are used as large-scale biological-control agents against locusts and grasshoppers. **Y.M. & F.R.**

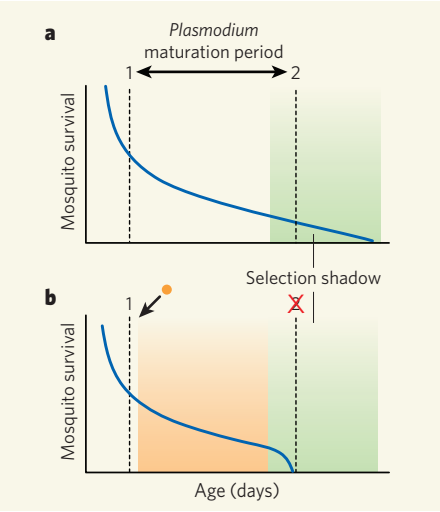

Figure 2 | The mutation-accumulation evolutionary theory of senescence and its application in malaria control. a, Survival of an adult female mosquito that was infected by *Plasmodium* during her first blood meal (1) and transmitted *Plasmodium* during a subsequent blood meal (2). The interval between the two meals corresponds to the *Plasmodium* maturation period, during which the female may have taken other blood meals without transmitting the parasite. For simplicity, we assume fecundity does not vary with age. Because survival declines with age, selection is much weaker against mutations with deleterious effects late in life — shown by the 'selection shadow'. (Modified after T. B. L. Kirkwood & S. N. Austad *Nature* 408, 233–235; 2000.) **b**, Survival of a female mosquito infected by *Plasmodium* and a fungus (orange dot) at her first blood meal. The fungus grows in the female (orange shaded area), which dies before she can transmit *Plasmodium*. In effect, the fungus acts as a late-life-expressed deleterious mutation.

FURTHER READING
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