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Genetic approaches for malaria control

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Abstract

The already unacceptable large burden of malaria continues to increase, indicating that the available means to fight the disease are insufficient. The genetic manipulation of the mosquito vectorial competence is a potential new promising weapon for the control of malaria. Considerable progress has been made in recent years towards this goal. It is now possible to introduce synthetic genes into the mosquito germ line, promoters have been identified that effectively drive gene expression in tissues and at times appropriate to target the parasite, and effector genes that impair parasite development in the mosquito have been identified. With these tools, proof-of-concept experiments have already demonstrated that it is possible to interfere genetically with the vectorial competence of the mosquito. At least some of the transgenic mosquito lines that have been created appear to be as fit as their wild-type counterparts. Presently, the major unresolved challenge is the development of methods to drive effector genes into mosquito populations in the field. While several approaches are under consideration, such as transposable elements, *Wolbachia*, meiotic drive and paratransgenesis, their relative feasibility remains to be demonstrated. Additional challenges are the resolution of safety concerns and satisfactorily addressing social, ethical and political considerations. Hopes remain high that the remaining challenges will be solved and that we shall be able to deploy this new genetic weapon in the foreseeable future.
Keywords: mosquitoes; malaria; transgenesis; effector genes; driving mechanisms; refractoriness

Introduction

Insect-transmitted diseases impose an enormous burden on the world population in terms of loss of life (millions of deaths per year) and morbidity. These diseases impose huge economic losses both in terms of health-care costs and lost productivity, mostly in countries that can least afford it. Three basic approaches have been attempted to contain these diseases: 1) treat infected people with drugs that kill the pathogen; 2) control insect vector populations; and 3) develop vaccines that prevent infection.

Drugs

Drugs have been at the forefront of the fight against many arthropod-transmitted diseases. For decades, chloroquine was successfully used against malaria. However,

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with time pathogens are selected for drug resistance, forcing the development of new drugs. Unfortunately, this cycle of drug discovery followed by resistance becomes more difficult to perpetuate with the passage of time. In principle, combination drug therapy should greatly alleviate this problem. In practice, other factors (mostly economic) make the implementation of this strategy difficult. While drugs are extremely useful in containing and treating diseases, they are not sufficient on their own for disease eradication. Clearly, a combination of strategies is needed.

Control of insect populations

On the contrary, reduction of vector insect populations will reduce disease transmission. This can be accomplished in various ways, for instance, with insecticides, by managing the environment (elimination of breeding sites) or by interfering with reproduction (sterile-insect releases). Recent technological advances suggest an alternative approach, namely genetic modification of the competence of the vector arthropod to transmit pathogens (vectorial competence), which is the main subject of this article.

Vaccines

The only successful vaccine in existence against an arthropod-transmitted pathogen is the yellow-fever vaccine. Even in this case, the disease has not yet been eradicated. Decades of intense research aimed at the development of other vaccines, notably for malaria and dengue fever, but this has yet to yield a viable product. At the heart of the problem, at least for malaria, is that during thousands of years of association with humans pathogens have been selected that efficiently evade the host immune system. High genetic diversity, variability of potential target molecules (e.g., *Plasmodium var.* genes), and intracellular sequestration are strategies frequently used by pathogens that allow them to elude immune attack. While the search for effective vaccines should continue, this has been an uphill battle.

Insecticides

Under appropriate circumstances, insecticides are powerful weapons to fight vector-borne diseases. For instance, they have been crucial in the eradication of malaria in Europe and of *An. gambiae* in Brazil, and they are important in controlling disease epidemics (e.g., dengue, West Nile) in urban areas. The introduction of DDT in the mid 1940s heightened the hopes of disease eradication. A case in point is the WHO campaign to eradicate malaria, which was successful at its inception (for instance, malaria was almost eliminated from the entire Indian subcontinent). However, problems such as development of insecticide resistance by mosquitoes, the discovery of the harmful effects of DDT to the environment and to non-target organisms, and the ‘letting down of the guard’ when disease was almost under control, led to the reversal of most initial successes. While judicious use of insecticides is still a powerful weapon to fight disease, one aspect of its use is frequently overlooked: insecticides usually leave intact the biological niche where the target insects reproduce. Therefore, insect populations rapidly return to pre-treatment levels as soon as application is halted, which is a very serious problem. For instance, one can hardly hope for large-scale mosquito-population reduction in Africa, especially if one considers that management of breeding sites (e.g. widely distributed small pools of water) would be required. While residual spraying of house interiors or use of bednets will lower transmission rates and reduce prevalence and incidence of

infections, the breeding sites will continue to generate mosquitoes and this cycle of breeding and killing is likely to hasten the development of insecticide resistance. Thus, insecticides are useful to bring temporary relief but cannot be considered as solutions by themselves. In future, insecticides are likely to become key weapons when used in combination with other approaches such as vaccines, drugs or when used for population replacement (see below).

Sterile-insect technique (SIT)

Insect populations can be controlled by the release of large numbers of sterile males. Thus, if a female mates with a male that has no sperm or whose sperm was rendered unviable, this female will have fewer or no progeny. When many sterile males are released, the local population tends to decline or become extinct. There are a number of cases of the successful local application of this technique, for example, in the control of the Mediterranean fruit fly in Latin America, the New World screwworm in the Americas and Libya, and for tsetse in Zanzibar, Africa. SIT also has been applied, on a limited scale, to *Culex* in India and *Anopheles albimanus* in El Salvador (see Curtis, Chapter 3).

For population control, the crucial parameter is the ratio of the number of released sterile males to the number of males in the local population, which ideally should be around 10:1. Therefore, sterile-insect control is only effective when the resident population to be controlled is small relative to the number of sterile males that can be mass-produced for release or when it can be reduced to very low levels with conventional control tools before the start of releases. It is highly desirable that only males be released for two reasons: 1) In most cases only females bite and transmit disease while, moreover, sterile females can also transmit; 2) Males would court and mate with the released sterile females (instead of local females), thus reducing the efficacy of the programme (Alphey and Andreasen 2002). Large-scale production in the laboratory of a pure male population by non-genetic means may be problematic. It may rely on sex-specific differences of pupal size (culicine mosquitoes) or adult eclosion times (tsetse), but these protocols rarely yield a 100% male population. Clearly, genetic sexing methods (see below) are far superior. The most commonly used technique for male sterilization is exposure to high doses of radiation, a procedure that damages chromosomes and results in unviable sperm. Sterilization by chemical means also has been employed. Because of the large numbers of insects that need to be released, it is crucial that the effectiveness of the sterilization procedure approaches 100%. However, the large doses of radiation and chemicals needed to achieve this effectiveness may reduce insect fitness, survival and mating competitiveness. These strategies can fail if the laboratory-reared males do not mate as effectively as their field counterparts.

The advent of germ-line transformation for a number of different insects has led to the development of genetic alternatives for production of sterile insects (Heinrich and Scott 2000; Thomas et al. 2000). In one version of this approach (Release of Insects carrying a Dominant Lethal or RIDL, Thomas et al. 2000), a conditional dominant lethal gene is introduced into the target insect genome. This gene has two important properties: 1) it is expressed only in females (or it kills only females); and 2) the gene is effectively repressed by a compound that does not occur normally in nature (e.g. tetracycline). Large insect populations are maintained by rearing them in the presence of tetracycline, which represses the dominant lethal gene and allows the survival of equal numbers of males and females. Prior to release, the insects are reared in the

absence of tetracycline, a condition that allows the expression of the dominant lethal gene and the death of all females. The resulting males can be released without further manipulation or treatment. Males carry two copies (homozygous) of the dominant lethal gene. When these males mate in nature, all female progeny will be killed and only males will be produced. Since these surviving males are heterozygous for the dominant lethal gene, the population-reducing effect is still manifest in the second generation.

It should be emphasized that the effectiveness of the SIT is dependent on population structure and dynamics. Furthermore, this technique leaves intact the biological niche in which the target insect is found. SIT is most likely to succeed in cases where target populations are small, the number of target insects is low, and the target area is sufficiently isolated, thereby reducing the likelihood of re-invasion. It is unlikely to be effective for controlling mosquito populations in highly endemic areas of Africa where the mosquito population consists of several vector species in high densities, where access to breeding sites is difficult and where poorly interbreeding mosquito populations co-exist.

Genetic manipulation of vectorial competence

Germ-line transformation

Drosophila melanogaster was the first multicellular organism to be stably transformed (Spradling and Rubin 1982). The same general principles that were used in this pioneering work are still employed today for all germ-line transformation work in insects (Atkinson and James 2002). Embryos are injected with two DNA constructs. One construct contains a gene encoding a dominant selectable marker (e.g., eye color, a fluorescent protein) and the gene of interest, each driven by a separate promoter, and both sequences are together flanked by the inverted repeats of a transposable element. The second construct encodes a transposase, which is an enzyme that recognizes the inverted repeats and catalyses the insertion of the intervening sequences into the genome of the host insect. It took from 1982 until the mid 1990s to develop two crucial technologies: an appropriate transposable-element system (at first scientists did not realize that the *P* transposable element is not active in non-*Drosophila* organisms) and a suitable transformation marker (e.g., GFP). Since then, germ-line transformation of many insects has been accomplished but mosquitoes (*Aedes*, *Anopheles*, *Culex*) are the only insects of medical importance in this list. Importantly, both *An. stephensi* and *An. gambiae* can be transformed, though the success rate in the latter case is still low. Improvement of the transformation efficiency of *An. gambiae* is a high-priority topic for future research. It would also be desirable to develop germ-line transformation procedures for other medically important insects such as sand flies and black flies. Current technology cannot be applied to germ-line transformation of tsetse because these do not lay eggs (that would need to be injected), only fully formed larvae. However, genetic modification of tsetse vectorial capacity could be achieved via genetic modification of one of its symbionts.

The net result of germ-line transformation is the integration into the genome of the host organism of a relatively large DNA sequence, flanked by inverted repeats of the transposable element. The inserted DNA contains at least two genes, the gene to be investigated and a transformation marker gene (e.g., eye color, GFP) that allows transformed individuals to be identified. The integrated DNA is usually stable and transmitted in a Mendelian manner from one generation to the next. In the following

text, we will consider transformation of insects with genes that affect their ability to transmit pathogens. We will start by considering promoters to be used for driving gene expression and then effector genes capable of interfering with parasite development.

Promoters

Promoters that drive the expression of effector genes in transgenic insects should be strong, that is, they should result in abundant transcription. This is because the effectiveness of the gene products is expected to increase with increased abundance. Two general types of promoters can be considered: ubiquitous and tissue-specific. Ubiquitous promoters are less desirable because general expression of a foreign gene product in all tissues of the insect and at all times is likely to impose a fitness load. Tissue-specific promoters have the advantage of being restricted to a tissue and are often developmentally and/or physiologically regulated (not constitutive). Strong tissue-specific promoters that have been characterized in mosquitoes include gut *carboxypeptidase* (Moreira et al. 2000), fat body *vitellogenin* (Kokoza et al. 2000) and gut *peritrophic matrix protein 1* (*PM1*; Jacobs-Lorena laboratory, manuscript in preparation). The *carboxypeptidase* promoter has the advantage of being induced by blood intake, and thus its activation coincides with parasite arrival in the gut. The carboxypeptidase signal sequence effectively promotes secretion into the midgut lumen, which is the compartment where the parasite initially resides. The *PM1* promoter and the protein's signal sequence direct synthesis and storage of the protein in vesicles of the midgut-epithelial cells prior to a blood meal. The vesicle contents are released into the midgut lumen immediately after blood ingestion. The *vitellogenin* promoter is also induced by the blood meal (expression peaks at ~24 h) and the peptide signal sequence promotes secretion into the mosquito body cavity (haemocoel), where the parasite later develops. This promoter is ideally suited for expression of effector molecules that target the ookinete soon after its crossing of the midgut epithelium and emergence into the haemocoel. Once it reaches the haemocoel, the ookinete transforms into an oocyst that is difficult to target because it is covered by a thick protective layer. Upon maturation (at about 10 days after the infective blood meal), the oocyst releases sporozoites that disperse though the haemocoel until they come in contact with, and invade, the salivary gland. It would be desirable to find promoters that drive synthesis of proteins secreted into the haemolymph at the time of sporozoite release. While sequences that direct secretion into the salivary-gland lumen have been identified, their expression levels of the corresponding promoters are low (Coates et al. 1999). It would be desirable to identify a strong salivary-gland promoter to target pathogens stored in the salivary glands. The pathogen usually is stored in the salivary-gland lumen for extended periods of time, and this increased period of contact may favour parasite inactivation by the effector-protein product. One should keep in mind though, that mosquito saliva (and any effector protein secreted into it) is transferred to its vertebrate (human) host, raising safety and ethical questions.

Effector genes

The term effector gene is used here for genes whose products interfere with the development of a pathogen. At least four classes of effector genes can be identified: 1) Genes whose products interact with insect host tissues crucial for parasite development: Examples of this class are SM1, a peptide that occupies putative salivary-gland and midgut receptors for the malaria parasite (Ghosh, Ribolla and Jacobs-Lorena 2001) and phospholipase A2 (PLA2), which is a protein that interferes

with the malaria ookinete invasion of the midgut (Zieler et al. 2001); 2) Genes whose products interact with the pathogen: Examples of this class are genes encoding single-chain monoclonal antibodies that bind to the parasite's outer surface thus blocking their development (Yoshida et al. 1999; De Lara Capurro et al. 2000); 3) Genes whose products kill the pathogen: Examples are peptides from the insect's innate immune system such as defensins and cecropins, and peptides from other sources that act as selective toxins to parasites but do not affect the host insect, such as magainins, Shiva-1, Shiva-3 and gomesin (Kim et al. 2004). Most published work on effector genes deals with effects on the malaria parasite and little is known about such genes for other pathogens. In particular, it is not clear what class of effector genes would be useful for nematodes (filaria). Since these may be encapsulated in certain mosquito strains, genes that activate encapsulation could be considered as possible effector genes. For viruses, genes of the first class (interference of host-tissue invasion) or genes that interfere with virus replication (Olson et al. 1996) are possible candidates. 4) Another possible strategy is to reduce vector competence by manipulation of its immune genes, for instance by using RNA interference or 'smart sprays' (Christophides, Vlachou and Kafatos 2004).

Another important strategic consideration is the stage of malaria parasite development to target. When a mosquito ingests an infected blood meal, it acquires thousands of gametocytes of which only few (usually less than ten) manage to cross the midgut and form oocysts. Later, each oocyst produces thousands of sporozoites, a significant proportion of which invade the salivary gland. Because the strong bottleneck at the level of midgut invasion, this stage of parasite development constitutes a prime target for intervention. Midgut invasion is also a strong bottleneck in the process of arboviral transmission.

Genetically modified mosquitoes

Successful development of the technology described above (transgenesis, promoter characterization and effector-gene identification), permitted the creation of genetically modified mosquitoes impaired in their ability to transmit the malaria parasite. An early example was the creation of an *Ae. aegypti* expressing defensin in the haemolymph (Kokoza et al. 2000). However, the effect of defensin on malaria parasite development has not been reported. At about the same time, the James laboratory reported that a single-chain monoclonal antibody that recognizes a sporozoite surface protein inhibits invasion of the salivary gland (De Lara Capurro et al. 2000). In this instance, the effector gene was transiently expressed from a viral vector that is not inherited by the mosquito progeny. The Jacobs-Lorena laboratory showed that a stably integrated gene encoding SM1 strongly inhibits parasite development in transgenic mosquitoes (Ito et al. 2002). In another example, transgenic mosquitoes expressing PLA2 also had much reduced vectorial competence (Moreira et al. 2002). Recently, it was demonstrated that the capacity to transmit the malaria parasite is reduced by about 60% in transgenic *An. gambiae* expressing cecropin from a *carboxypeptidase* promoter (Kim et al. 2004). Thus, it is clear that mosquitoes can be genetically modified to reduce their vectorial competence. To date, most reported experiments have been done with non-human malaria parasites. An important next step is the transfer of this technology to human pathogens.

Insect fitness

For the introduction of an effector gene into populations, it is important that it confers the least possible detrimental effect on mosquito survival or reproduction

(fitness load). This parameter can be initially tested in the laboratory by use of population cages. For instance, *SM1*-transgenic mosquitoes do not seem to have any load, while *PLA2*-transgenic mosquitoes lay significantly fewer eggs and therefore carry a significant fitness load (Moreira et al. 2004). In contrast to the apparent lack of fitness load of *SM1*-transgenic mosquitoes, Cateruccia, Godfray and Crisanti (2003) reported that transgenic mosquitoes expressing GFP from an *actin* promoter may have a fitness disadvantage. It appears however that in these experiments, loss of fitness was mainly due to inbreeding (the experiments were conducted with homozygous transgenic mosquitoes that may have been subject to the 'founder effect') and perhaps to generalized foreign gene expression from a ubiquitous promoter (see above). Moreover, Irvin et al. (2004) have detected a fitness load in transgenic *Ae. aegypti* that express an eGFP marker gene. However, as for the experiments by Cateruccia et al., they used homozygous transgenic mosquitoes to measure fitness and these experiments cannot determine whether the fitness load originates from nearby recessive genes that were homozygosed with the transgene ('hitchhiking effect') or from a true fitness load imposed by the transgene itself.

Another consideration is that the malaria parasite itself reportedly imposes a fitness load on the mosquito (Hogg and Hurd 1997). In agreement with this observation, the Jacobs-Lorena laboratory has preliminary results indicating that in cage experiments, transgenic mosquitoes expressing SM1 out-compete wild-type mosquitoes with the same genetic background when fed on *P. berghei*-infected mice, presumably because the transgenics have a lower parasite load (unpublished observations).

Eventually tests will have to be devised that measure insect fitness in the field (as opposed to laboratory cages) because other factors may come into play. Moreover, laboratory mosquitoes may not compete well with their field counterparts (important for release studies). One possible solution to this issue may be to cross the effector genes into wild-caught local mosquito populations prior to release.

Between now and field release

While genetic modification of mosquitoes to resistance to the malaria parasite is clearly feasible in a laboratory setting, many issues remain to be addressed before implementation of this approach in the field can be envisioned. Examples of unresolved issues follow.

i) Parasite resistance and multiple effector genes

Parasites tend to have a heterogeneous genome that favours selection of individuals able to overcome barriers such as drugs or possibly effector gene products. It will therefore be crucial that transgenic mosquitoes incorporate more than one (ideally several) effector genes, each of which blocks parasite development by a different mechanism.

ii) Driving effector genes into field populations

This is undoubtedly the major unresolved technical issue. Several approaches have been suggested.

Population replacement by inundatory release. A possible scenario would be to start with an isolated area (e.g., an island) where malaria is prevalent, and reduce to the maximum extent possible the mosquito population by use of insecticides. As discussed above, this would leave an empty biological niche. The next step would be the release of transgenic mosquitoes to occupy this niche. New transgenic releases could follow periodically with the expectation that the original mosquito population will eventually be replaced by the transgenic one. While waiting for the development of an effective driving mechanism (see below), comparison of malaria transmission

before and after population replacement should provide valuable data to assess effectiveness of the transgenic approach. It should be noted however that while population replacement is conceivable for research purposes in small physical or ecological islands, its implementation on a large country- or continent-wide scale is not feasible.

Transposable elements. There is an excellent example in *Drosophila* of how an element can spread through wild populations. In a matter of a few decades, the *P* element spread through virtually all *D. melanogaster* in the world. Presumably, this happened because the transposase causes the element to multiply in the genome, resulting in non-Mendelian transmission. Unfortunately, *P* elements are not active in non-drosophilid insects and more importantly, to date no transposable element with similar properties has been identified in mosquitoes. Even when such elements are identified, there will be major issues to be addressed. One relates to 'cargo' size. As mentioned in the preceding text, it will be critical to use at least two different effector genes. In addition, a gene encoding the transformation marker (e.g., GFP) and a gene encoding the 'driver' (transposase) will be needed. Note that it is crucial for cargo and driver to be tightly linked. Thus, a minimum of four genes are needed representing a substantial cargo of 12~16 kb (calculated at 3~4 kb/[gene + regulatory sequences]). In nature, transposable elements are known to become truncated as they hop from one position to another, and the probability of truncation can be expected to increase with the size of the cargo (for comparison, a typical transposable element has about 3 kb). Thus, cargo damage and consequent inactivation of the genes carried by the element is a major concern. Another consideration is that in some instances, insects carrying a transposable element accumulate a repressor of the transposase, precluding introduction of a different gene with the same transposable element and into the same population. In other words, this could be a 'one-shot' proposition: should one discover that the wrong transgenes were used, that resistance developed or that gene inactivation occurred, there would not be a second chance to spread another set of genes with the same element.

Wolbachia. *Wolbachia* are intracellular bacteria that inhabit the germ line of a number of insects and distort reproduction by killing progeny that do not contain it, by a phenomenon known as cytoplasmic incompatibility (CI). Compelling evidence in favour of *Wolbachia* as a drive mechanism comes from *Drosophila*. Turelli and Hoffmann (1991) observed that *Wolbachia* swept through the *D. simulans* population in California at the rate of 100 km per year. In principle, *Wolbachia* could provide a powerful driving mechanism. However, no *Wolbachia* have yet been identified in anopheline mosquitoes (these are the exclusive vectors for human malaria), although they have been observed in culicine mosquitoes. A major limitation of *Wolbachia* is that it inhabits the germ line while the pathogen develops in the soma. Thus, it is difficult to target parasites with genes introduced into *Wolbachia*. A possible solution to this problem is the identification of genes that cause CI. Currently little is known at the molecular level about how CI functions or how many genes are involved. When identified, such gene(s) could conceivably be used to create a driving mechanism via their insertion into the mosquito genome.

Meiotic drive. Population replacement can be driven by certain genes, such as the *Drosophila segregation distorter* gene, that favour its inheritance over individuals not containing the gene. Unfortunately, very little is known about such genes in insects of medical importance. One complication is that at least in model systems (*Drosophila*, mouse), the drive mechanism depends on multiple genes (e.g., distorter and responder) and this could complicate the implementation of this system in

mosquitoes. Moreover, if such genes were to be employed to drive effector genes into populations, all meiotic drive and effector genes would have to be tightly linked to avoid loss of effectiveness due to recombination.

Paratransgenesis. An alternate approach to spread effector genes through mosquito populations is to introduce effector genes into bacteria that inhabit the mosquito gut, rather than introducing them into the mosquitoes themselves. This approach (known as paratransgenesis) has been successfully tested in another vector/parasite system to disrupt the transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease, by the triatomid bug *Rhodnius prolixus*. *R. prolixus* harbours an obligate bacterial gut symbiont that lives in close proximity to the *T. cruzi* parasite. When this symbiotic bacterium was transformed with an effector gene encoding cecropin A, and fed to naive *R. prolixus* nymphs, *T. cruzi*'s ability to survive in the nymphs was significantly reduced (Durvasula et al. 1997). Preliminary experiments from the Jacobs-Lorena laboratory suggest that the same principle can be used for reducing the capacity of mosquitoes to vector malaria parasites. When *E. coli* displaying the inhibitory SM1 peptide (Ghosh, Ribolla and Jacobs-Lorena 2001) or PLA2 (Zieler et al. 2001) on its surface was fed to *An. stephensi* followed by an infectious blood meal, significantly fewer *P. berghei* parasites developed as compared to control mosquitoes fed on wild-type bacteria. Several considerations argue in favour of the paratransgenic approach: 1) Bacteria live in the same compartment where the initial stages of *Plasmodium* development occur; 2) In principle, bacteria should be easier to introduce into mosquito populations than transgenes. One possible scenario is that baits containing the modified bacteria, a source of sugar and mosquito attractant(s) would be placed in strategic locations (e.g. huts) around a village where malaria transmission occurs. Moreover, genetic modification of bacteria is straightforward and efficient. Bacteria can be easily and cheaply grown in large quantities. This facilitates the introduction of multiple effector genes into mosquito populations, each bacterium being transformed with a different transgene. Unlike mosquito transgenes, inactivation of bacterial transgenes after many generations in the field is not a major concern because of the likely easier logistics of introducing freshly transformed bacteria. Moreover, if an effector gene fails to perform as promised, introduction of alternate transgenes is relatively simple. 3) A paratransgenic approach also poses fewer risks compared with a large-scale release of genetically modified vectors. Not only does a large-scale mosquito release cause increased nuisance to the local population, but there may be an increased health risk if the mosquitoes are capable of vectoring other pathogens. However, the paratransgenic approach is not without concerns: 1) It is not known how adult mosquitoes acquire their bacterial flora. Thus, we do not yet know how to implement a plan to control *Plasmodium* transmission with genetically modified bacteria; 2) Introduction of a genetically modified organism of any kind into the field needs to be approached with much caution because of the unknown consequences. In particular, bacteria are known to be able to spread genes by horizontal transfer (especially if present in plasmids). It is not known whether the modified bacteria could populate the guts of non-target organisms. If so, the consequences need to be assessed.

iii) Population structure

It is quite clear that at least for anopheline mosquitoes, population structure is complex. This is because a number of morphologically identical but chromosomally different (cytotypes) mosquito populations can co-exist in any given area. Importantly, these populations may not freely interbreed and this may seriously affect

efforts to spread effector genes through populations. A better understanding of population structure and mosquito ecology should be given a high priority.

iv) Safety concerns

While there is no reason to believe that any of the effector genes identified to date have any effects on non-target organisms, concerns are being raised by the scientific and lay communities regarding the safety of transgenic mosquitoes. For instance, it has been suggested that these mosquitoes might be better vectors for other (non-malaria) pathogens. While there is no evidence to suggest that this is the case, caution should be used and these possibilities should be tested. Another concern is that of horizontal gene transfer from the transgenic mosquitoes to other organisms, even to humans. Again, this possibility is remote. Moreover, the possibility of horizontal transfer of the effector gene to the germ cells of another organism is even more remote. Finally, most (if not all) effector genes being considered should be innocuous to higher organisms.

v) Political, social and ethical considerations

In addition to scientific concerns, it is important to address concerns of public perception. The issues and debates over genetically modified crops and other attempts of insect releases provide a useful precedent from which we should learn. It will be important to inform the public at large about the benefits and risks of the transgenic technology. The time to start is now, because full public awareness of these issues may take an entire generation to accomplish. The targets of such a campaign must include residents of the affected countries and their government officials. The results of safety tests should be openly and broadly divulgated. It is important to emphasize that no approach will be entirely risk-free and that the balance between potential benefit and risk should prevail in deciding about implementation of a new genetic strategy. It is also crucial to emphasize that any single approach is unlikely to be completely effective on its own, and that a final solution will have to incorporate a combination of several weapons, such as drugs, insecticides, bed nets, and hopefully vaccines and genetic modification of vector competence.

Priorities

Following are some current research priorities.

- First and foremost, a method to drive effector genes into field mosquito populations needs to be devised.
- The efficiency of *An. gambiae* transformation needs to be improved.
- Anopheline mosquitoes cannot be stored frozen or desiccated. Establishment of repository centres for transgenic lines is desirable.
- Whenever possible, work should be conducted with the organisms that are most relevant to human disease (*An. gambiae*, *P. falciparum*).

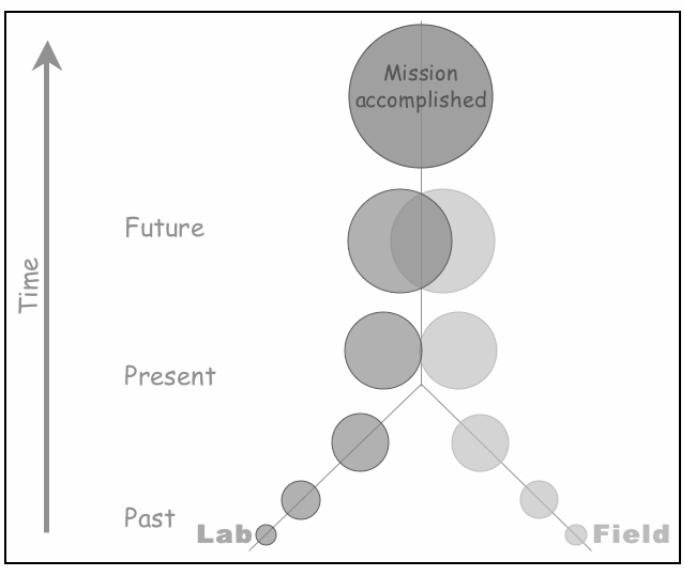

Figure 1. Laboratory and field scientists have mostly worked independently of each other in the past. However, the task of implementing malaria control via genetic manipulation of mosquitoes is so large that only close interactions and collaboration among investigators in the different lab and field disciplines will allow the final goal to be attained.

Prospects

During an historical 1991 meeting sponsored by the MacArthur Foundation and the WHO/TDR in Tucson, Arizona, a consensus was reached that the genetic manipulation of mosquito vectorial competence is an important and attainable goal. Hard work by an emerging community of mosquito molecular biologists delivered the first germ-line transformation in 1998, followed in rapid succession by the development of the necessary tools and by the proof-of-principle demonstration that the approach is feasible. At the same time, field researchers have made fundamental discoveries concerning population structure and vector ecology. Clearly, implementation of the genetic modification of vector competence that was considered in this article will depend on close collaboration among many groups of scientists with a broad range of lab- and field-based expertise (Figure 1). The task ahead of us is so big that it is inconceivable to proceed otherwise. While major lab and field issues remain to be solved, the rapid progress made to date gives one reason to be optimistic that this important new weapon (genetic modification of vector competence) will be ready for field testing within the next decade or so.

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