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Review

Towards genetic manipulation of wild mosquito populations to combat malaria: advances and challenges

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Summary

Malaria kills millions of people every year, yet there has been little progress in controlling this disease. For transmission to occur, the malaria parasite has to complete a complex developmental cycle in the mosquito. The mosquito is therefore a potential weak link in malaria transmission, and generating mosquito populations that are refractory to the parasite is a potential means of controlling the disease. There has been considerable progress over the last decade towards developing the tools for creating a refractory mosquito. Accomplishments include germline transformation of several important mosquito vectors, the completed genomes of the mosquito *Anopheles gambiae* and the malaria parasite *Plasmodium falciparum*, and the identification of promoters and effector genes that confer resistance in the mosquito. These tools have provided researchers with the ability to engineer a refractory mosquito vector, but there are fundamental gaps in our knowledge of how to transfer this technology safely and effectively into field populations. This review considers strategies for interfering with *Plasmodium* development in the mosquito, together with issues related to the transfer of laboratory-acquired knowledge to the field, such as minimization of transgene fitness load to the mosquito, driving genes through populations, avoiding the selection of resistant strains, and how to produce and release populations of males only.

Key words: *Plasmodium*, genetic engineering, paratransgenesis, genetic drive mechanisms, genetic sexing, fitness, mosquito.

Introduction

Malaria is a devastating disease that kills between one and three million people annually and causes massive economic losses. Moreover, the number of cases is rising, due to the emergence of drug-resistant parasites and insecticide-resistant mosquitoes, although intensive research to develop new drugs and insecticides is ongoing. Furthermore, despite promising developments, no effective vaccines have yet been developed and existing control measures are inadequate. Mosquitoes are obligatory vectors for the disease and this part of the parasite cycle represents a potential weak link in transmission. Therefore, control of parasite development in the mosquito has considerable promise as a new approach in the fight against malaria.

Development of the parasite in the mosquito is complex (Fig. 1; Ghosh et al., 2003) and for the most part occurs in the midgut (gamete to oocyst stages). Although thousands of gametocytes are acquired with the blood meal, only a few successfully mature into oocysts, but each of them produces thousands of sporozoites (Ghosh et al., 2001). Because oocyst formation is a bottleneck in sporogonic development, targeting pre-sporozoite stages could be a more effective strategy to block parasite transmission.

In recent years, methods for the genetic modification of mosquitoes have been developed, and effector genes whose products interfere with *Plasmodium* development in the mosquito are beginning to be identified. While many of the initial hurdles have been overcome, major questions remain to be answered, foremost among which is how to introduce refractory genes into wild mosquito populations. Here we review strategies to alter mosquito vector competence and consider issues related to translating this knowledge to field applications.

Strategies to interfere with *Plasmodium* development in the mosquito

Transmission blocking vaccines

Transmission blocking vaccines consist of antibodies that are ingested by the mosquito with the blood meal and interfere with parasite development. Proteins expressed on the surface of gametes (e.g. Pfs47/48, Pfs230) and ookinetes (e.g. Pfs25 and Pfs28) have been tested for such vaccines (Carter, 2001; Healer et al., 1999; Duffy and Kaslow, 1997). Antibodies

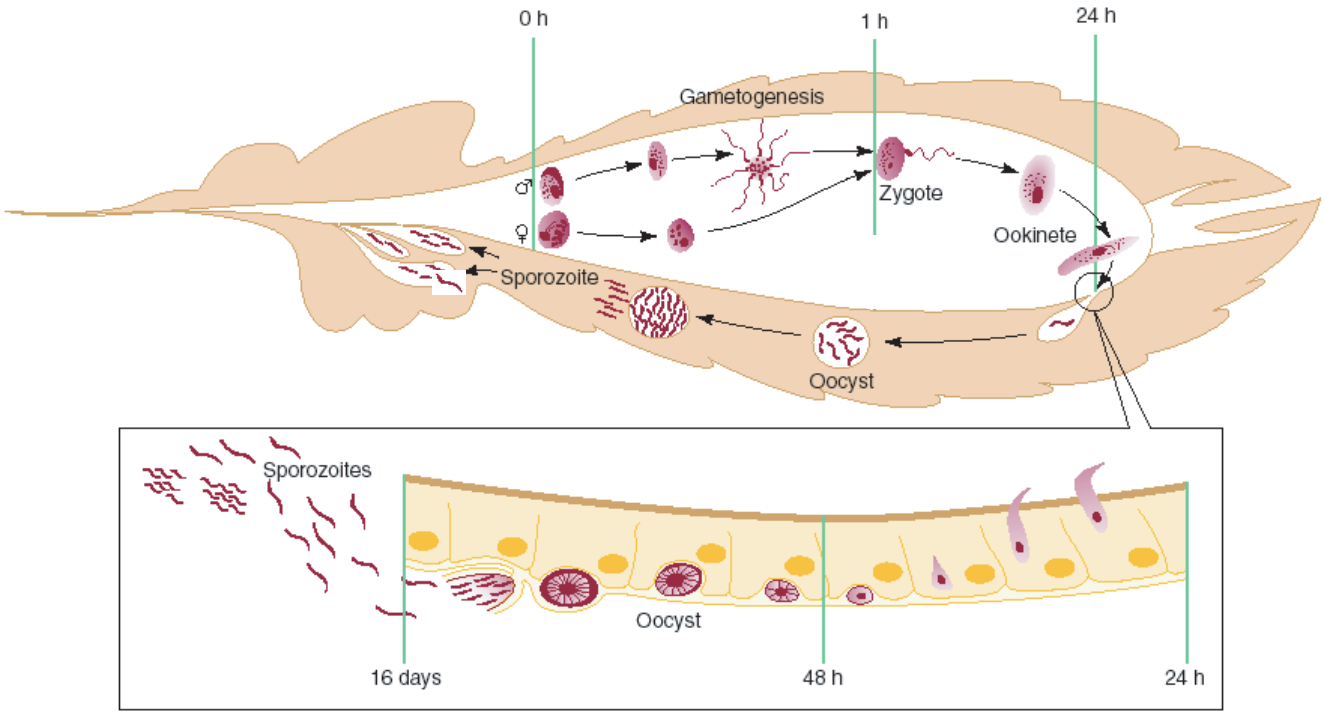

Fig. 1. Life cycle of *Plasmodium* in the mosquito. The approximate developmental time at which each stage occurs in *Plasmodium berghei* (maintained at 20°C) is indicated. Transmission starts when the mosquito ingests an infected bloodmeal (0 h). Within minutes, gametocytes develop into gametes (the star-shaped figure illustrates exflagellation, which is the formation of male gametes) that fuse to form the zygote. At 24 h, the motile ookinete invades the midgut epithelium and differentiates into an oocyst. About 2 weeks later, the oocyst ruptures, releasing thousands of sporozoites into the mosquito body cavity. Of all the tissues that sporozoites come in contact with, they can invade only the salivary gland. When the mosquito bites another vertebrate host, transmission is completed by release of sporozoites from the salivary glands (not shown). Reprinted from Ghosh et al. (2003), with permission from Elsevier Science.

against these proteins bind to the parasite and presumably block ookinete invasion of the midgut epithelium. Alternatively, mosquito midgut antigens could be targeted. Various reports that polyclonal antibodies against mosquito midgut proteins interfere with *Plasmodium* oocyst formation have been published (Ramasamy and Ramasamy, 1990; Srikrishnaraj et al., 1995; Lal et al., 1994, 2001), but in no case have the relevant antigens been identified (Jacobs-Lorena and Lemos, 1995). It should be noted that transmission blocking vaccines do not protect the immunized individual but act by preventing infection of people in the surrounding community. Thus, for ethical reasons and for increased effectiveness, transmission blocking antigens will have to be incorporated into conventional vaccines that target the vertebrate stages of the parasite.

Paratransgenesis

Paratransgenesis, the genetic manipulation of commensal or symbiotic bacteria to alter the host's ability to transmit a pathogen, is an alternative means of preventing malaria transmission. Bacteria can be engineered to express and secrete peptides or proteins that block parasite invasion or kill the parasite in the midgut. This strategy has shown promise in controlling transmission of *Trypanosoma cruzi* by *Rhodnius prolixus* under laboratory conditions (Beard et al., 2002). Furthermore, symbiotic bacteria in the tsetse fly have been isolated, transformed with a reporter gene, and reinserted into the fly (Beard et al., 1998). For this strategy to be used in malaria control, bacteria that can survive in the mosquito's midgut must be identified. Gram-positive and -negative bacteria, including *Escherichia*, *Alcaligenes*, *Pseudomonas*, *Serratia* and *Bacillus*, have been identified in the midgut of wild anopheline adults (Demaio et al., 1996; Straif et al., 1998). These bacteria are easily cultured in the laboratory and may be suitable targets for genetic manipulation. Whether these bacteria are stable or transient residents of the midgut of adult mosquitoes remains to be determined.

To successfully control malaria the refractory proteins or peptides expressed by the bacteria must act on the midgut stages of the malaria parasites, maintain their bioactivity in the midgut environment, and be expressed in sufficient quantities. When *An. stephensi* mosquitoes were fed *E. coli* that express a fusion protein of ricin and a single-chain antibody against Pbs21 (a *P. berghei* ookinete surface protein), oocyst formation was inhibited by up to 95% (Yoshida et al., 2001). Other effector molecules, such as SM1 and PLA2, are considered below (see 'Genetically modified mosquitoes'). The use of paratransgenesis for the control of malaria will require the development of methods

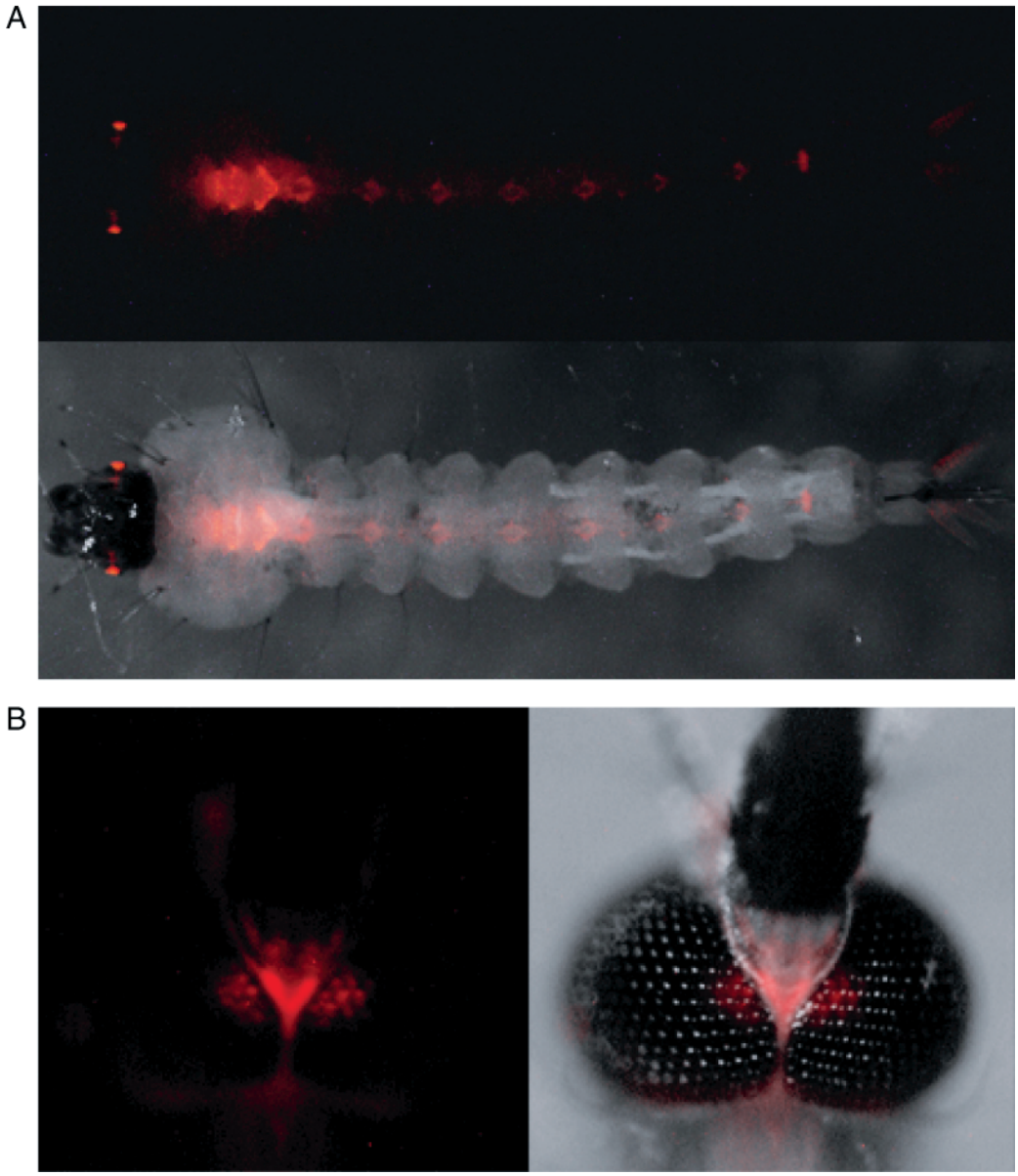

Fig. 2. Transgenic *An. stephensi* mosquitoes expressing the dsRED marker. (A) Fluorescence photomicrographs of transgenic fourth instar larva, viewed from the ventral side. Top, dsRED fluorescence alone; bottom, fluorescence superimposed on a light micrograph. dsRED expression is regulated by the 3xP3 promoter, which is active in the eyes and nervous system. (B) Fluorescence photomicrographs of an adult head viewed from the ventral side (left, fluorescence only; right, merged fluorescence and light micrograph). Note that all ommatidia express the dsRED marker, but because fluorescence depends on the angle of the incident (activating) light, only a few are visible in the photo.

to introduce genetically modified bacteria into field mosquitoes.

Genetically modified mosquitoes

Another promising approach is to genetically modify mosquitoes to express proteins or peptides that interfere with *Plasmodium* development. Methods to produce transgenic culicine (Jasinskiene et al., 1998) and anopheline (Catteruccia et al., 2000; Grossman et al., 2001) mosquitoes are now available (Fig. 2). Promoters to drive the transgenes and effector molecules whose products hinder parasite development are considered below.

Promoters

An essential step in engineering mosquitoes with reduced vector competence is the identification of suitable promoters to drive the expression of anti-parasitic genes. During its development in the mosquito, the parasite occupies three compartments: midgut lumen, hemocoel and salivary gland lumen. Thus, promoters that drive synthesis and secretion of

proteins into these compartments need to be identified. In addition to spatial considerations, the time of protein synthesis relative to arrival of the parasite in each of these compartments needs to be considered.

As argued above, control of transmission has the best chance of success if pre-sporozoite stages in the midgut lumen are targeted. Studies in our laboratory demonstrated that carboxypeptidase, a digestive enzyme, and AgAper1, a peritrophic matrix protein, are activated in response to a blood meal and the proteins secreted in the midgut lumen (Edwards et al., 1997, 2000; Moreira et al., 2000; Shen and Jacobs-Lorena, 1998). Interestingly, transgenes driven by the AgAper1 promoter produce proteins that are stored in secretory vesicles of midgut epithelial cells prior to a blood meal and are released immediately after blood ingestion (E. G. Abraham, M. Donnelly-Doman, H. Fujioka, A. Gosh, L. Moreira and M. Jacobs-Lorena, unpublished observations). This makes AgAper1 an ideal promoter to target the earliest stages of parasite development.

Later stages of parasite development can be targeted in the hemocoel and salivary glands. The vitellogenin promoter and signal sequences were shown to drive strong gene expression in the fat body and protein secretion into the hemocoel (Kokoza et al., 2000). However, this gene has a restricted temporal profile of expression that peaks around 24 h after a blood meal and returns to basal level by 48 h. Soon after traversing the midgut epithelium, the ookinete transforms into an oocyst that is covered by a thick capsule. Sporozoites are liberated from the oocyst as early as 10 days later. These characteristics of parasite development limit the choice of effector genes that can be used in conjunction with the vitellogenin promoter to those encoding proteins with exceptionally long half-lives. Re-activation of the vitellogenin promoter by additional blood meal(s) may lessen this shortcoming. The availability of a strong promoter with peak expression in the hemolymph at about the time of sporozoite release from oocysts would be ideal. Two salivary gland promoters have been characterized in transgenic mosquitoes: Maltase-I and Apyrase (Coates et al., 1999). However, expression from both promoters was rather weak, which may limit their usefulness. Considering that sporozoites can reside in the lumen of the salivary gland for extended periods, the identification of a strong salivary gland promoter would be desirable. Finally, mosquito promoters induced by the presence of the parasite, such as those of immune genes, are of potential usefulness (Dimopoulos et al., 2002; E. G. Abraham, M. Donnelly-Doman, H. Fujioka, A. Gosh, L. Moreira and M. Jacobs-Lorena, unpublished observations).

Strong and ubiquitous promoters could also be used to drive the expression of effector genes. However, such promoters are not ideal because generalized expression may impose a fitness load on the mosquito (this point is also considered below) and even promoters considered ubiquitous (e.g. actin) are not equally expressed in all cells.

Effector genes

The quest for anti-parasite molecules has been directed towards identification of gene products that hinder transmission by either killing or interfering with parasite development. For example, transgenic mosquitoes expressing defensin, an anti-microbial peptide, effectively killed gram-negative bacteria, though defensin's action on malaria parasites has not been documented (Kokoza et al., 2000). Alternatively, single-chain antibodies that recognize parasite surface proteins show promise in interfering with parasite development. As mentioned above, an anti-Pbs21 single chain antibody inhibited oocyst formation by up to 95% (Yoshida et al., 2001). A single-chain antibody against *P. gallinaceum* Circunsporozoite protein (CSP), a major surface protein of sporozoites, expressed from a Sinbdis virus vector, reduced the number of parasites in the salivary glands by 99% (de Lara-Capurro et al., 2000).

Although its mode of action is unknown, phospholipase A2 (PLA2) from a variety of sources significantly inhibited ookinete invasion of the mosquito midgut epithelium when mixed with an infected blood meal (Zieler et al., 2001). Moreira et al. (2002) have shown that transgenic mosquitoes expressing bee venom PLA2 from the carboxypeptidase promoter reduced *P. berghei* oocyst formation by 87%. Transgenic *An. stephensi* expressing PLA2 from the AgAper1 promoter inhibited oocyst formation to about the same extent (E. G. Abraham, M. Donnelly-Doman, H. Fujioka, A. Gosh, L. Moreira and M. Jacobs-Lorena, unpublished observations).

An alternative strategy is to use synthetic molecules to interfere with parasite development. Ghosh et al. (2001) screened a phage display library for protein domains that bind to the midgut and salivary gland epithelia and identified a short peptide, named SM1, which inhibited parasite invasion. Furthermore, *An. stephensi* engineered with a synthetic gene expressing a SM1 tetramer under the control of the carboxypeptidase promoter were impaired in supporting *P. berghei* development (average 82% inhibition of oocyst formation). Moreover, in two out of three experiments, parasite transmission by the transgenic mosquitoes was completely blocked (Ito et al., 2002). This inhibition is thought to occur by peptide binding to epithelial cell surface proteins (putative receptors) required for parasite invasion. The effectiveness of SM1 in inhibiting the development of parasites that cause malaria in humans remains to be demonstrated.

Although major advances have been accomplished in recent years, it is important that the search for new effector molecules and promoters continue for two reasons. First, considering how easily parasites acquire drug resistance, it is likely that parasites will be selected that can overcome the barrier imposed by the effector molecules. Secondly, maximum efficiency of blocking parasite development (ideally 100%) is important for the transgenic mosquito strategy to have a significant impact on disease transmission. Furthermore, while many of the tools for genetic modification of mosquitoes have been developed, an extensive gap exists in our ability to transfer this technology to the field for the control of malaria.

Challenges facing a successful field release

The fitness cost of refractoriness

To maximize the likelihood of successfully introducing refractory genes into a wild mosquito population, transgenes should impose minimal fitness load. We assessed fitness of transgenic *An. stephensi* expressing the SM1 and the PLA2 transgenes by a variety of criteria, including measurements of longevity and fertility, and use of population cages (L. A. Moreira, J. Wang, F. H. Collins and M. Jacobs-Lorena, manuscript submitted for publication). The SM1 transgene did not impose a detectable fitness load, but transgenic PLA2 mosquitoes had much reduced fertility and competed poorly with non-transgenics in cage experiments. The reasons for this reduced fitness remain to be investigated. Catteruccia et al. (2003) reported that four different transgenic mosquito lines expressing fluorescent reporter proteins from an actin promoter are less fit than the wild type. However, reduced fitness was most likely due to inbreeding. They isolated homozygous lines soon after transgenic mosquitoes were obtained, which may have caused recessive deleterious genes residing near the point of transgene insertion to become homozygous ('hitchhiking effect'). Conversely, in the experiments of Moreira et al., the transgenic mosquitoes were kept as heterozygotes, being continuously crossed to mosquitoes from laboratory population cages. This demonstrates the importance of mosquito outcrossing. In addition, the experiments of Catteruccia et al. (2003) used a transgene driven by the strong and ubiquitous actin promoter. The abundant synthesis of a foreign protein throughout the organism may conceivably have deleterious effects on fitness (Liu et al., 1999). For this reason, SM1 expression was restricted to posterior midgut cells for only a few hours after a blood meal and the protein was secreted from the cells, thus minimizing fitness load. Absolute absence of fitness load may not be essential for introducing genes into wild populations. Theoretical modeling suggests that given an appropriate drive mechanism, a gene could have a significant fitness cost and still be driven through the population (Ribeiro and Kidwell, 1994; Boete and Koella, 2003). This is fortunate, since this same model suggests that any released mosquitoes would need to be nearly 100% refractory to have any impact on malaria transmission, necessitating multiple refractory genes that may incur greater fitness costs.

Developing an effective drive mechanism

Two general strategies can be considered for introducing transgenic mosquitoes in the field: population replacement or a genetic drive mechanism. Population replacement, or inundatory release, requires a significant reduction of the resident mosquito population (for instance, with insecticides), followed by the release of large numbers of refractory mosquitoes to fill the vacated biological niche. This strategy is promising as a research tool and as a field test to assess the effectiveness of the transgenic mosquito approach for interrupting malaria transmission. However, this strategy cannot be considered for large-scale control purposes, because it is not possible to produce sufficient numbers of mosquitoes to achieve population replacement on a country- or continent-wide level.

An efficient genetic drive mechanism is helpful because a manageable number of genetically modified mosquitoes can replace the wild population, even if the effector gene(s) imposes some fitness cost. A crucial requirement for this approach is a tight linkage between the effector gene(s) and the drive mechanism. Any dissociation between the two would cause the effector gene to be lost. In contrast to insecticide control, this strategy involves replacement of one population by another, meaning that in principle, it needs to be accomplished only once. Transposable elements are an attractive mechanism to drive genes into populations. Unfortunately, little is known about their effectiveness. Compelling evidence exists in *Drosophila* that transposable elements can efficiently spread through largely distributed populations. For instance, the *P* transposable element spread through the *D. melanogaster* world population in the short span of about 30 years (Anxolabehere et al., 1988). Transposable elements such as *piggyBac*, *minos*, *mariner* and *hermes*, which have been successfully used to transform mosquitoes, need to be examined for their usefulness as drive mechanisms. For example, the *piggyBac* element is thought to have an efficient cut-and-paste mechanism and jump nonreplicatively, in a manner similar to *P* elements (Lobo et al., 1999). When *P* elements are excised the organism typically repairs the double stranded break in the chromosome using a recombinational repair system and uses the uncut chromosome as a template (Engels, 1997). In individuals homozygous for the *P* element this leads to a duplication of the element as it transposes to a new location in the chromosome. If a similar duplication mechanism exists for *piggyBac* it would act as an efficient and useful drive mechanism.

Transposable elements may incur a substantial fitness cost. Transposition causes random integration across the genome, some of which may disrupt genes and lead to mutations that could be lethal, reduce fecundity or decrease fitness. Predictive models suggest that transposable elements would be able to drive refractory genes from a small number of transgenic mosquitoes into the wild population even if a fitness cost was present (Ribeiro and Kidwell, 1994; Boete and Koella, 2003). However, there is considerable lack of experimental data to corroborate or disprove the models. Another consideration is that mobility of the transposable element may be negatively regulated by a repressor. For instance, mobility of the *P* element in *D. melanogaster* decreases after several generations because an inhibitor of transposition gradually accumulates and the fly is said to acquire the *P* (refractory) cytotype. This is of practical importance because in such cases the gene(s) can be driven through a population only once. If the effector gene(s) acquires mutations or the parasite becomes resistant to the effector gene product another gene cannot be driven into the same population with the same transposable element.

A second possible drive mechanism is the use of so-called 'selfish genes'. These genes drive themselves through a population by using the host cell DNA repair machinery (Burt,

2003). One such selfish gene, homing endonuclease gene (HEG), encodes an endonuclease that in heterozygous individuals cleaves the sister chromosome within a 20–30 bp recognition sequence. The chromosome encoding the HEG is not cut because the selfish gene disrupts the cleavage site. As described for the *P* element above, the cell's repair machinery subsequently duplicates the HEG by using the HEG^{+} chromosome as a template for recombination. A major advantage of using HEG as a drive mechanism is that it should allow the reversal of gene dispersal by introducing an HEG that targets and disrupts the anti-parasitic gene(s). Furthermore, the possibility of horizontal transmission is minimized because, unlike transposable elements, the gene is never excised from the chromosome. However, no precedent exists for the engineering of homing endonucleases to recognize the desired target sequence. For a detailed review of selfish genes as drive mechanisms see Burt (2003). While on theoretical grounds the approach is attractive, it is not ready for implementation.

A third potential drive mechanism to introduce an effector gene into a vector population involves the use of the symbiotic bacterium *Wolbachia*, which exerts its drive mechanism primarily through cytoplasmic incompatibility. When a male infected with *Wolbachia* mates with an uninfected female, most of the fertilized eggs perish. However, when an infected male mates with an infected female, the eggs hatch normally and the bacteria are transovarially transmitted to the next generation. This effect can drive *Wolbachia* through a population very effectively by giving infected individuals increased reproductive success. For example, a strain of *Wolbachia* was discovered in *Drosophila simulans* and found to have advanced across the state of California at a rate of 100 km per year (Turelli and Hoffmann, 1991). *Wolbachia* may have a wide tissue distribution in insects, allowing refractory genes to target the various *Plasmodium* life stages throughout the mosquito (Dobson et al., 1999). Three methods have been suggested to drive a transgene into a vector population using *Wolbachia*: (1) the refractory gene could be introduced directly into the *Wolbachia* genome, (2) the genes for cytoplasmic incompatibility plus the refractory gene could be integrated into the vector's genome, or (3) the refractory gene could be engineered into a second maternally transmitted organism that could 'hitchhike' with *Wolbachia* (Turelli and Hoffmann, 1999). Unfortunately, a *Wolbachia* has yet to be identified in wild anopheline mosquitoes, although they have been isolated from their culicine relatives (Ricci et al., 2002). If anopheline mosquitoes are physiologically unable to harbor *Wolbachia*, identifying the genes conferring cytoplasmic incompatibility and inserting them into the vector's genome might be feasible. If however, anophelines tend not to be infected because they have rarely been exposed to *Wolbachia*, all three strategies hold potential.

Regardless of the driving mechanism employed, it will be essential that the effector gene (that interferes with *Plasmodium* development) be tightly linked to the driving element. A dissociation of the two (for instance, by recombination) will cause the driving gene to continue to spread through the population alone while the effector gene is lost.

Mass production of transgenic insects and genetic sexing mechanisms

Transgenic-based methods to reduce or eradicate vector populations, such as the release of insects carrying a dominant lethal (RIDL; Thomas et al., 2000), show promise for some species. However, their use as a malaria control program in Africa would be difficult to implement due to reproductively incompatible subspecies and migration of mosquitoes among villages. Even if successful, this approach would leave an ecological vacuum that another malaria vector could quickly fill. Therefore, replacement of wild populations with transgenic mosquitoes carrying refractory genes instead of population suppression or eradication methods would be more appropriate.

Unfortunately, this approach still requires the release of vast numbers of biting insects, which is ethically questionable due to their nuisance factor and potential role as vectors for secondary diseases. Thus, widespread release of genetically modified mosquitoes is best done using only non-biting males, necessitating an efficient system for male selection. Moreover, the ability to release only males would provide a more realistic prospect of making the use of transgenic mosquitoes acceptable to the local communities and to the public in general.

Males of a few insect species can be separated by physical methods, based on sexual dimorphism (Sharma et al., 1976; Alphey and Andreasen, 2002). However, these techniques do not easily translate to most vector species, including anopheline mosquitoes, and rarely give 100% sex separation. An alternative approach is to use genetic techniques for sex-sorting, known generically as genetic sexing mechanisms (GSMs). It allows males and females to be produced under one set of conditions to allow propagation and males only under selective conditions. GSMs based on the radiation-induced translocation of semi-dominant genes for insecticide resistance onto the Y chromosome were developed in *An. gambiae* and *Anopheles albimanus* (Curtis et al., 1976; Seawright et al., 1978). This system was used for the production of a million *An. albimanus* sterile males per day (Dame et al., 1981), but introducing insecticide resistance into the wild populations is too risky for field applications. Moreover, genetic crossing over between the relevant genes may occur, disrupting the production of a single sex population (Curtis, 2002).

Transgene-based GSMs using conditional female-specific lethality systems, based on the tetracycline-repressible expression system of Gossen and Bujard (1992), show great promise and were successfully tested in *Drosophila* (Thomas et al., 2000; Heinrich and Scott, 2000). In this system, two genes are engineered into the insect: one that expresses a tetracycline-repressible transcriptional activator protein (tTA), and a tetO promoter element linked to a lethal gene. In the absence of tetracycline, tTA binds to tetO leading to expression

of the lethal gene and death of the insect. When tetracycline is added to the diet tTA fails to activate the tetO promoter and allows the insects to survive and propagate. Female-specific lethality can be achieved in two ways: the lethal gene itself can be female-specific, or a female-specific promoter can be used to drive tTA expression, in turn inducing expression of the toxic gene. Unfortunately, using a repressible system to generate males for a large-scale field release would incur a massive fitness cost, since refractory females possessing the female-specific lethality system would be immediately killed in the wild and thus would not contribute to the gene pool. For this reason, a better approach would be to use an inducible system rather than a repressible one.

An inducible female-specific lethality system prevents expression of the lethal gene in the absence of induction, allowing male selection prior to release, but permitting subsequent generations of females to survive in the wild. A tetracycline-inducible expression system developed by Baron and Bujard (2000) has the same components as the tetracycline-repressible expression system, except a mutated version of tTA that binds to tetO only induces the expression of the lethal effector gene in the presence of tetracycline.

Avoiding resistance to the refractory genes

Parasites facing a refractory mosquito population would be under strong selective pressure, similar to the one posed by anti-malarials, and thus resistance may develop. Engineering a mosquito with multiple refractory genes that target different aspects of parasite development could minimize resistance to the refractory genes. For example, a transgenic mosquito might be engineered to express a peptide to disrupt midgut and salivary gland invasion, have an enhanced encapsulation response to target the oocyst, and express defense peptides to target the sporozoites. Furthermore, chances of success will be greatly increased if each refractory element is as close to 100% effective as possible and if introduction of the refractory genes is coupled with traditional control methods, such as reduction of wild populations with insecticides prior to a transgenic release, drug treatment of infected individuals, and use of bed nets.

The effectiveness of transposable elements may decrease with time after field release. Immediately after the introduction of a novel transposable element into a population the element enjoys a period of unrestrained activity and spreading. Eventually, individuals with mutations in the transposase or those that have enacted regulatory inactivation of the element will be selected. Transposase silencing has been well studied in the mariner family and has been hypothesized to occur by several mechanisms, including overproduction inhibition whereby an increase in transposase activity correlates with decreased transposition or random transposase mutations. Random transposase mutations may lead to open reading frame disruptions and inactive transposases that compete with active transposase for substrate (competitive inhibition) or reduce the activity of wild-type transposase (dominant negative complementation; Hartl et al., 1997a,b; Tosi and Beverley, 2000). The mechanism of transposable element silencing will need to be well understood before transposable elements are used in the field.

Conclusions

Major advances in recent years, including successful germline transformation and characterization of promoters, are allowing researchers to test putative refractory genes. One important task for the near future is the identification of additional effector genes, and this will be greatly facilitated by the availability of the *An. gambiae* and *P. falciparum* genome sequences. This knowledge can be used to engineer a mosquito that inhibits or kills the malaria parasite during multiple developmental stages. With this ideal mosquito on the horizon, the most important task is to begin laying the groundwork for its introduction into the wild. A high priority should be devoted to the topic of how to introduce the relevant genes into wild mosquito populations. Also needed are ecological studies to evaluate population structure and gene flow. In addition, we must grapple with the ethical and political concerns involved with a large-scale release of a genetically modified organism. Considerable challenges lay ahead but there are reasons to be optimistic that we will be able to add genetic modification of mosquitoes to our arsenal in the fight against malaria.

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