13

Evaluation of drive mechanisms (including transgenes and drivers) in different environmental conditions and genetic backgrounds

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Abstract

Three major objectives, develop viable gene drive mechanisms, identify the epidemiologically significant vectors of pathogens in specific transmission zones, and introgress effector genes into specific populations, must be met in order to move to the field laboratory advances in genetic control strategies.
Keywords: genetic control; mosquitoes; gene drive; population replacement

Current state of the art

A long-term plan was put forward some years ago to research the potential of genetically engineered mosquitoes for the control of disease transmission (Meredith and James 1990; WHO 1991; James 1992). The hypothesis for this research is that increasing the frequency of a gene (or allele) in a population of mosquitoes that interferes with the development or propagation of a pathogen will result in the reduction or elimination of transmission of that pathogen to humans. Furthermore, it is expected that a reduction in transmission will result in less disease. This hypothesis derives from the concept of population replacement in which vectors competent to transmit a specific pathogen are replaced or displaced by ones that cannot transmit (Curtis and Graves 1988). This hypothesis is to be tested by using molecular-biological tools to synthesize genes that result in non-vector phenotypes when incorporated into the genome of mosquitoes. These genes are to be coupled to a drive mechanism such that release of the genetically engineered mosquitoes results in the spread of the anti-pathogen gene through a target vector population. Following

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implementation of this strategy, there should be measurable decreases in transmission and disease.

Research in three areas was identified as being important to test this genetic-control hypothesis (James et al. 1999). First, in laboratory-based work, it must be shown that it is possible to engineer mosquitoes that are refractory to the transmission of parasites. Second, methods must be identified for moving genes made in the laboratory into wild mosquito populations. Strategies that rely on Mendelian genetics to increase gene frequencies in populations require fitness advantages tightly linked to the anti-pathogen gene and are likely to be too protracted in time to sustain support and interest (Collins and James 1996; Braig and Yan 2002). However, concepts have been developed of genes spreading in an infection-like wave through mosquito populations, and there is enthusiasm for using transposable elements or other mobile nucleic-acid vectors (Kidwell and Ribeiro 1992) and bacterial symbionts (Curtis and Sinkins 1998) to drive genes. The third research area provides the information about target vector populations and disease transmission dynamics that is needed to model and predict how anti-pathogen genes will affect the epidemiology of a disease in a specific endemic region. This is important for both the introduction of genes and for establishing the parameters by which the success of introductions will be measured. The genetic structure, population migration indices, gene flow and other population-genetic factors of the vectors will influence the outcome of a genetic strategy, and the effects of these factors on introduced genes in the target population must be anticipated and integrated. Threshold values for entomological inoculation rates, the fraction of the vector population that must be made pathogen-resistant, and the effect of immune states of people living in disease-endemic areas also must be considered. As a final measure, the most significant outcome of research efforts in all three of these areas will be a reduction or elimination of human disease as a result of interrupting transmission of the target pathogen.

The demonstration of the function of isolated promoters (Coates et al. 1999; Kokoza et al. 2000; Moreira et al. 2000), the development of transgenesis technologies (Jasinskiene et al. 1998; Coates et al. 1998; Catteruccia et al. 2000; Allen et al. 2001; Grossman et al. 2001; Kokoza et al. 2001; Lobo et al. 2002; Perera, Harrell II and Handler 2002) and the identification and synthesis of potential anti-pathogen effector genes (Olson et al. 1996; De Lara Capurro et al. 2000; Ghosh, Ribolla and Jacobs-Lorena 2001; Moreira et al. 2002; Ito et al. 2002; Osta, Christophides and Kafatos 2004; Blandin et al. 2004) complete the proof-of-principle for the laboratory-based research area. What is required next for this area is to transfer the methodology and approaches developed in animal models of disease to mosquito–pathogen interactions relevant to humans, and this work is ongoing. Important for the context of this book, the challenges of the second research area must be engaged by designing safe (low risk-to-benefit ratio) and efficient mechanisms for driving genes through vector populations.

Issues and challenges

Movement of laboratory-developed genes into wild populations requires addressing three major issues that need innovation and research. The first is to develop viable gene drive mechanisms that can be used to move genes into target populations. Strategies based on competitive displacement, reduced heterozygote fitness and meiotic drive have been analysed and arguments favouring some approaches over others have been made (Braig and Yan 2002). Properties of an ideal

gene-driving system are being identified (Braig and Yan 2002; James 2005) and these have generated robust debate. For example, whether or not a transgene inherently confers a fitness load on the mosquito is controversial. While Catteruccia, Godfray and Crisanti (2003) and Irvin et al. (2004) detected fitness load in mosquitoes *homozygous* for a transgene, Moreira et al. (2004) detected no significant fitness load in *heterozygous* transgenic mosquitoes expressing a tetramer of the SM1 dodecapeptide. The experiments in the former two reports could not distinguish whether the fitness load was imposed by homozygosity of nearby deleterious recessive genes (cf. 'founder effect', 'hitchhiking effect') or by the transgene itself. This is not an issue in the Moreira et al. (2004) experiments because transgenic mosquitoes were backcrossed at each generation, with wild-type mosquitoes. Thus, transgenic and wild-type controls had similar genetic backgrounds. Specific options for gene drive mechanisms include extracellular and intracellular symbionts, viruses and transposons. Extracellular bacterial symbionts ingested during coprophagy have proven remarkably efficient in spreading exogenous genes through reduviid bugs in an effort to control Chagas' disease (Beard, Cordon-Rosales and Durvasula 2002). In principle, a similar approach could be used to deliver effector genes to the mosquito midgut. In preliminary experiments, the Jacobs-Lorena laboratory constructed recombinant *E. coli* expressing an SM1-Omp (Outer membrane protein) fusion protein and showed that mosquitoes that had ingested these bacteria were impaired in transmission of the malaria parasite *P. berghei*. Advantages of such 'paratransgenesis' approach include: 1) logistics are simpler (bacteria can be genetically manipulated with ease, they can be cheaply grown in large quantities); 2) the approach is compatible with the concomitant use of insecticides in the target area; 3) many effector genes can be delivered simultaneously using a mixture of transgenic bacteria; and 4) the nature of the effector genes can be changed at any time during the control programme. A major unresolved issue is that no one knows how adult mosquitoes acquire their bacterial flora and therefore, a strategy to deliver the genetically modified bacteria to the mosquitoes in the field has yet to be developed. Intracellular symbionts, such as the *Wolbachia* species (Curtis and Sinkins 1998), and the endosymbionts of tsetse (Aksoy 2000), hold some promise, but a current lack of efficient *ex vivo* manipulation presents challenges to their further development for mosquitoes (Noda, Miyoshi and Koizumi 2002; O'Neill et al. 1997). However, the extent to which these agents cause cytoplasmic incompatibility may make them useful in population reduction strategies of genetic control (Curtis and Sinkins 1998). A number of viruses have been identified that may provide the basis of a gene drive system. Mosquito densoviruses appear particularly attractive because of their apparent inability to infect non-target organisms (Afanasiev and Carlson 2000). Many of these viruses are lethal to insect larvae and seem more appropriate to develop as a population reduction tool to be used in conjunction with other anti-vector strategies for local eradication of a target vector population. Class-II transposable elements (those that transpose via a DNA intermediate (Finnegan 1985)) also are attractive as the basis for a gene drive mechanism because of their ability to excise and insert (mobilize) in DNA. Transposons are spread through populations by replicative transposition in the germ line and this process circumvents standard Mendelian inheritance (Kidwell 1983; Collins and James 1996). Thus, we have a number of requirements and candidate mechanisms available for gene drive mechanisms.

The second major issue is to recognize that not all mosquitoes are the same, and that this has a significant influence on the transmission dynamics of specific pathogens. For example, some of the most efficient vectors of malaria, *An. gambiae*

and *An. funestus*, each consist of a group of closely related, highly anthropophilic sibling species that adapted genetically to different ecological niches (Coluzzi et al. 2002). These mosquitoes have different larval habitats with the former exploiting transient water sources created by seasonal rains or agricultural irrigation, and the latter favouring permanent sites found in pools in stream beds, in small ponds, marshes and long-term flooded rice fields. The complementary life cycles of these two mosquitoes are sufficient to maintain malaria transmission throughout the year in many areas. *Aedes aegypti* is by far the most important and efficient vector of dengue viruses also because of its affinity for humans (Gubler 1998). Within a specific transmission environment, little intraspecific variation is observed. Immature mosquitoes develop primarily in man-made containers near human dwellings and adult females rest indoors where they feed frequently and preferentially on human blood. Extended flight can be limited because food, mates and oviposition sites are readily available within or near human habitations (Edman et al. 1998; Harrington et al. 2001). This makes possible explosive dengue epidemics even when mosquito population densities and DV entomological thresholds are low (Kuno 1995; 1997; Focks et al. 2000; Scott et al. 2000). Thus, before it is possible to expect a positive outcome of a genetic intervention strategy it is necessary to define for a specific locale the exact mosquitoes that are targets of the intervention. If malaria transmission is observed in different environmental conditions, it is likely that different species or subspecies (genetically isolated sympatric populations) may be the primary vectors. Furthermore, different vectors are important at different times of the year and, therefore, a comprehensive approach for utilizing population replacement strategies must target all the important vectors within a given area.

The third major issue is that it is likely that laboratory-derived mosquitoes will differ significantly from the target insects in the field. Fitness factors that affect fecundity, fertility and mate choice are likely to drift as they adapt to selection pressures in the laboratory. Therefore, it is preferable to develop procedures that introgress the important genes into strains of mosquitoes that are as similar as possible to the targets in the field. Following up on the discussion above, particular attention must be paid to the time of year that the release will take place so that the release mosquitoes match as much as possible the correct seasonal target. Thus the major challenges are to be able to develop a viable gene drive mechanism, define as much as possible what are the important vectors of a the target disease and determine how the drive mechanisms and effector molecules are going to be introduced into those specific populations.

Research and control opportunities

The research opportunities follow directly from the challenges listed above. The research of drive mechanisms will follow a path of studying natural or synthetic genetic phenomena that alter segregation ratios to spread specific genes or alleles through populations. From these phenomena, specific drive systems must be designed that incorporate features that mitigate concerns about the release of genetically modified organisms. Such drive systems must be demonstrated first to work in laboratory experiments and progress to large cage trials in the field. The systems then must be introgressed into field-derived mosquito strains and evaluated for their reproductive and competitive success. This can only be done based on a description of the target mosquitoes in the proposed release area.

Future directions for research and capacity/partnership building

The future direction of research in this area overlaps those described in Chapter 12. All future work should emphasize the enlistment and involvement of DEC personnel to foster the level of awareness required for workable guidelines for the evaluation and application of genetic control programmes. Furthermore, a research agenda that requires a DEC component should include experiments to demonstrate that drive sstems can spread an effector through caged populations of mosquitoes, introgress laboratory-derived gene drive systems into target populations recently derived from the wild, demonstrate drive systems in field cage settings, determine the effects on life-history traits of drive systems in field cages, and determine the possibility of movement in field cages of drive mechanisms among closely related species.

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