Green light for mosquito control

Peter W Atkinson

Testes-specific expression of green fluorescent protein allows automated sex sorting of mosquitoes for population control.

Pest insects have a seemingly infinite ability to frustrate technologies used to control them. A powerful alternative to chemical insecticides, the Sterile Insect Technique (SIT)[1] is an area-wide control strategy based on the release of hundreds of millions of sterile insects that can wipe out an insect population through 'sterile mating,' a process that results in no offspring. In this issue, Catteruccia *et al.*[2] describe an improved method for sexing mosquitoes, a critical step in mosquito SIT programs, that demonstrates the utility of applying genetic engineering in mosquito control programs. Their achievement moves biotech approaches to pest insect control several steps closer to reality.

Insects, with their large numbers and ability to disperse, provide an ideal setting in which natural selection can generate new strains resistant to chemical insecticides. We, on the other hand, have been quite slow in developing alternative technologies for insect control. Although the application of genetic technologies would appear to hold much promise, until recently there has been a lack of genetic tools for direct use in pest insects. Indeed, this task represents a significant engineering challenge. Gene delivery, gene expression, gene stability and other components must each be optimized and then integrated to produce a modified pest strain that performs as expected in the field. Risks associated with genetically modified organisms and their release into the field must be minimized, and their benefits, such as increased farm productivity or decreased disease transmission, must be apparent, sustainable and rapidly achieved.

Peter W. Atkinson is in the Department of Entomology, University of California, 900 University Avenue, Riverside, California 92521-0314, USA.
e-mail: peter.atkinson@ucr.edu

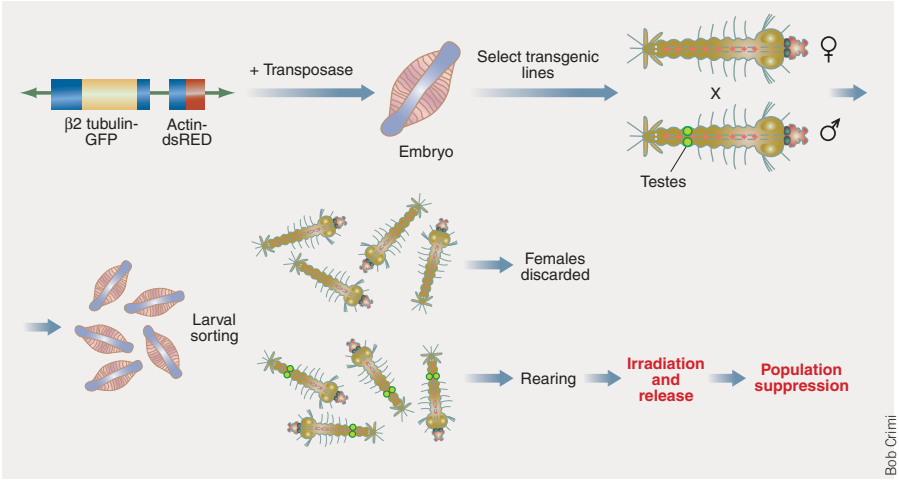

Figure 1 A transposable element containing the β2-tubulin-GFP gene together with the dsRed genetic marker placed under the control of the actin promoter is microinjected into *A. stephensi* embryos in which the transposable element integrates into the germ line. Adults developing from microinjected embryos are backcrossed, and progeny are screened for expression of the dsRed marker, which is constitutively expressed in most tissues. Typically less than 10% of progeny are transgenic. Transgenic lines are then established and sex-specific expression of GFP examined. All GFP-expressing individuals are male; all non-GFP–expressing larvae are female. Transgenic larvae can then be automatically sorted on the basis of testes-specific GFP expression using a fluorescence-activated sorter. Male larvae are saved for further rearing whereas female larvae are discarded. Future incorporation of this technology in SIT programs would require sterilization of the males and then release, resulting in pest population suppression or eradication.

None of these challenges is by itself insurmountable, but the trick lies in integrating the solutions.

SIT is a potent area-wide genetic control strategy that can reduce and even eliminate insect populations through the mass rearing and releasing of hundreds of millions of sterile insects to mate with the wild population. Initially proposed by Knipling for controlling New World screwworm in the Americas[1], it has achieved outstanding success, eliminating this devastating livestock and medical pest from the United States, Mexico and much of Central America to Panama. The same approach has since been used to control Mediterranean fruitfly outbreaks in many regions of the world.

In SIT programs such as these, in which males mate repeatedly while females mate only once, release of sterile males is all that is required to drive a population to extinction. The accidental release of sterile females has little impact on the efficacy of these programs but can still be detrimental, as irradiated, sterile Mediterranean fruitflies lay eggs in fruit, leading to spoilage.

In the case of mosquito SIT programs, release of even sterile females is to be avoided at all costs because sterile females are still capable of transmitting pathogens among

human hosts. One therefore seeks to rear and release only males, a process called 'sexing.' The elimination of females as early as possible in the rearing process has the additional advantage of reducing rearing costs.

Catteruccia *et al.* propose a simple and elegant solution to efficient mosquito sexing (**Fig. 1**). First, using transgenic lines of the human malaria vector *Anopheles stephensi*, they show that the expression of a reporter gene encoding green fluorescent protein (GFP) can be localized to the testes and that the sexes can be identified as early as late third instar larvae. Testes-specific expression intensifies during fourth instar and is strongest in pupae. Manual scoring of adults emerging from GFP-sorted pupae is 100% accurate. This differential expression of GFP between males and females is further used to allow high-throughput automated sorting of the sexes, opening up the possibility of using this genetic technology to eliminate females during rearing in mosquito population control programs.

Sex-specific expression of GFP is achieved by placing this reporter gene under the control of β2 tubulin upstream and downstream regulatory sequences borrowed from another mosquito species, the African malaria mosquito *Anopheles gambiae*. The β2-GFP transgene is introduced into *A. stephensi* by transposable element–mediated genetic transformation. β2 tubulins are members of the tubulin gene family, which are essential components of the cellular cytoskeleton and the mitotic and meiotic spindles. Two types of tubulin, α and β, combine to form complete microtubulin fibers. There are several isoforms of β2 tubulins in *Drosophila melanogaster*, with the β2 isoform expression confined to the testes, where it participates in axoneme structure and most likely in the structure and function of the meiotic spindle[3]. Tubulins are conserved within and between species and, despite a 250-million-year divergence time between *Drosophila* and *Anopheles*[4], the role of β2 tubulin in spermiogenesis appears to be conserved.

The definitive sex-specific expression of GFP in late larvae and pupae achieved by Catteruccia *et al.* represents an enormous stride towards the goal of irradiating and releasing only male mosquitoes in any SIT program in the numbers required. This work should stimulate efforts to extend it to other mosquitoes that are vectors of debilitating diseases, such as malaria and dengue.

The nexus between testes-specific expression of GFP and practical applications is provided by modern fluorescence-activated sorting technology. Catteruccia *et al.* use a high-throughput sorter developed for identifying fluorescent *D. melanogaster* embryos to determine whether sex-specific larval expression can be used to automatically sex mosquito larvae[5]. The principle is similar to that of fluorescence-activated cell sorting. Although late-instar mosquito larvae are very different in size and shape from *D. melanogaster* embryos, initial attempts to separate fluorescent male larvae from nonfluorescent female larvae reached a speed of a few larvae per second. Although this is far less than the maximum speed of approximately ten embryos per second achieved for *D. melanogaster*, it is clear that with optimization, fluorescence-based automated sorting will be able to efficiently separate male from female mosquito larvae. A rate of five embryos per second would allow 18,000 larvae to be sexed per hour, which approaches the requirements of mosquito control programs.

Several issues remain. Catteruccia *et al.* conclude that transgenic males producing transgenic sperm are as competitive as their wild-type siblings in laboratory cages, but the question of competitiveness in more complex and realistic environments remains open. SIT programs can be bedeviled by lack of mating competitiveness between irradiated males and their field counterparts, biasing mating towards the later. Should transgenic sperm be less able to compete for fertilization than wild-type sperm, then the number of males needed to be reared and released to overcome this increases.

Opportunities also abound. Catteruccia *et al.* report that GFP-expressing sperm are easily detected in spermathecae of inseminated females, opening up the possibility of examining questions of sperm competition and storage during multiple gonadotrophic cycles in mosquitoes. Confinement of transgene expression to the gonads also raises the possibility of limiting expression of homing endonucleases and transposases to the germ line, and, in so doing, driving genes linked to these systems through insect populations.

A promise of molecular entomology has been to bring the tools of contemporary molecular genetics to applied entomology. The control of mosquito vectors of human disease is one area in which the tools of molecular genetics could have a profound impact on human welfare. The demonstration that some of these tools can be simple and effective is thus a significant step towards fulfilling this promise.

1. Knipling, E.F. *J. Econ. Ent.* **48**, 459–462 (1955).
2. Catteruccia, F., Benton, J.P. & Crisanti, A. *Nat. Biotechnol.* **23** 1414–1417 (2005).
3. Kemphues, K.J., Raff, E.C., Raff, R.A. & Kaufman, T. C. *Cell* **21**, 445–451 (1980).
4. Gaunt, M.W. & Miles, M.A. *Mol. Biol. Evol.* **19**, 748–761 (2002).
5. Furlong, E.E.M., Profitt, D. & Scott, M.P. *Nat. Biotechnol.* **19**, 153–156 (2001).

In vivo veritas

Laurence Zitvogel & Thomas Tursz

Dendritic cells transplanted into patients for cancer immunotherapy can be tracked by MRI.

Dendritic cell immunotherapy, introduced into oncology as early as 1995, has proven feasible, innocuous and even effective in some patients. Yet most patients have not shown objective clinical responses. Clearly, optimal methods for loading dendritic cells with antigen, maturing them and applying them clinically remain to be worked out[1]. In this issue, de Vries *et al.*[2] address one aspect of this challenge—the hitherto unrecognized problem of accurately delivering dendritic cells to their site of action. They show for the first time that dendritic cells labeled *ex vivo* with iron oxide particles can be tracked after transplantation in humans by magnetic resonance imaging (MRI), allowing the cells' migration *in vivo* to be monitored.

Dendritic cells are professional antigen-presenting cells of the immune system with the unique ability to pinocytose or phagocytose blood-borne or tissue-resident antigens, to process antigenic proteins into suitable peptide fragments, to incorporate them into MHC class I and II molecules and to present them to T cells[3]. Dendritic cell immunotherapy is based on the simple idea that T-cell antigens and/or NK/NKT receptor ligands presented by dendritic cells will prime T-, NK- and NKT-cell effectors that will counterattack tumor cells.

Laurence Zitvogel & Thomas Tursz are at ERM0208 INSERM, Institut Gustave Roussy and Université Paris XI, 39 rue Camille Desmoulins, Villejuif, France.
e-mail: zitvogel@igr.fr