Institution: University of Oxford

Unit of Assessment: UOA5

Title of case study:

An innovative GM approach to the control of insect pests and mosquito vectors of human disease

1. Summary of the impact

Professor Luke Alphey at the University of Oxford has developed a new and highly effective technique for the control and eradication of insect pests and carriers of disease. This groundbreaking approach involves the introduction of a dominant lethal gene into an insect's DNA at the egg stage. Since 2012, the method has been successfully applied in Brazil to control *Aedes aegypti*, the worldwide vector of the dengue fever virus. The regulatory framework for genetically modified insects has also changed substantially as a result of Alphey's work. The spin-out company Oxitec has attracted investment in the region of £13.4 million since 2008, reflecting the huge potential of this approach.

2. Underpinning research

The Sterile Insect Technique (SIT) has typically used irradiation or chemosterilants to generate chromosomal aberrations in the sperm of adult male insects, which are released in large quantities to mate with wild females. Few viable offspring are produced, and over a sustained period the wild population can be suppressed or eliminated. SIT has been used successfully to control some insect pests, but it has disadvantages: irradiation damages the whole insect and can affect an insect's sexual competitiveness; SIT is difficult to use in some insect species, notably mosquitoes; and it does not enable easy sex-separation (for various reasons it is preferable to release only male insects for successful SIT programmes).

Research undertaken by Professor Luke Alphey at the University of Oxford's Department of Zoology has resulted in a radically new approach to SIT. A groundbreaking paper published in 2000 described a technique called 'Release of Insects carrying a Dominant Lethal' (RIDL). A dominant lethal gene, repressible in the lab by use of the antibiotic tetracycline, is introduced into an insect's DNA at the egg stage. When the genetically modified (GM) adult insects are released, the gene begins to manufacture a protein that disrupts the normal functioning of the cell, killing the insect. Males carrying the mutation mate with wild females and offspring inherit the lethal gene. The genetic modification can be sex-specific (only female offspring die) or non-sex-specific (all offspring die). This was the first time that transgenic methods had been proposed as a replacement for conventional SIT, and the paper demonstrated that both methods worked in Drosophila flies[1]. Models predicted that RIDL would be at least as effective as SIT, and would have additional benefits: transgenic males were likely to have a fitness advantage over irradiated males, and sex-separation could be facilitated by the use of female-specific lethality. A spin-out company, Oxitec Ltd, was created in 2002 to support the research.

A paper published in 2005 described the first use of the RIDL technique in a serious insect pest: the Mediterranean Fruitfly (Medfly). In this case the dominant lethal gene killed both male and female offspring[2]. Because Medflies cause most damage as larvae, the gene was designed to cause lethality at an early stage in development. A genetic marker causing fluorescence was included to allow discrimination of wild type and engineered insects – a key component of effective monitoring of populations. The paper showed that RIDL could provide a replacement or back-up for radiation sterilisation. A paper in 2007 explored another RIDL method: using a novel GM technique (sex-specific alternative splicing) to introduce a female-specific genetic mutation into the Medfly[3].

Concurrently Alphey and colleagues were working on *Aedes aegypti*, the key vector worldwide of the yellow fever and dengue fever viruses. A 2007 paper described OX513A, the GM mosquito subsequently used in field trials, again designed to carry a dominant lethal gene. The paper

investigated how late-acting lethality could be used to offset density-dependent effects (the tendency for surviving offspring to survive better owing to reduced competition for resources). Conventional SIT induces lethality at the embryo stage; this paper showed how RIDL could potentially allow the lethal phase to be tailored to improve cost-effectiveness[4].

By 2011 Alphey and colleagues had constructed a number of transgenic variants of *Aedes aegypti* and other insects. The first cage trials of one such strain resulted in elimination of caged wild mosquito populations within 10-20 weeks and demonstrated that RIDL could be used to drastically reduce mosquito populations in the laboratory. In 2011 a paper reported the first ever field trials in 2006-8 of a GM insect, the pink bollworm (a serious moth pest of cotton); 15 million insects were released over 2500 acres. This was followed in November 2011 by a paper reporting the first field trials of OX513A, the GM mosquito described above. The study demonstrated that transgenic males mated successfully with wild females in an area of the Cayman Islands[5]. Another field trial was also conducted in an uninhabited forest area in Malaysia. In 2012 a paper reported on the second phase of the Cayman Island trials, showing an 80% reduction in the target mosquito population relative to untreated areas over a release period of several months, indicating the strong potential of RIDL to control *Aedes aegypti* and thus massively reduce the incidence of dengue fever[6].

3. References to the research

1. Thomas DD, Donnelly CA, Wood RJ, Alphey LS. (2000) Insect population control using a dominant, repressible, lethal genetic system. Science 287: 2474–2476. doi: 10.1126/science.287.5462.2474 ***First paper describing how a female-specific transgenic technique (RIDL) could be used in insect control as an alternative to the classical irradiation-based Sterile Insect Technique.***

2. Gong P, Epton MJ, Fu G, Scaife S, Hiscox A, Condon KC, et al. (2005) A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat Biotechnol. 23: 453–456. doi: 10.1038/nbt1071 ***First use of one variant of the new RIDL technique to control Medfly, a serious insect pest; all offspring are killed by the dominant lethal gene passed on by transgenic males.***

3. Fu G, Condon KC, Epton MJ, Gong P, Jin L, Condon GC, et al. (2007) Female-specific insect lethality engineered using alternative splicing. Nat Biotechnol. 25: 353–7. doi: 10.1038/nbt1283 ***First use of the RIDL variant described in the original 2000 paper; only female offspring are killed by the dominant lethal gene passed on by transgenic male Medflies.***

4. Phuc H, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, et al. (2007) Late-acting dominant lethal genetic systems and mosquito control. BMC Biol. 5: 11. doi: 10.1186/1741-7007-5-11 ***First description of OX513A, the transgenic mosquito strain subsequently used in field trials; analyses the use of late-acting lethality as a way of offsetting density-dependent effects.***

5. Harris AF, Nimmo D, McKemey AR, Kelly N, Scaife S, Donnelly CA, et al. (2011) Field performance of engineered male mosquitoes. Nature Biotechnology 29: 1034–1037. doi: 10.1038/nbt.2019 ***First field trials of the transgenic mosquito strain OX513A, demonstrating successful mating of transgenic males with wild female mosquitoes.***

6. Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, et al. (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nature Biotechnology 30: 828–830. doi: 10.1038/nbt.2350 ***Report on the second field trial of OX513A, demonstrating suppression of the wild mosquito population.***

Funding for research: Grants of around £4.7M have been received for this research, predominately through the Grand Challenges in Global Health initiative.

4. Details of the impact

The research described above has created a new and highly effective technique for the control and

eradication of insect pests and carriers of disease. Professor Alphey is the world leader in non-proliferative GM interventions and the only person so far to have designed transgenic insects that have been approved for release in the wild. As well as leading to successful suppression of insect populations and demonstrating significant advantages over conventional SIT, Alphey and Oxitec Ltd have been instrumental in 'forcing the pace' in terms of regulation of GM insects, and Oxitec has attracted substantial investment as a result of the potential of RIDL.

The biggest impact since 2008 has been in the control of the mosquito *Aedes aegypti*, the dengue fever vector. The World Health Organization ranks dengue as the most important mosquito-borne viral disease. In the last 50 years *Aedes aegypti* has spread from Africa across the world, leading to a 30-fold increase in the incidence of dengue, which now threatens an estimated 2.5 billion people worldwide. There are as many as 390 million new infections per year and 25,000 deaths, mostly children. The economic consequences are grave; dengue is estimated to cost the equivalent of 83 - 658 lost years of human life per million of the population every year. No vaccine or therapeutic drug treatment is available, and the only current approach is to control the populations of *Aedes aegypti*. This is currently done mainly through the use of insecticides; but because *Aedes aegypti* frequents human habitations in urban areas this has a serious impact on human health, as well as creating mosquitoes that are increasingly resistant to insecticides.

SIT trials were conducted against mosquitoes in the 1970s with some success, but these have not been pursued since, partly at least because SIT in mosquitoes is very hard to implement. The GM mosquito OX513A represents a huge improvement over both SIT and conventional control by insecticides. Following the successful Cayman Island trials [5,6], in 2012 Oxitec Ltd established a new project in Brazil with the University of Sao Paulo and a local company, Moscamed. The project had full backing and regulatory approval from the Brazilian government, as well as strong support from local communities. In one and a half years the trials have progressed to a stage equivalent to that of phase III trials in drug testing. Phase II trials demonstrated major success in controlling *Aedes aegypti* in a densely populated suburban area, reducing the wild mosquito population by over 85%[7]. In another more isolated area, with less immigration of mosquitoes, a 96% reduction of the mosquito population was achieved after only six months, and maintained for a further seven months[8]. Full-scale local production of OX513A mosquitoes is now in place and in June 2013 a project was launched in an entire town of 50,000 people, demonstrating the confidence that the Brazilian authorities have that this approach represents their best chance of bringing dengue under control[9]. Other advantages are that the approach has a much lower impact than insecticide use in terms of damage to human health and the environment, and there is also no need for potentially dangerous facilities with strong gamma-ray sources for irradiation.

Owing to Professor Alphey's success in designing a range of effective GM insects in a very short period of time, there have been significant policy changes worldwide. Prior to the field trials of the GM pink bollworm in 2006-8, it was generally considered that release of GM insects was nowhere near being realised, and consequently there was little or no regulatory framework in place anywhere in the world. Alphey's research has had a major impact on the development of such frameworks, necessitating a thorough consideration of the issues involved in GM insect releases. In 2006 the Animal and Plant Health Inspection Service, part of the US Department of Agriculture, initiated what became the first-ever Environmental Impact Statement on any GM organism, triggered by Alphey's successful GM designs for pink bollworm and fruitfly; the final version of this statement was issued in October 2008[10]. This has been followed by:

- The 2009 World Health Organization consultation report on genetically modified mosquitoes (GMM): this states that 'Recent studies on RIDL *Aedes aegypti* mosquitoes in Malaysia [i.e. Alphey's work] stimulated requests from countries for WHO guidance on GMM use'[11]. A WHO working group on international guidance for GMM was set up following these consultations, and a draft Guidance Framework for the testing of GMM has recently been issued[12].
- The approval in Brazil in 2009 of the importation and release of OX513A by the national regulator, CTNBio, after a local review of the regulatory process, which had not previously addressed GM insects[13].
- The May 2013 European Food Standards Authority guidance on the environmental risk

assessment of GM animals, which includes nearly 40 pages on insects. The guidance refers specifically to GM insects used to control vectors of human disease and manage agricultural pests, both of which developments have been spearheaded by Alphey[14].

Standards for the regulation of GM insects in general have thus been drawn up, or are in the process of being drawn up, as a direct result of Professor Alphey's work in designing and trialling specific types of GM insect, and this is facilitating developments in the field as well as helping to ensure quality control and proper risk assessment. The huge potential of RIDL to control both insect pests and vectors of disease is reflected in the success of Oxitec Ltd, which has been a thriving company for over a decade. Since 2008 the company has received around £13.4M in convertible loans and equity investment, and employee numbers have grown from 30 at the end of 2009 to 37 in mid-2013[15].

5. Sources to corroborate the impact

7. Oxitec Ltd. Moscamed prepares for next phase in the development of Oxitec's transgenic mosquitoes in Brazil (2012). Available from: http://www.oxitec.com/press-release-moscamed-prepares-next-phase-development-oxitecs-transgenic-mosquitoes-brazil/ ***Oxitec press release, quoting Dr Margareth Capurro of the University of Sao Paulo discussing the successes of Phase II of the project.***

8. Thompson T. Oxitec report 96% suppression of the dengue mosquito in Brazilian trials Oxitec Ltd. 2013. Available from: http://www.oxitec.com/press-release-oxitec-report-96-suppression-of-the-dengue-mosquito-in-brazilian-trials/ ***Oxitec press release confirming the 96% mosquito suppression rate.***

9. Oxitec Ltd. Moscamed launches urban-scale project in Jacobina (2013). Available from: http://www.oxitec.com/oxitec-newsletter-august-2013/ ***Oxitec press release confirming the extension of the project to a town of 50,000 people.***

10. United States Department of Agriculture (USDA). Use of genetically engineered fruit fly and pink bollworm in APHIS Plant Pest Control Programs: Final Environmental Impact Statement. Animal and Plant Health Inspection Services (APHIS); Oct 2008. Available from: http://www.aphis.usda.gov/plant_health/ea/downloads/eis-gen-pbw-ff.pdf ***Final version of the first ever Environmental Impact Statement for any GM organism, triggered by Alphey's designs for GM pink bollworm and fruit fly.***

11. World Health Organization. Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission: Geneva, Switzerland, May 2009. Available from: http://www.who.int/tdr/publications/documents/gmm-report.pdf ***Report on the link between WHO's consultation on GM mosquitoes and Alphey's work.***

12. World Health Organization. Guidance framework for testing of genetically modified mosquitoes [DRAFT]. Available from: http://www.who.int/tdr/news/2012/GMM_Guidance_2012.pdf ***WHO Consultation Report and Draft Guidance on Testing for genetically modified mosquitoes.***

13. http://www.ctnbio.gov.br/index.php/content/view/13971.html http://www.ctnbio.gov.br/index.php/content/view/15761.html ***CTNBio's approval of the import of OX513A mosquitoes to Brazil and first release in phase II of the project.***

14. European Food Standards Authority (EFSA); 2010 Sep. [Internet] Available from: http://www.efsa.europa.eu/en/efsajournal/doc/3200.pdf ***EFSA report 'Guidance on the Environmental Risk Assessment of Genetically Modified Animals'. The section on GM insects is from page 73-111.***

15. Email with information from the Chief Financial Officer at Oxitec Ltd, confirming investments and employee numbers (held on file).