Insect Population Suppression Using Engineered Insects

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Abstract

Suppression or elimination of vector populations is a tried and tested method for reducing vector-borne disease, and a key component of integrated control programs. Genetic methods have the potential to provide new and improved methods for vector control. The required genetic technology is simpler than that required for strategies based on population replacement and is likely to be available earlier. In particular, genetic methods that enhance the Sterile Insect Technique (e.g., RIDL™) are already available for some species.

Introduction

Suppression of vector populations has for many years been at the forefront of efforts to control vector-borne diseases. This was one of the earliest deliberate control measures—the ancient Romans drained swamps to control malaria. More recent successes include the elimination of the malaria vector Anopheles gambiae from Brazil in the 1930s by the program led by Soper and the Rockefeller Foundation, the control of mosquito-borne diseases in South America by the Pan-American Health Organization, and the large-scale malaria control and eradication programs of the 1950s and 60s. All of these mosquito control programs combined the use of insecticidal chemicals, breeding site restriction and physical barriers such as screens and bed nets, but with a heavy emphasis on insecticides. Increased prevalence of insecticide resistance in vector populations, combined with greater awareness of the negative environmental impacts of widespread insecticide use, means that equivalent programs are probably impractical today. However, vector control remains a key component of any integrated program intended to control vector-borne disease. It is therefore imperative to develop new and improved methods for vector control, especially ones that do not depend on the use of toxic chemicals.

Several molecular genetic methods have been proposed for the suppression or eradication of pest insect populations. This chapter will focus on genetics as applied to the pest insect itself, but there may also be uses for engineered plants and microbes in this context, for example for enhanced versions of microbial biocontrol.

Suppressing or eradicating a target pest population means reducing its reproductive capacity, in other words the average number of progeny from each adult that themselves survive to reproduce. If the average reproductive capacity can be brought below unity, then the population is not self-replacing and will decline. Most natural populations are regulated by density-dependence; in this case a moderate reduction in reproductive capacity will lead to the
population reaching a new, lower equilibrium level—this is suppression, but not eradication. However, any population is limited in its ability to accommodate excess mortality or sterility. This ability is related to the basic reproductive rate in the absence of density-dependent effects; if it can be overcome, then the population can indeed be eliminated. There are numerous precedents for this, both for disease vectors and for agricultural pests. Most methods of population control act independently or even synergistically, and can be combined to give a greater impact on the target population, so an effective program is likely to involve several different approaches. In turn, vector control is only one route to reducing the intensity of transmission, and so vector population control methods are themselves likely to form one part of a larger integrated disease control program.

Two broad strategies for genetics-based population suppression have been proposed. In one of these, released engineered insects would produce nonviable progeny, either immediately or within a few generations, thereby tending to suppress the target population. Implementation of such a program would require the release of large numbers of engineered insects, and would resemble the currently-used sterile insect technique (SIT), to a degree that depends on the precise nature of the genetic system used. This is discussed further below. The aim of the other proposed method is to impose a genetic load on the population by spreading within it one or more genetic elements that will reduce the average fitness of the insects that carry them. This second method has no obvious analog in present pest control methods. One potential way to do this is to introduce genetic elements that will drastically reduce the fitness of the target population. There are a number of different types of genetic element that might have the potential to do this. Several variant schemes have been proposed for using one such class of element, homing endonucleases. However, practical use remains a distant prospect as daunting technical and regulatory issues remain to be solved.

In contrast, sterile insect methods have a long history of successful field use. The SIT depends on the rearing, sterilization and release of large numbers of insects. These sterile insects compete for mates with the wild population. Mating to sterile insects reduces the reproductive potential of the wild population; if sufficient sterile insects can be released for a sufficient period of time, the target population will collapse and may even be locally eliminated. A ground-breaking experiment in the 1940s by Vanderplank eliminated a tsetse population through hybrid sterility following mass-release of a sibling species. Later, radiation was used to sterilize mass-reared insects of the target species itself. One early program, and the paradigm for this approach, was the complete elimination of the New World screwworm (Cochliomyia hominivorax) from North and Central America, and the successful prevention of reinvasion from South America by regular release of sterile insects in a barrier zone in Panama. Large-scale programs based on the SIT have also been successfully conducted against tsetse and against a range of agricultural pests, e.g., tephritid fruit flies.

The SIT has a number of significant advantages as a control method. It does not depend on the use of toxic chemicals in the environment, and it uses the insects’ own mate-seeking behavior to find the wild females. It can therefore work well against targets which are difficult to find, for example because of low prevalence, or resting or breeding behaviors that make adults or juveniles difficult to reach with less target-seeking methods, e.g., toxic chemicals. Since it depends on mating with released insects, the SIT is exquisitely species-specific, with minimal nontarget impacts.

Though it has been very effectively deployed against some agricultural pests, the SIT has had limited impact on disease vectors, the elimination of tsetse from Zanzibar being one of rather few examples. Following the success of the New World screwworm program, a number of trials were conducted in the 1970s, with some success, but there are no large-scale SIT programs in operation today against any mosquito, indeed the number of pest species against which SIT programs are currently deployed is quite limited. These early trials, taken together, indicated that the SIT had considerable potential for the control of mosquitoes, but that the technology then available was not quite adequate to provide a cost-effective...
intervention except in special circumstances. Problems with mass-rearing, sex-separation, sterilization and distribution, and maintaining the competitiveness of the insect through all of this, tend to reduce the cost-effectiveness of the SIT. For some of these key issues, especially sex-separation and sterilization, modern genetic methods could potentially provide dramatic improvements in the cost-effectiveness of the SIT, particularly for some vector species. This is likely to improve the cost-effectiveness of the SIT to the point where it becomes an attractive option for vector control in a range of contexts, allowing SIT to fulfill its potential as an effective, environmentally friendly vector control system.

Genetic Sexing and Genetic Sterilization

It is highly desirable that the released sterile insects are all male. There are two distinct reasons for this. Firstly, for insects such as mosquitoes, the adult females are potentially dangerous—even sterile females will bite and might transmit disease—whereas males do not bite. Secondly, the presence of females in the release population may distract the sterile males from seeking out wild females. Since mating to wild females is the basis of SIT, this could have a significant impact on the program. Indeed, for the Mediterranean fruit fly (Ceratitis capitata, “Medfly”), eliminating females from the release population (“male-only release”) was found to give a 3- to 5-fold improvement in effectiveness per male. Even if females were merely neutral to the program, there would still be a considerable financial benefit in eliminating them from the release population, by eliminating the costs of rearing and distributing these females. For some species there may be sufficient sexual dimorphism, for example in size or time from pupation to eclosion, to allow efficient sex-separation on the basis of this trait. This has been used for tsetse, and may also be possible for some mosquitoes. However, for many insects no such method is available. In these cases a genetics-based sex separation system would be highly desirable.

Several genetic sexing systems have been constructed by classical genetics. These were all based on the translocation of a dominant selectable marker to the Y chromosome. Unfortunately, the chromosome aberrations of these systems tend to reduce the performance of the flies that carry them, making them less effective agents for the SIT. Despite much effort to minimize the problem, the translocations are also unstable and reversions are therefore a potential problem when large populations of insects are grown for release. This problem has been greatly mitigated by the introduction of “clean filter rearing systems” to maintain strain integrity; indeed such a system would seem advisable for any nonwild type strain, whether made by classical genetics or recombinant DNA methods. The most successful of the classical systems, the temperature-sensitive-lethal (tsl) based system developed for Medfly, is now widely used; almost all sterile Medfly production facilities have converted to this strain. However, the absence of equivalent strains, even for other species of fruit fly, highlights another problem with the classical approach—genetic tools developed in one species by classical mutagenesis cannot be transferred to another species. So, despite the success of the Medfly tsl strains, development of a sexing system remains a high priority for other species, including the New World Screwworm, various fruit flies (e.g., the Mexican fruit fly, Anastrepha ludens) and mosquitoes, and has also been advocated for some moth species.

Recombinant DNA methods offer the prospect of simpler systems for genetic sexing, and ones more readily transferable to new species. One potential method would be to arrange for the sex-limited expression of a fluorescent protein. Following several earlier studies in Drosophila melanogaster, this was recently demonstrated in the laboratory for Anopheles stephensi, using a promoter from a testis-specific gene to give testis-specific, and therefore male-specific, expression of DsRed, a red fluorescent protein. This allowed males and females to be separated on the basis of their fluorescence, either manually or using an automated fluorescence-based sorter. Testis-specific expression has several potential advantages—testis-specific promoters are relatively easy to identify, and labeled sperm may allow the determination of the mating status of females. However, expression is limited to a small tissue,
can be detected only relatively late in development, and may not be practical on the scale required. On the other hand, labeled sperm have other uses, for example to determine the mating status of trapped females, and thereby monitor the mating success of released males.

Differential expression of a marker, such as fluorescence, requires that every insect be individually examined and sorted. Given the large scale of SIT programs—the Medfly facility at El Pino, Guatemala, has a production capacity of 3.7 billion sterile male Medfly per week—individual inspection may not be practical. It would be highly preferable to use a system in which females are eliminated by a sex-specific lethal system without any need for individual sorting.

Unlike a sex-limited visible marker, a female-lethal system has to be conditional. The females must survive under some "permissive" conditions, to allow the strain to be propagated, but die under some other "restrictive" conditions, to allow elimination of females from the population intended for release. One could arrange that "normal" rearing conditions are permissive, with the lethal system being induced by some change to an abnormal condition. This would be an "inducible" system; the trigger might be heat, for example, as is used in the tsl-based system. Alternatively, one could arrange that "normal" rearing conditions are restrictive, so that the lethal system needs to be suppressed, for example by the addition of a chemical "antidote" to the diet; this would be a "repressible" system. For a sexing system, one would then simply omit the antidote from the diet of the last generation before release, thereby automatically eliminating the females. The feasibility of this "repressible" approach has been demonstrated in *Drosophila melanogaster*. A female-specific fat body enhancer was used to drive expression of a tetracycline repressible transcription factor, which in turn controlled the expression of a lethal gene product. Under "permissive" conditions—when flies were raised on media containing tetracycline—equal numbers of male and female progeny were found (376 males: 342 females). When tetracycline was removed from the rearing media to generate "restrictive" conditions no female progeny were recovered in comparison to >5,000 males. We have recently been able to generate equivalent genetic systems and strains for two pest fruit flies, the Mediterranean fruit fly *Ceratitis capitata* and the Mexican fruit fly, *Anastrepha ludens* (unpublished). Several female-specific promoters are now available from vector species, for example, Actin4 from *Aedes aegypti*, or the carboxypeptidase genes from *Anopheles gambiae* and *Aedes aegypti*. The range of female-specific promoters now available should enable the construction of repressible female-specific lethal systems in a wide range of insect species.

A repressible system has several potential advantages. A suitable repressible lethal system could potentially remove the need for a physical sterilization step, in other words it could remove the need for irradiation. This would work as follows: an insect strain homozygous for a repressible female-specific lethal would be reared to large numbers in the presence of the repressor. Then, for the last generation, before release, the strain would be reared in the absence of the repressor; all the females would therefore die, giving a male-only population for release (Fig. 1). These males would be released into the environment without irradiation. They would seek out and mate wild females. The progeny of such matings would each inherit one copy of the female-specific lethal. Lacking the repressor, the female progeny would die. This is the key to this method of population control—eliminating these females reduces the reproductive capacity of the target population. The male progeny would inherit the female-lethal system, so that half of their daughters would also die (Fig. 2). This gives a modest additional benefit, however it should be obvious that the female-lethal gene will disappear from the population extremely rapidly, by natural selection, if is not maintained by repeated release of large numbers of engineered insects. A repressible lethal system would therefore provide a genetic containment system as a back-up to conventional physical containment (Fig. 1C). In this respect, and operationally, this system—known as RIDL—still closely resembles conventional SIT, but with both genetic sexing and genetic sterilization being provided by a single genetic system. Mathematical modeling predicts that this system can be more effective for population control than is conventional SIT, especially if the female-killing alleles are present at several loci (Fig. 3).
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The system outlined above combines both genetic sexing and genetic sterilization, but of course this is not essential. Fryxell and Miller proposed that a dominant conditional (cold-sensitive) lethal mutation could be used in place of radiation, in a system that they called autocidal biological control (ABC). This system would not provide genetic sexing, but would kill all of the affected progeny, rather than just the daughters. This may be desirable under some circumstances, particularly where both males and females are released (“bisex” release). A similar system was demonstrated more recently, using an engineered lethal and
with tetracycline rather than temperature as the repressor (a version of RIDL affecting both males and females). This was further refined by Horn and Wimmer to give embryonic lethality. Radiation sterilization also typically kills the affected individuals as early embryos, before they hatch into larvae and start eating the crop; this is highly desirable for many agricultural pests but unimportant for most disease vectors, where only adults can transmit disease.

A cold-sensitive system, such as that proposed by Fryxell and Miller, could be used in a different manner. If summer temperatures do not drop below the critical point, the gene may spread through the target population for several generations before being activated by lower winter temperatures. The population dynamics of this sort of control are rather different
Figure 3. Female-specific lethality as “genetic sterilization”. The reproductive potential of an insect population depends primarily on the number of females—increasing the number of females will typically increase the number of progeny, but increasing the number of males normally will not. Killing both sexes, or specifically killing females, might therefore be expected to have equivalent effects on the size of the target population. We used a deterministic discrete-generation population genetics model (based on that of ref. 32) to predict the effects of releasing into a target population nonirradiated males homozygous at one or more loci for a female-specific lethal gene and to compare this with the effectiveness of conventional SIT. Assuming that the lethal gene acts on the developing insect before they reach reproductive maturity, for example at an embryonic or larval stage, a non-sex-specific version of the RIDL system that kills both males and females is equivalent in this model to conventional SIT; negative effects of irradiation or genetic engineering are ignored. The population is assumed to be closed and homogeneous, with a natural sex ratio of 1:1 and females selecting mates in proportion to their relative abundance. The panels show the number of females in the population in each generation, relative to the initial number. The key in panel d applies to all panels. A constant number of adult males are released per mosquito generation; this “release ratio” is relative to the initial wild male population. $R_0$ denotes the number of female offspring produced by each female that would survive to adulthood if there were no intervention and no density dependent mortality. Panels a and b assume no effects of pest density and $R_0 = 1.5$, and compare release ratios of 0.45 (A) and 0.6 (B). Without any intervention, the population would experience exponential growth (not shown). Panels c and d assume density dependent mortality of larvae (using formulae from refs 39 and 40) and $R_0 = 4$, and compare release ratios of 1 (A) and 3 (B). The population is assumed to start at carrying capacity and will therefore remain at the initial level if there is no intervention. Panels a and c illustrate marginal situations where SIT does not control the population but the RIDL release strategies do. In the density dependent case (C) SIT can actually increase the equilibrium size of the adult female population. Panels b and d show that, with a sufficiently high release ratio, SIT and the RIDL release strategies all control the population.
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from SIT—the target population is allowed to grow unchecked for several generations. On the whole this method is likely to be less effective than RIDL or conventional SIT, but may have applications in specific circumstances.38,43

Molecular Biology of Repressible Lethal Systems

In principle, the condition could be any controllable feature of the environment—temperature, photoperiod, diet components, etc. In practice, few suitable regulated gene expression systems are available around which such a system could be built. Two well-known ones are heat-shock (temperature regulation) and tetracycline-regulated systems (diet component). The feasibility of using temperature as the regulated condition is shown by the Medfly til-based system, in which heat treatment of embryos is used to kill females. However, these are inducible systems, in which a specific treatment needs to be applied in order to produce the desired effect, i.e., kill females. As discussed above, there are distinct advantages to using a repressible system rather than an inducible one. A repressible system confers a “fail-safe” property—females can then only survive in the presence of an antidote; if any escape, either they or their progeny will die for lack of the antidote. This system has the additional significant advantage that it can be adapted to provide genetic sterilization as well (see above).

The tetracycline regulated gene expression systems harness the properties of a bacterial protein, TetR. This protein binds to a specific DNA sequence (tetO) in the absence of tetracycline, but not in its presence. TetR does not confer tetracycline resistance; its function in the bacteria is simply to regulate the expression of another gene. Gossen and Bujard developed a eukaryotic gene expression system by fusing TetR to a eukaryotic transcriptional activator.44 This system has several desirable properties—it has a very good on/off ratio (ratio of expression under induced and repressed conditions), works in a very wide range of species, and responds to low concentrations of tetracycline, a very well-characterized, safe and inexpensive chemical.45-48 Other eukaryotic expression systems regulated by specific chemicals have been developed, but these features of the tetracycline regulated systems have made them the most widely used.49

The tet system is conventionally used as a two-component system. One component expresses the tetracycline-repressible transactivator (tTA); the other has the effector molecule under the control of a tTA-responsive element containing multiple copies of tetO. This is similar to the GAL4/UAS bipartite expression system that has been widely used in Drosophila melanogaster.50,51 Recently, we demonstrated that this system could be condensed into a single component system by placing the tTA coding region under the transcriptional control of the tTA-responsive element (Fig. 4).48 In the absence of tetracycline, a positive feedback loop is established in which tTA drives the expression of more tTA. We showed that this could provide a repressible lethal system with various desirable characteristics in a major pest insect (Medfly)48 and have also developed a similar system for the mosquito Aedes (Stegomyia) aegypti (unpublished).

One advantage of the “positive feedback” system is that it does not require any species-specific promoters to be characterized. Koukidou and colleagues exploited this to make a genetic marker, by using a nonlethal positive feedback system to drive expression of a fluorescent protein.52 They showed that this system could give detectable expression of the fluorescent protein in insects (Drosophila melanogaster and Ceratitis capitata), a nematode (Caenorhabditis elegans), a plant (Nicotiana tabacum) and a human cell line, thus illustrating the very broad applicability of this approach.

Regulatory Issues and Concluding Remarks

Some applications of insect genetics to disease control require the long-term persistence of novel DNA sequences in wild vector populations, for example sequences that prevent the insect transmitting a particular pathogen. There are daunting technical difficulties involved in arranging this, particularly the “gene driver” technology required to spread the pathogen-resistance gene through a wild population. There are likely also to be significant regulatory and political issues, particularly where the proposed modification would be irrevers-
uble, or would tend to spread across national boundaries. In contrast, the use of recombinant DNA methods to improve the SIT appears to be relatively straightforward from a technical perspective; the first potentially suitable strains are already available, both for agricultural pests and for at least one disease vector. The regulatory and political hurdles may also be less severe for this approach. Such use would be readily reversible, in that the introduced DNA sequences are not designed to persist, rather they tend to eliminate their hosts and so would rapidly disappear from the wild unless maintained by frequent, large-scale reintroduction in an SIT-like program. Some applications, for example the use of a genetic marker and/or genetic sexing with radiation-sterilization, would not have any significant introgression of recombinant DNA sequences into the wild population at all. Repressible lethal systems would have the additional advantage of a “fail-safe” action not found in present SIT strains.

Despite the above comments, it is important not to focus exclusively on the genetic modification technology; genetic control strategies should be assessed as one would any other novel intervention (such as a new drug or vaccine, or use of treated bed nets). This means focusing on questions of cost-effectiveness, affordability, accessibility, equity and sustainability. In particular, it is important to compare the proposed genetic strategies with current technologies, and to consider what role genetic methods may play as part of an integrated vector control and disease control program.

Recombinant DNA methods clearly have the potential to make substantial improvements to the SIT as currently practiced, and thereby to provide new and effective tools for vector control. This is not a panacea; these new methods will not be cost-effective in all circumstances or against all species. However, the threat from exotic vector-borne diseases is increasing, and the range of other control options is narrowing as insecticide resistance and environmental concerns reduce the range of available treatments. The use of SIT-like genetic control strategies

![Figure 4. Tetracycline-repressible lethal systems](image)

A) In the absence of tetracycline (Tc) tTA binds tetO, drives expression of an effector molecule leading, in the case of a lethal effector, to death. B) In the presence of Tc, tTA binds Tc; the Tc-bound form of tTA does not bind DNA, it therefore does not activate expression of the effector and the system is inactivated. C, D) Simplified one-component system. C) In the absence of Tc, basal expression of tTA leads to the synthesis of more tTA, which accumulates to high level. This level can be regulated by modifying the stability and translational efficiency of the tTA mRNA. At the highest levels, expression is lethal, so tTA is both the driver and the effector. D) In the presence of Tc, tTA is inactivated by Tc and is therefore expressed only at basal levels. (Adapted from ref. 48).
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has considerable potential in the control of major human and livestock diseases and this potential needs to be tested and explored. Unlike other proposed genetic strategies, the necessary technology, and in some cases actual strains, already exist to allow experimental trials in the very near future.

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