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Studies on Genetics Based Methods for Agricultural Insect Pest Management: A Review

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Abstract

Genetics has long played a role in the history of insect pest management, Beginning from simple collections of mutations and the development of chromosome linkage maps, many of the early applications of genetic technology were focused on the development of new strains for the sterile insect technique as a form of biological control. Since that time, and as the field of genetics underwent a true merger with molecular biology, the focus shifted almost entirely to methods using molecular technology. These methods had the advantage of being more broadly applicable to control of a wide range of pest species, from the frustrations often associated with attempts to transfer genetic tools developed in Drosophila directly to these species. The latest applications of molecular genetic technologies in the area of genetically based control methods now also include cutting-edge systems for genome editing and the use of RNA inhibition for selectively knocking out the expression of individual genes. Finally, as the field of genetics has shifted its focus from the analysis of individual genes to that of entire genomes, We outline some policy considerations for taking genetic insect control systems through to field implementation.

Keywords: Pest Management SIT; Transgenic Organisms Genome Editing.

INTRODUCTION

Genetics has played a significant role in the history of efforts to manage insect pests. As the pests and their activities have evolved over time, the genetic methods used for management have changed as well. To some extent, of course, these changes mirror changes that the field of genetics itself has under gone. Genetics began as a field focused primarily on investigating heritable changes in organisms without even knowing the true physical nature of genes. It was also among the first to recognize the importance of chromosomes in relation to heredity, (robinson, 2002)[5] Boetel et al., 2015)[6] and later the field of genetics led the way to merge with the biochemical discipline of molecular biology to enhance the fundamental understanding of the nature of genes and inheritance. It has now become a field focused almost entirely on analyses done at the level of the genome as opposed to individual genes (Dale et al. 2012)[1] genetic

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methods for pest management followed a similar pattern. The earliest methods used relatively slow and labour intensive methods based on breeding or selection strategies to achieve heritable genetic changes such as for the development of new strains for the sterile insect technique (SIT), which will be described in more detail later in this review. The more contemporary methods for strain construction use techniques of molecular biology that can produce virtually 'instant' modifications of individuals through applications of transgenic technology.(James, 2000).[2]

In short, information and ideas from both classical and contemporary genetics have been applied to the management of insect pests. Each has played valuable roles in the development and applications of new methods for pest control and will continue to do so for the future. This review will focus on genetic applications in the management of insect pests of agricultural importance, but it is worth noting that many parallels can be found in efforts to use genetic methods to control insect pests such as mosquitoes, ticks and other species of medical importance. (Robinson 2002)[25]

Early basic genetic research on insect pests

The field of genetics, many of the earliest efforts incorporating genetic methods into pest management practices relied heavily on conceptual thinking, but were some what passive in nature. For example, in the 1980s, considerable effort went in to collecting interesting mutations and developing chromosome based linkage maps for some insects of agricultural importance such as the Mediterranean fruit fly, Ceratitis capitata (reviewed in and the apple maggot fly Rhagoletis pomonella (review in). These efforts were intended to parallel some of the massive body of work already in place for the genetics of the vinegar fly, Drosophila melanogaster Around the same time, some very talented and dedicated cytologists began focusing on the development of maps of polytene chromosomes found in many pest species. (Zatucki 2012)[72] (Hoy 2000)[45]

Genetics merges with molecular biology

This work was progressing, the field of genetics itself inevitably shifted towards incorporation of more molecular based approaches, in particular those based on the use of DNA. Using these methods, specific types of DNA sequences, in particular repetitive DNA sequences, could potentially be directly isolated from previously uncharacterized genomes without the requirement of homology or sequence similarities to isolate and characterize specific genes (or DNA sequences) when moving from one species to another. (Hoy, 2013)[14] Sagn, 2014)[42]

Two of the most common repetitive DNAs that could be directly isolated using these techniques included sequences known as microsatellites and minisatellites The isolation of these sequences took advantage of the vast reservoir of information about genetic variation present in portions of the genome such as the centromeres and telomeres of chromosome that are known to contain extensive tracts of such highly repetitive DNA Consequently, the number of species of agriculture importance that became amenable to the development of basic tools such as chromosome linkage maps incorporating these types of DNA based markers expanded dramatically to include other flies (Foster 1980)[11] as well as beetles and bees species under consideration as agents for biologically based control programmes of pest species. (Lampe, 2000)[40] Werren 2008)[69]

Genetics and applied research for pest management

Applications of research in the area of pest management, one of the areas where genetics played a clear role was in the development of new strains for improvement of the SIT. The basic concept of SIT, which had been first described more than 50 years previously is that large numbers of male flies of a pest species are reared, sterilized and then released to mate with wild females. When this method works, wild females are effectively removed from the breeding population, and over time the target pests should simply disappear through a lack of reproductive success. The use of this strategy has steadily grown and continues to be employed on a global basis to control a wide range of pest species. (Hurit 1995)[19] (Wise et al. 2011)[10]

Sterile insect methods

Relatively soon after being conceived, SIT had already been effectively employed for control programme targeting certain species in various localities around the world. However, it was also recognized early on that there was a clear need for new strains that could improve the efficiency of this method. These strains were often euphemistically referred to as 'genetic sexing' strains (reviewed in (Alphey 2002).[31]

One major area for improvement involving the concept of genetic sexing had to do with the females.

As described, the goal of the SIT method was to have the released, sterilized males mate with wild females. During the initial rearing phases, large numbers of females were of course needed to build the populations up to the millions of flies per week typically required for releases covering reasonably large areas. Indeed in the early days of SIT, strains were selected for rearing primarily based on female fecundity and egg productivity in the laboratory.

At the final stage though, females were collected and released along with the sterile males because there was no efficient way to selectively remove them. This was a problem because even though they were sterile, the released females would inflict extensive damage in the form of fruit stings when they attempted to lay eggs. The damage would also serve as entry points for mould and bacterial infections, etc. and was largely considered to be unacceptable. Furthermore, the females would draw the sterile males into mating with them instead of the wild females that were the intended targets. Clearly, if the females could be selectively eliminated just prior to release, the efficiency of the whole method could be dramatically improved. (Scali et al 2005)[50]

Chromosome translocations and SIT

One early genetic approach to achieving the desired separation was to link visible or selectable markers to sex determination mechanisms, in particular to the Y chromosome This was feasible because in many of the Tephritid species considered to be amenable to SIT, it was already known the presence of at least part of the Y chromosome was sufficient for male sex determination. This system was functionally similar to that of mammals, and was in sharp contrast to the Drosophila system that depended on the ratio of sex chromosomes to autosomes for sex determination. Fortunately also, a number of the visible mutations that had been more or less passively collected in the early genetic studies of different species were potentially usable for this purpose. For example in the medfly, which normally produce pupae with brownish colour, a strain carrying a recessive mutation producing white coloured pupae had been established. A chromosome translocation was generated that linked the wild type allele of this gene to the Y chromosome This translocation was crossed into the strain producing white pupae to produce males with the normal, brown coloured pupae that were easily distinguishable from the females with white pupae. Using this strain in mass rearing, large numbers of pupae could be produced and run through machines using photoelectric sensors to sort the different coloured pupae. With this technology, 99% or better separation of the sexes could be achieved at the pupal stage of development. The female pupae could be recycled back into the rearing system, while the male pupae were packaged, sterilized and released[30] for the control programme. (Meinnis et al. 2004)[30] Franz, 2011)[29]

Integrating pest management methods

Genetic insect control methods need not be directly aimed at population suppression. The female lethal, or male selecting, versions could in principle be used to help manage resistance to other control methods. First, consider an example of another plant pest control method using GM technology: insecticidal crops. (Alphey, 2002)[31] Franz 2011)[29]

Transgenic Bt crops are engineered to express insecticidal toxins derived from Bacillus thuringiensis, causing mortality to susceptible insects eating the plant (Tabashnik et al., 2013). Effective Bt crops are valuable and there is a strong economic threat from the propensity of insects to evolve resistance. The primary approach used to slow the evolution of resistance is known as the high dose/refuge strategy and this is mandatory in some countries. The effectiveness and dominance of resistance to toxins is often dose dependent. Commercial crop varieties are designed to express a ‘high dose’ of the relevant Bt protein, so that, if any allele in the population is able to confer resistance, the amount of toxin expressed will be sufficient to kill resistant heterozygotes. If this is achieved, the resistant allele is functionally recessive. Planting high dose Bt crops across an entire landscape would likely lead to the rapid spread of resistance because the only individuals that could survive would be homozygous. The ‘refuge’ part of the strategy provides an area of nontransgenic plants (either a conventional variety of the crop or an alternative host plant species) to serve as a safe harbour for susceptible insects. This acts as a source of susceptible alleles and helps to dilute and slow the evolution of resistance by providing susceptible mates for resistant insects so that their progeny are heterozygous and are killed by the toxin (Tabashnik et al., 2008, 2013).

In terms of the genetics, consider a resistant alleler, which is initially rare. The dominant allele S is susceptible to Bt. If the high dose assumption is achieved, only some rr individuals can survive a full life cycle on transgenic plants and emerge as adults to mate. In the refuge, most emerging adults will be susceptible SS, especially if the r allele has fitness costs in the absence of the Bt toxin. If the refuge is located so that the two sub populations are well mixed, most resistant rr survivors will mate with susceptible SS insects from the refuge. Their resulting Sr progeny cannot survive on the Bt crops and so will not pass on the resistant allele to future generations. Unfortunately, a few insect species, such as the economically important pests Helicoverpazea (Boddie) and Helicoverpa Armigera (Hübner) (both species, confusingly, known as both bollworm and corn earworm), have been identified in the past as 'moderate dose pests', where the toxins were unable to kill all heterozygotes (EPA, 1998; Tabashnik et al., 2008). Even where its main assumptions appear to hold, the high dose/refuge strategy is predicted only to delay resistance and, after two decades of commercially grown Bt cotton and Bt corn (maize), some field evolved resistance has Kuhn 2000)[49], Zalucki, 2012)[73] Vargas et.al, 2005)[67] Childress, 1969)[10]

Agricultural pest management: mathematical modelling

Starting with the USDA in the 1950s (Knipling, 1955)[24], mathematical modelling has long been used to understand the potential effect of sterile insect methods on an insect population (Alphey & Bonsall, 2014b). Models can address research questions relevant to a particular context, whether the target insect is a plant pest that causes damage when ovipositing, through feeding or by transmitting plant pathogens, or is a vector of human, livestock or wildlife diseases. Those research questions can serve a range of purposes, including helping to understand underlying biological processes, designing appropriate traits, predicting the impact of fitness costs, informing the design and evaluation of experiments, or exploring potential benefits.(Handier, 2004)[36]

A common theme in this work is to combine ecology and genetics. For example, modelling the effects of larval competition and exploring late acting lethal phenotypes in mosquitoes predicted that this could be substantially more effective at population control than an early acting (e.g. embryonic) lethality or radiation induced sterility (Atkinson et al., 2007; Phuc et al., 2007; Alphey & Bonsall, 2014a). Indeed, if density dependent juvenile competition were over compensatory, genetic lethality that occurred at an earlier stage, there by freeing survivors from regulation by intense competition, could push adult insect numbers higher than in the natural uncontrolled population (Yakob et al., 2008; Alphey & Bonsall, 2014a)[72] This multidisciplinary approach can be broad; population dynamic models incorporating density dependent competition were combined with epidemiological models to investigate the potential effect of releases on a mosquito borne disease in a human population (Atkinson et al., 2007; Alphey et al., 2011a)[5] and linked with bio-economic and health economic models to estimate the potential cost effectiveness of this novel vector control (Alphey et al., 2011a). Similar multicomponent modelling approaches could be applied to plant pests, to explore potential for cost effective population control to limit crop yield losses.(Mcinnib 1984)[35]

In a simple deterministic population dynamic model with density dependent regulation (Alphey et al., 2011a), pest numbers approach a natural equilibrium, or oscillate around it (Fig. 2). Genetic control using modified males can be incorporated by scaling reproductive growth by the fraction of matings that produce viable progeny (the number of fertile males divided by the total number of males, assuming a well mixed, randomly mating population) (Alphey & Bonsall, 2014b). The density dependence term in the formula is adjusted according to whether the genetically induced mortality occurs before or after the competition takes effect (Alphey & Bonsall, 2014b). Critical thresholds, or tipping points, are a common feature of (Knipling E (1955)[24], Franz G(2011)[29] Rosethele (2001)[8] been surprising that this approach failed to be broadly applicable. Even within D. melanogaster, extensive evidence showed that the P system of transposable elements, based on the concept of hybrid dysgenesis, was not universal. The mobilization of the P elements only occurred when a male from a 'P' strain was crossed with a female from an 'M' strain. Injection of P elements constructs into any strain that was not an 'M' strain suppressed mobilization. Gould (1980)[2] Franz (2005)[32]

Transgenesis in non-drosopholid species

The interest in the use of transposable elements for transformation systems with broader potential continued to grow as new elements, such as the Minos, Mariner, Hermes and other elements were identified in different species. These were the first transposable elements shown to be capable of achieving transformation at a reasonable frequency in species outside of D. melanogaster. Further down the road, what may be the closest thing to a universal system for insect transformation was developed based on the use of another transposable element known as 'piggybac'. This transposable element was based on a gene originally isolated from the cabbage looper Trichoplusia Handler adapted it into a vector that could be injected into insect embryos. Since its introduction in early 2000, this system has been used to genetically transform a wide variety of insect species, (Handler, 2000)[33] Homcjhan 2014)[20] Klassen 2005)[23]

In terms of genes that might exhibit a sex specific pattern of expression, a number of genes involved in the sex determination pathway of Drosophila had been identified, and complete DNA sequences were available for many of them. These included genes such as double sex (dsx) and transformer (tra) and that exhibited some type of sex specific pattern of expression during development. However because of fundamental differences in the mechanisms of sex determination and the extensive evolutionary divergence between these insects it was not clear that the same sex specific type of expression would be seen in pest species such as the Tephritids. Nonetheless, the extensive DNA sequence information available for these genes made them clear targets for isolation and characterization in other insect species qualitatively similar findings to the original proportional release model under the alternative, and arguably more practical assumption, that a fixed number of modified males is released into each pest generation. (Thompson, 2015)[66] (Aikin son 2000)[45]

Gene editing

Heritable genetic 'sterility' is not the only genetics based method being developed to control insect populations (Alphey, 2014; Burt, 2014). Recent advances in genetic modification have focussed on techniques of gene and genome editing. Molecular methods, including CRISPR ('clustered regularly interspaced short palindromic repeats') approaches, have been developed with the aim of precisely modifying genes (Esvelt et al., 2014; Kim & Kim, 2014).[31] These techniques have the potential to drive genetic constructs through a population, incorporating 'gene drive' mechanisms that confer greater than Mendelian inheritance even if the construct has fitness costs.

These gene editing approaches have been developed in mosquitoes either to suppress vector populations, by affecting female fertility (Burt, 2003; Deredec et al., 2008; Hammond et al., 2016), or to modify a population, by spreading a trait that affects the ability to harbour pathogens (Gantz et al., 2015). Gene editing approaches could also be used to suppress agricultural pests and/or manage resistance; for example, CRISPR gene editing has been used in a functional study to identify suitable gene targets in diamond back moth (Huang et al., 2016). However, considerable technical, ecological, regulatory and social engagement work remains to be carried out as these approaches move towards scalable field implementation. Atkinson, 2002)[3]

Interdisciplinary research: theoretical, laboratory and field

Developing genetic approaches to insect control through to field applications is an inter disciplinary endeavour. Theoretical analyses such as those described in the present review are part of a much bigger picture, a composite of varied elements that must work together to achieve real change. Laboratory science is crucial for the creation of appropriate strains, particularly molecular biology and insect genetics. Applying this technique successfully to populations in nature is largely an exercise in applied ecology. For example, how many insects are in the target population? This is hard to measure, although it is a key element of the effective release ratios that will be achieved, and so influences the impact, duration and cost of a control programme. How might the effects of identified fitness costs scale up to population level? Insect behaviour is important; where do they mate and lay their eggs, and how far can they disperse? Released engineered males must be able to reach a significant proportion of females in the target population and be reasonably competitive for mates when they find them. Evolutionary biology and behavioural ecology must be understood, for example, to ensure that mass reared insects retain appropriate mating behaviours, and to inform future resistance management strategies for self sustaining genetic traits that will be designed to persist in the environment. (HSU P.et al (2014).[54]

A variety of performance measures are critical to success, including a lethal phenotype's conditionality (do transgenic insects survive on the antidote containing diet?), lethality (do close to 100% of target insects die in absence of the repressor?) and sex, tissue or stage specificity (does a female lethal construct produce any detrimental fitness effects in males?), in addition to the longevity, flight ability, dispersal, mating behaviour and competitiveness of the released males. (Gaceone, 2011)[46]

Candidate lines are selected and tested, assessing all these crucial performance measures in a stepwise series of experiments and trials progressing from test tube, through small cages, then large cages (semi field conditions), to open release. Technology development, pilot studies and control programmes involve other disciplines beyond science; there are also regulatory, social and ethical dimensions with respect to implementing this approach.(Lavery et al., 2008; Esvelt et al., 2014)[26] He et al. 1995)[47]

Policy and regulation of genetic insect control

Policy and regulations surrounding genetic insect control have developed and expanded in the last few years and continue to receive attention (e.g. House of Lords Science and Technology Committee, 2015). Based on existing environmental risk legislation, in most jurisdictions that have regulatory frame works for these, the deliberate release of genetically modified insects requires proportionate assessment to ensure that wider biodiversity and/or human health is not adversely affected. Simultaneously, the benefits of suppressing agricultural pests, reducing harm and improving plant yields impinge on cost benefit analysis in using particular control technologies.

Across the European Union, Directive 2001/18 requires Member States to evaluate risks of releasing GM organisms (GMOs, whether plants, vaccines or animals). This is a risk (cost) based approach to the deliberate release of GMOs, which is based on the use of recombinant DNA technology (i.e. genetic modification) as the trigger for regulation. Contrasting regulatory processes exist. In Canada, for example, the legislation for 'plants with novel traits' outlines that the phenotypic effects (novel traits) of the plant are the basis for regulation, which is a more 'product based' approach to triggering an environmental risk assessment (Canadian Food Inspection Agency, 2016).

For GM insects, several guidance frameworks have been produced in recent years. The European Food Safety Authority (2012) published a regulatory frame work for GM animals and the World Health Organization (WHO/TDR and FNIH, 2014) issued guidance for the testing and regulation of GM mosquitoes. Both of these asserted that a tiered approach from laboratory studies (focussed on the molecular biology and simple ecological processes) through to contained or confined field trials to commercial implementation should underpin environmental risk assessment in support of the development of GM insect technologies. At each point, risk assessment, risk management and risk communication ensures the validity of the emerging technology.

Unlike plants, where genetic modifications are compared with an unmodified (conventional) plant, defining harm and identifying appropriate comparators for genetically modified insect pests requires more nuanced approaches. Proportionate to the technology and logically consistent with other pest intervention methods, appropriate ways of assessing the environmental risk might (Sander 2014)[55] Schetelig, 2009)[60] Tan, et al, 2013)[64] polymerase chain reaction to align with specific regions of the genome for targeted amplification. As described Hsu et al. this targeting system can also be thought of as similar to the search function of contemporary word processors that can identify a specific string of letters in a lengthy word document.

The pairing of the complementary sequence in the sgRNA genomic sequence. These changes include double strand breaks and repairs, modification of terminal sequences and other editing type functions (reviewed in).[56] In short, this is a system that can modify the DNA of a genome in vivo, without the introduction of exogenous segments as is currently done in transgenic systems.

There are two Drosophila-based systems that have been most widely used to make these modifications. One is based on the injection of plasmids into early embryos, either together or separately, containing the two parts of this system (the CRISPR and the gene encoding the Cas9 enzyme). A second approach uses strains that have been engineered separately to contain each of the two different components. (Haymer, 1994)[15]

Genome level approaches

Gene editing

One great advantage of working at this level is that the genome of each organism can be analysed de novo. In other words, these are purely molecular methods that can be carried out newly for each genome, and there is no dependence on gene homology or the transfer of technology from Drosophila. Genome level comparisons are still often made to Drosophila to facilitate the annotation of data derived from these studies, but this is not strictly a requirement for functional analysis of a new genome. For example, to identify genes involved in insecticide resistance in the oriental fruit fly, Bactrocera dorsalis, used a de novo assembly of the transcriptome (the transcribed sequences of the genome) of this species to identify several scores of genes actually or potentially involved in chemical resistance. Some genes from Drosophila were used during the annotation process, but the raw data were generated without any reliance on direct homology from Drosophila genes. (Wise et al)[71]

Another example of a genome level approach relevant to insect pest management, and one that was also developed independently of Drosophilacentric work, involves the use of RNA interference (RNAi) technology to carry out functional studies of several thousand genes identified in genome of the flour beetle Tribolium castaneum. The RNAi method is designed to either completely eliminate, or at least knockdown, the expression of genes to a point where the functional properties of the gene can be clearly identified. This method uses small segments of RNA to inhibit expression by interfering with the translation of RNA transcripts of individual genes. Using this approach, Schmitt-Engel developed an extensive database of individual genes important in the early development of Tribolium. Many of the genes they identified have great potential for control applications, and many would likely have escaped identification using traditional candidate gene approaches.

CONCLUSION

In conclusion, the use of genetic tools in pest man- agement is likely to increase dramatically in the future, especially in the realm of biologically based control methods. The advent and increasing use of genome level tools holds great prospects for novel approaches to achieve this, and for moving away from the need to transfer Drosophila-based technologies to pest species. Drosophila will continue to serve as a model organism in many realms of biology, and will no doubt continue to contribute to the genetic understanding of pest species. However, given the fact that insects are among the most diverse organisms found on the planet, for the future it is clear that it will be to everyone's advantage to use technologies that consider each species independently and without the need to impose constraints for understanding the biology of each species. (Thompson 2015)[66]

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