FUTURE NEEDS IN RESEARCH ON GENETIC SEXING OF *Ceratitis capitata*

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Abstract

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The author makes suggestions on the direction of research for genetic sexing over the next several years and prepared the paper as a guide for discussion. The literature of genetic and cytogenetic studies on insects as a whole is the basis for most of the approaches that the genetic control community has used, but only a tiny fraction of the literature is directed at genetic sexing and most of that is limited to small scale laboratory studies. The effort to use genetic sexing strains on the scale of mass rearing of medflies is unprecedented, and it is not surprising that a few problems have been encountered during implementation. Consideration of this fact leads to the conclusion that it is necessary to 'think big' and target the research.

1. BACKGROUND

Genetic sexing of the Mediterranean fruit fly, *Ceratitis capitata*, was set as a worthwhile goal about fourteen years ago by a Consultants Meeting convened by the Joint FAO/IAEA Division. The economic advantages of being able to kill females selectively during mass rearing of sterile flies were obvious. Large scale programmes for eradication of the medfly were anticipated, and a method was being sought to avoid having to rear the females and to eliminate oviposition stings by sterile females. At that time there was a general lack of information on the genetics of this species. The literature was so sparse that a complete literature survey could be accomplished in one afternoon at the library. On the basis of the information available for the medfly and other insects (for which genetic sexing was documented), priorities were outlined and a plan was formulated for a balanced programme of research and development aimed at both the accumulation of basic information and studies for applied objectives.

In 1980 most of the existing genetic sexing systems were based on genetic markers such as dominant insecticide resistance or recessive colour variants that had been rendered male linked by the use of translocations and inversions. There were a

few innovative systems that employed recessive, temperature sensitive lethals or other recessive balanced lethal combinations. At that time, eukaryotic molecular biology was also underdeveloped. For example, there were no methods for germ line transformation for any insect. Cloning and characterization of genes were still fairly difficult, and this whole area, although potentially of great promise, was not well advanced enough to offer any obvious solutions to genetic sexing of the medfly. Therefore, the original blueprint for R&D aimed at genetic sexing of the medfly was a combination of classical genetic and cytogenetic techniques based on the successful application of such efforts with other insects. Although some early work was directed towards insecticide resistance, this was discouraged to some extent because of the possibility of the accidental release of resistance into field populations.

Over the course of these past fourteen years, a great deal of progress has been achieved. An extensive list of mutants (and genetic map) and detailed maps of polytene chromosomes from trichogen cells and salivary glands are available. Significant advances have been made in radiation genetics and population genetics of this fruit fly. Our current understanding of the medfly is continuing to accumulate at an advanced level. This total effort should provide a sound basis for future genetic manipulation of the medfly and also provides a useful plan of action and expected results for other tephritid flies. The overall prospects for future breeding programmes are certainly enhanced by the intensive efforts of those scientists involved in basic studies over the past several years.

Genetic sexing strains have been assembled with a white-pupa mutant and a temperature sensitive lethal (*tsl*) as the means for the automated separation (with seed sorting machinery) of males and females and the selective killing of females, respectively. Other efforts, e.g. based on alcohol sensitivity, were promising but complicated by mitigating factors of the alcohol dehydrogenase system in the medfly that led to interesting results but no sexing system. A dieldrin resistance factor was abandoned because of cross-resistance and difficulty in establishing a clear-cut inheritance. A system based on purine sensitivity was also discarded.

Two major problems were encountered with the system assembled for the pupal mutant. First, the machinery used to separate the wild type from the mutant is not completely effective, which means that a minor fraction of the released insects are females that will impart oviposition stings, with accompanying blemishes, on the fruit. This problem is not too important if eradication, without subsequent reinvasion, of the medfly is achieved, because under those circumstances the damage caused by the females occurs in only one growing season. However, where the sterile insect technique is used as a control measure or for quarantine purposes, the damage to fruit will be an ongoing process that will invariably be accompanied by complaints from growers.

The other problem was simply breeding a strain that did not deteriorate under conditions of mass rearing. Because the males of these strains are heterozygous for a

holandric translocation, any contamination of the strain with a normal male, either through an accidental introduction from a wild type strain or via genetic recombination, both meiotic and mitotic, will destroy the ability to maintain the genetic sexing strain in a usable state. The release of a small fraction of females has not been overcome, but the complication of recombination has been circumvented by the isolation of translocations that are more stable. Large scale field tests with the white-pupa strain were a resounding success, with the very promising result that a males-only release led to a much better performance of the sterile males. Elimination of females from the releases increased the effectiveness of the sterile males in mating with wild females. The original idea of saving half of the rearing costs is further enhanced by this development, and there is now no doubt of the importance of devising a female-killing system that will produce only males for sterilization and release.

Two problems also surfaced with the effort to make sexing systems by using temperature sensitivity. The early embryonic stage and neonate larva are normally sensitive to and also adversely affected by temperature changes, and this situation has led to a sexing strain in which some of the males are also killed by a discriminating temperature that kills the females. It might be possible to isolate a more effective lethal gene. The second problem is related to genetic stability under the conditions of mass rearing, but by the time the *tsl* was being implemented, the experience with translocation and recombination in the pupal mutant strains was used to good advantage to construct a stable strain. This strain is currently being tested for its suitability for mass production.

After germ line transformation methods were developed for *Drosophila melanogaster*, several groups of scientists tried to adapt P element to the medfly and other insects. These efforts were not successful, because the transposon vector seems to be useful for only closely related species. Investigations on the molecular biology of the medfly and other fruit flies are continuing, with important data being collected. Current efforts on germ line transformation are aimed mostly at the other *Drosophila* transposons, viz. *mariner* and *hobo*. Recently, a possible hybrid dysgenesis system was described, and developments such as this might expose a useful transposon.

2. THE FUTURE

At this point in time, the recommendation for future work on development of genetic sexing obviously involves a heavy emphasis on molecular biology; however, there is no way to predict when useful technology will be available. There are perhaps a few immediate improvements that can be made with classical genetic and cytogenetic methods. One important fact must be emphasized, and that is that the goal in research on the medfly is genetic sexing and sterilization for use in eradication

programmes. Restraint should be exercised to avoid unnecessary research; in other words, avoid the 'drosophilazation' of the medfly as a laboratory insect for basic studies that are not critical to getting the job done.

2.1. Classical genetics

To begin with, potential improvements can be envisaged for strains based on white pupae. After overcoming the restraint of genetic recombination, which caused strain deterioration, via the synthesis of stable translocations, the main problem now is to eliminate females that slip through the machine used for separation. Probably, it will be impossible to obtain perfect separation, but we could include mutants in the genetic sexing strains that would render the females harmless. Scientists with good skills in mutagenesis and radiation genetics could set about to develop strains in which the females are homozygous for white pupae and some other trait, e.g. an eye colour mutant that causes blindness or a flightless mutant, both of which already exist in the medfly. New translocations would probably be necessary, but the techniques for inducing and screening translocations are available. Perhaps a better idea would be to induce a mutant condition (more than likely affecting the muscles) whereby the females could not insert their ovipositor into a fruit, or a modification of the sensory organs so that the flies would not be able to identify fruit. These latter mutants may already exist in the large factory strains, since those strains do not have to pierce fruit to reproduce under the conditions of the factory or laboratory.

For strains based on temperature sensitivity, there should be a new effort to induce and study additional *tsl* mutants, with the hope that a better system can be assembled. The linkage and cytogenetic maps for the medfly and the accompanying knowledge on translocations and inversions are much more extensive since the initial work on *tsl* mutants. With the proper tools in hand, the work of a breeder is much less complicated. Schemes for inducing, detecting and evaluating *tsl*s should be much easier and more sensitive than in the original work, where only one stable mutant was isolated. The use of temperature as a discriminating condition is still a very attractive approach, and there may be some gems of wisdom gained that will be useful later when genetically engineered systems based on heat shock promoters are assembled.

Genetic leakage is a problem that will continue to plague the use of genetic sexing systems, and this will probably be true for genetically engineered strains as well as the current strains. A better knowledge of genetic and cytogenetic maps will be invaluable as a means for tracking what is happening when a strain begins to break down. For that reason, traditional genetic and cytogenetic studies should continue for the near future. This work should include the development of deletions and inversion balancers and can be integrated with molecular markers, so that mutants, microsatellites and restriction fragment length polymorphisms are used to map the genome and study genomic organization.

2.2. Population genetics

The most important aspect of population analysis that should be pursued is within the area of searching for hybrid dysgenesis, because presumably this phenomenon would signal the existence of usable transposons. For surveillance purposes, the identification of DNA sequences that differ from one major population to another would be very useful in tracking the source of reinfestation after the medfly is eradicated from an area.

2.3. Molecular genetics

The field of molecular genetics is wide open, but until a good technique for germ line transformation is a reality, there will be no substantial advance in genetic engineering of genetic sexing systems. P element, which is so effective in *D. melanogaster,* has been tested and abandoned, and current efforts are aimed at *mariner* and *hobo.* If *mariner* and *hobo* are not useful, then the prudent approach would be to pursue transposons in the medfly. In a recent report, a condition that looks like hybrid dysgenesis was described for the medfly, and the strains reported there would be a good starting point for screening for an active transposon. There are other ways to look for transposons, e.g. screening highly mutable strains, cloning and sequencing mutants, screening repetitive elements, and cloning the DNA around naturally occurring chromosomal aberrations.

Recently, there was a report on a simple approach of using restriction enzymes to transform a slime mould. Whether this method would work on insects is a big question. Perhaps a cleverly executed combination of first irradiating embryos with a low dosage, to cause minor damage to the chromosomes and DNA, followed by injecting a restriction enzyme and a plasmid containing a suitable reporter gene should be tried. This strategy would take advantage of the natural repair mechanisms and the ability of restriction enzymes to cut DNA.

The cloning of reporter genes and construction of plasmids that could be used whenever germ line transformation is finally worked out would be useful. Cloning and sequencing of the genes could be a part of the screen for transposons. Easily identifiable phenotypes, e.g. body or eye colour, are probably the best target genes.

Whenever transformation is possible, then decisions have to be made on what genes to engineer. From the standpoint of genetic sexing, the gene should be a conditional female-killing trait. The condition could be any physical variable (e.g. temperature) or any toxic substance. For the near future, work should be done on characterizing the genome in terms of sex determination (Y chromosome) and promoters for genes that are expressed in only one sex. Any information developed will be useful, but the key element in deciding what to work on should be whether the data will be useful for genetic sexing and sterilization.