CRP Title:

Molecular and genetic approaches to develop sexing strains for field applications in fruit fly SIT programmes

Section/Division: NAFA

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Period Covered: 1994 - 2001

Objectives of CRP

Overall

To promote the use of genetic sexing strains in fruit fly SIT programmes.

Specific

- To optimize genetic sexing strains (GSS) as regards their genetic composition, productivity and application.
- To develop third generation GSS using nuclear and molecular methods.
- To develop of GSS in fruit flies other than medfly.

Outputs

a) ***Research***

Competitive medfly GSS has been demonstrated

Many medfly GSS have been evaluated in field cages in competition with wild insects. In no case has a GSS performed less well than any other mass reared strain. The genetic changes used to construct the GSS do not impact negatively on the fitness of the strain. Much of this work was done in collaboration with participants in CRP D.4.10.12

All male releases were more effective than the release of males and females

Studies following the open field release of both bisexual and all male sterile medflies have shown that the release of males only is 3-5 times more effective at inducing sterility in the field population. Sterility induction in the field females is the key determinant of the efficiency of an SIT programme.

Genetic instability during mass rearing of medfly GSS was analysed and solved

Two types of instability have been documented during the large scale rearing of GSS. They have different origins and impact in specific ways on the pattern of instability. An understanding of the genetic and cytological basis of the instability has enabled improved strains to be designed. It is impossible to mimic one of these types of event in the laboratory as it is exceedingly rare. In addition, the introduction of the Filter Rearing System has provided added security to the mass rearing of these strains.

New selectable genes and chromosomal rearrangements were isolated in medfly and *B. tryoni*

In medfly, there are now three mutations being used to develop GSS. Two of them are being used in operational programmes whilst a third is undergoing evaluation. The latter could be used to isolate female larvae for the production of parasitoids. Inversions have also been induced and studied in medfly for the first time. One of these rearrangements has already been introduced into a GSS and is undergoing evaluation for competitiveness and mass rearing profile. Other inversions have been used to construct a balancer chromosome that will be very useful in the isolation of new mutations. In *B. tryoni*, several translocations and 2 selectable markers have been studied. In addition, a potential GSS has been developed in *B. dorsalis* using a white pupal colour mutation.

In situ hybridisation of genes to polytene chromosomes and the development of DNA markers for *B. tryoni* was achieved.

In medfly, many DNA sequences both coding and non-coding have been hybridised to polytene chromosomes and their positions mapped. This, together with the genetic data, has confirmed that there is a great deal of conservation of the organisation of the genome in flies. Fluorescent in situ hybridisation (FISH) has been developed for medfly and used in the analysis of transformed lines. Polytene chromosome analysis and in situ hybridisation was also developed for *B. tryoni*. In *B. tryoni* a collection of DNA microsatellites has been isolated and they are currently in use to identify the origin of outbreaks and help improve SIT interventions.

b) ***Others***

- Development of molecular markers, including species specific ones, to analyse field populations.
- Identification of hybrid dysgenesis in medfly, the development of transgenic techniques and the importance of repetitive DNA in genome.
- Definition of the sex determination system in medfly, Queensland fruit fly and olive fly.
- Defined levels of male recombination in various populations and provided evidence for premeiotic recombination in males.
- Improving the level of male mating competitiveness through the use of ginger oil and through selective breeding.
- Identification of possible modifiers of the ts1 mutation in some genetic backgrounds.

Effectiveness of CRP

a) ***In reaching Specific Objective***

Specific Objective 1. To optimize the composition, productivity and application of GSS.

The first three research outputs listed above have been essential in reaching this objective. GSS were shown to be competitive in field cage tests and to induce significantly higher levels of sterility in wild females than when bisexual strains were used. The knowledge gained from the analysis of stability has been used directly to improve GSS for field operations.

Specific Objective 2. To develop 3rd generation GSS using nuclear and molecular methods.

Many transformed lines are now available in medfly and have been studied in relation to this objective. The biological reagents required for medfly transformation are now available. The

demonstration that there are mobile elements in field populations of medfly has injected a note of caution into this approach.

Specific Objective 3. To develop GSS in fruit flies other than medfly.

The last 2 outputs listed above have made a major contribution to this objective. In *B. tryoni* and *B. dorsalis* potential GSS have been isolated. There is still however some way to go before these strains can be considered as bona fide sexing strains.

b) In contributing towards Overall Objective

All medfly rearing facilities, with the exception of the plant in Tapachula, Mexico, are now using GSS and there are plans being made to convert Tapachula to GSS rearing in the near future. This development has come about because GSS are competitive, they provide increased efficiency to SIT programmes and the strains themselves can be effectively and predictably mass reared. The first 3 outputs listed above have contributed directly to achieving this objective.

c) Factors, if any, which adversely affected the effectiveness of the CRP

Appropriateness of the CRP

The CRP filled a need to increase the efficiency of medfly operational SIT programmes. The increasing use of GSS protocols is a direct result of information and technology generated in this CRP. The technology has been mainly transferred through several TC projects.

Formulation of the CRP

The success of the CRP is partly due to the rather broad expertise basis of the participants. The CRP had participants from diverse backgrounds that complemented each other- i.e. basic and applied, lab and field, and small and large scale. This was deliberately done so that researchers working in the development of GSS in an academic environment would be able to interact with experts in the field of mass rearing SIT. This ensured that the activities remained focussed on the development of "relevant" technology.

Management problems

In some cases, participants were quite slow in replying to requests for administrative information. This makes arranging travel more difficult than it need be. Perhaps the official letter that is sent to participants could include more forceful requests for responses.

Expectation met

In a minority of cases, the intellectual input was below that expected despite many attempts by participants to provide sound advice. This resulted in the contract of one participant being not renewed for the final year.

Impact of the CRP

Improved medfly GSS have resulted in increased effectiveness of SIT operational programmes as evidenced by the wide take up of the technology. This will contribute to the wider use of the SIT and perhaps to its commercialization. The expansion of GSS technology for other pest species will follow closely that developed for medfly.

Relevance of the CRP

There are still several areas where GSS for medfly will need improvement based on their increasing use in operational SIT programmes. It is very important that the Agency maintains its leading role in the field. This can be done by continuing research in this area at the Seibersdorf Laboratory and by following some of the recommendations given below. In other insect control programmes where SIT currently plays a role and where the use of GSS would be advantageous. The Agency can collaborate on the development of these strains as is currently the case with a new CRP on genetic sexing in the screwworm.

Recommended future action by Agency

The use of molecular techniques to further enhance the usefulness of medfly GSS should be encouraged, especially in the following areas:

- Use genetic transformation systems to develop new GSS and evaluate stability of transformation strains.
- Develop genetically or molecularly marked GSS for medfly and improve stability.

For other fruit flies where SIT programmes are being carried, it will be important that the following activities are supported:

- Transfer of current medfly technology to other species of fruit flies and support of field testing and mass rearing of alternative medfly GSS.
- Evaluation of all male releases in other fruit fly species, initially using field cage evaluation.

The following general activities need to be supported in relation to improving the use of GSS:

- Evaluation of the optimal radiation dose when all male releases are used in SIT.
- Use the filter rearing system to introduce desired traits mass rearing.
- Support the development of cryo-preservation for strain maintenance.

Other Outputs:

- Transfer of GSS technology from medfly to Queensland fruit fly and other *Bactrocera* through increased networking.
- Positive impact of participation in the CRP in relation to other funding applications.
- Provision of opportunities for two-way interaction between operational activities and academic research.
- Development of increased skills following exchange of staff and scientific discussions.
- Fellowship support and the provision of essential supplies.