77

BARD

United States - Israel Binational Agricultural Research and Development Fund

FINAL REPORT

PROJECT No. US-98-79

Pseudo-Y Chromosome Meiotic Drive in the Mediterranean Fruit Fly

S.H. Saul, Y. Rossler

1984

Date June 20, 1983

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. US-98-79

Title of Research Project:

Pseudo-Y chromosome meiotic drive in the Mediterranean fruit fly

Names of Investigators:

Principal Investigator: Stephen H. Saul

Cooperating Investigator(s): Yoram Rossler

Name and Address of Affiliated Institutions:

Principal Institution: Univ. of Hawaii-Dept. of Entomology, 2500 Dole St.

Honolulu, Hawaii 96822

Cooperating Institution(s): "Israel Cohen" Inst. for Biological Control

CMBI- 27 K.K.L. Street, Rehovot 76345

Project's starting date: June 20, 1979

Type of Report: Initial Comprehensive Final X

Signatures:

Principal Investigator

Stephen H. Saul

Institution's Authorizing Official

M. Ghali
Moheb A. Ghali, Associate Dean
Office of Research Administration

TABLE OF CONTENTS

Summary of the research project	
Methods and Results	1
Conclusions	3
Evaluation of results with respect to the original proposal	3
Description and results of cooperation	4
Benefit to agriculture	4
Publications	4
Abstract	5

This project had as its ultimate goal the development of genetically based autocidal techniques. As intermediate goals were the accumulation of basic genetic and cytogenetic knowledge and the construction of a genetic sexing system for use in mass rearing sterile insect release (SIRM) programs. We have been able to produce or isolate several new visible and electrophoretic mutants and carry out genetic studies including linkage analysis. Our most significant result, however, was the production and testing of several Y-autosome translocations involving the rosy eye mutant locus which produce 85-100% males when reared on purine containing medium. This system which can have great value in future and present control programs was about to enter larger scale laboratory rearing trials when the project terminated.

METHODS and RESULTS

a) Electrophoresis. We used 2 basic techniques; continuous gradient and ultra thin layer isoelectric focusing (IEF) polyacrylamide to examine the populations in Hawaii. There is little variation within the Hawaiian populations and only 3 loci (mannose 6-phosphate dehydrogenase, aconitase, and esterase-2) were variable enough to provide useful information.

Standard techniques of electrophoretic analysis (starch gel, single concentration polyacrylamide gel) had led us to believe that Hawaiian populations are genetically depauperate. The technique of continuous concentration gradient polyacrylamide gel electrophoresis (which we have adapted to use with the Mediterranean fruit fly during the past year) separates proteins on the basis of both charge and size and has revealed hitherto unsuspected genetic variation. Two biochemical loci were newly discovered to be polymorphic using this technique: 1) mannose 6-phosphate isomerase (E.C. 5.3.1.8.), monomeric, autosomal; and 2) aconitase (E.C. 4.2.1.3.) also monomeric and autosomal. A third locus which we have been studying in detail, the esterase-2 (an alpha esterase), has 4 alleles segregating in a natural field population from Maui. Particularly striking is the high frequency (over 60%) of a null (in vitro inactive) allele. The USDA laboratory population studied was very similar in gene frequencies to the wild population except for the loss of the least common of the 4 alleles. The significance of the high frequency of null alleles in the Hawaiian populations (we have also examined populations from Oahu and Hawaii) is not yet known and must await results from other populations around the world, especially the presumed ancestral (to Hawaii) Australian populations and South African populations from the center of the species range. The results from these analysis could shed light on problems of population adaptation to, and change under laboratory colonization, and help us to trace the origins and spread of Ceratitidis populations around the world.

b) Mutant analysis. We screened large numbers of inbred lines of flies derived from natural and laboratory populations and used EMS and formaldehyde as chemical mutagens and a cobalt-60 irradiator for physical mutagenesis. Eye color mutations have turned out to be the most common and useful and we have carried out extensive genetic and linkage analysis of these mutants. Purple (Pr) eye is an autosomal dominant and the first described in this species. It is proving especially useful in linkage studies since it can be recognized in the heterozygous state with most other alleles. It should also be very useful in future studies as we attempt to construct balanced lethal systems.

We have carried out further analysis of the mutants isolated by chemical and radiation mutagenesis. The "light" mutant described earlier has been confirmed as an autosomal recessive easily distinguished from the wild type. It has excellent viability and fertility and we have found that the eye color difference is easily distinguished from the wild type even 1-2 weeks after death while caught in a standard sticky trap. This makes this mutant a good candidate for use in release studies in which the released flies must be distinguishable from the native flies i.e., as in a sterile insect control program. The field mating behavior of the mutant should be studied.

We have further studied a mutant which we isolated last year and have named PCV (posterior crossveinless). This mutant is expressed as a break or disappearance of the medial-cubital crossvein. From preliminary results this allele appears to be recessive and the locus is sex-linked. If this proves to be true it will be the first sex linked locus found in the Mediterranean fruit fly ("double chaetae" is sex limited) and should be of great value in our future genetic studies.

c) Translocations and genetic sexing. We found that 4500-5000 rad of cobalt-60 radiation on 1-2 day old flies was the most productive of translocations. Using this dose and our rosy mutant stock we were able to produce 4 separate translocations between the Y chromosome and the autosome containing the wild type allele at the rosy locus. These lines were recognized by the fact that they only produce wild type males and rosy females when raised on normal media. We found that when raised on media containing 0.06% of purine the lines produced 85-100% males. The lines differed in frequency of males and viability and it appeared that a continuing search for new translocations would produce new lines with different and perhaps more suitable properties. Selection for greater tolerance to purine in the male heterozygous flies and therefore greater differences between males and females when reared on purine were underway.

d) Cytogenetic analysis.

Cytogenetic analysis of salivary gland polytene chromosomes in C. capitata has proved to be exceedingly difficult. Using the standard lacto-aceto-orcin staining techniques for Drosophila as a starting point, we have found that C. capitata larvae seem to show less polyteny. In slide preparation, they consequently often appear like young D. melanogaster larvae (2nd instar rather than the optimal 3rd instar), with the concomitant problems of chromosome fragmentation and poor spreading. Moreover, C. capitata shows many chromosomal regions of what appears to be amorphous heterochromatic material. This also interferes with proper spreading and band analysis. Attempts to circumvent these problems by providing a rich larval food medium (to increase chromosome size); by heating the preparations (to spread the chromosomes more); or by reducing the fixing and staining time (to avoid excessive deterioration of chromosomal material), have not removed these problems. However, we have made some significant observations; 1) C. capitata chromosomes take up lacto-aceto-orcin stain almost immediately (one or two minutes is enough for adequate staining); and 2) at least some of the amorphous heterochromatic regions are constant in position, suggesting that they are real variations in structure along the chromosomes, rather than artifacts of the staining process. We hope that eventually maps of at least the polytene bands between such regions can be constructed.

e) Insect growth regulators as control agents

As part of our survey of genetic variation in populations of C. capitata in Hawaii, we sampled flies several times from the peach orchards on Maui. While there, in cooperation with personnel from the USDA/ARS Tropical Fruit and Vegetable Research Laboratory and with expenses for supplies and manpower donated by the Zoecon Corp. of California, we ran trials on the effectiveness of the insect growth regulator (IGR) methoprene, as well as several other insecticides, as soil treatments for the control of C. capitata larvae and pupae. Under the high UV light conditions of the test site methoprene seemed to be rapidly degraded and the most effective treatment was diazinon 50 WP at 3.6 kg of a.i./ ha.

CONCLUSIONS

The slow, but, steadily increasing progress in gathering of basic genetic information and the development of a laboratory prototype genetic sexing system are the major results of this project. As a foundation for future genetic studies and eventual autocidal methodology and as a useful tool in current control programs for this important agricultural pest these results makes us confident in claiming that this project appears to have been successful.

Evaluation of results with respect to the original proposal.

The primary goal of the original proposal was the development of a genetic control technique based on the use of pseudo-Y chromosome meiotic drive or any other genetic methodology. The probability of success in the search for meiotic drive systems, temperature lethals, translocations, etc. is a function of the effort expended in that search, since it requires the irradiation of hundreds, preferably thousands of flies, pair mating for 2 generations, and testing. Rather than put all our resources into this screening program, with no guarantee of positive results until thousands of cultures can be examined, we split our resources by keeping a screening program going at relatively modest levels at all times, while at the same time investigating other areas (also part of the original proposal) in which we can be more certain of results, i.e., electrophoretic analysis, mutant analysis, and to a lesser extent, cytogenetic analysis and the development of a genetic sexing method. The work with methoprene required minimal time and no expense to the BARD grant and seemed like a worthwhile, albeit, not closely related research area.

It is possible that a different strategy, namely to put all resources into the screening for genetic control methodologies would be a worthwhile gamble. However, we chose a more cautious approach which may, or may not, delay the finding of a genetic control system, but, which enables us to collect basic genetic information about C. capitata and help to create a broader base of knowledge from which to build our control programs.

We feel that the results from the genetic sexing work alone would justify the approach we took. Even though not part of the original project description the isolation of the rosy mutant and subsequent development of a laboratory prototype system was ample justification for diverting a major portion of our resources to this work.

DESCRIPTION AND RESULTS OF COOPERATION

There were 3 components to the cooperation on this project: 1) visit by Cooperating Investigator to Hawaii; 2) visit by Principle Investigator to Israel, and; 3) shipment of genetic stocks from Israel to Hawaii. The visits were important as exchanges of technology with each investigator able to learn those techniques which were most developed in the other laboratory. In the case of the Hawaii laboratory this was the advanced electrophoretic methods and the important role of parasites. In the case of the Israel laboratory this was the genetic and recombination analysis of mutants, induction of translocations, and the role of the Mediterranean fruit fly in an highly coordinated integrated pest management program.

Undoubtedly, the most important results of the cooperation followed from the shipment of genetic stocks to Hawaii. This was the first importation of live flies into the U.S. for genetic studies. Unfortunately, delays with quarantine facilities prevented shipment until 5 months before the end of the project. Even in that short time the combining of stocks from both laboratories and subsequent analysis has led to one important manuscript which places genetic markers on all 5 autosomes. This is a major step and will be a foundation for all future genetic studies of this species.

BENEFIT TO AGRICULTURE

If the basic genetic studies of this project can be followed up they could represent a major step along the way to a genetically based autocidal methodology. Even if this is not possible, the genetic sexing prototype system developed can find immediate use in the many sterile insect release (SIRM) programs in use or planned in Mexico, Hawaii, Egypt, and, if needed, California.

PUBLICATIONS (in print, reprints enclosed)

- Saul, S.H. 1982. Some sexually dimorphic characters in the Mediterranean fruit fly *Ceratitis capitata* and their variations. *Proc. Hawaiian Entomol. Soc.* 24:115-21.
Saul, S.H. 1982. Rosy like mutant of the MFF and its potential for use in a genetic sexing system. *Ann. Entomol. Soc. Am.* 75:480-483.
Saul, S.H. et al. 1983. Lab and Field trials of soil applications of methoprene for control of the MFF. *J. Econ. Entomol.* 76:174-177.

PUBLICATIONS (in journal review, manuscripts will be supplied when accepted)

- Saul and Rossler. Further studies of the dc locus in the Mediterranean FF. submitted to *Ann. Entomol. Soc. Am.*
Saul, S.H. Two new eye color mutants in the MFF. submitted to *J. Heredity*.
Saul and Rossler. Linkage groups of the MFF. submitted to *Ann. Entomol. Soc. Am.*
Saul. Genetic sexing in the MFF: Conditional lethal translocations that preferentially eliminate females. submitted to *Science*.

ABSTRACT

The basic genetic research under this project isolated and studied new mutants including the first dominant mutation. Markers were identified on all 5 autosomal linkage groups. A laboratory prototype genetic sexing system using the rosy eye mutant, Y-autosome translocations, and purine medium was developed.