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Genetic Sexing of the Mediterranean Fruit Fly

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STUDIES CARRIED OUT IN THE U.S.A.

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PART 2 OF THE REPORT INCLUDES STUDIES CARRIED OUT IN ISRAEL

AN EXPLANATORY NOTE TO THE REVIEWERS OF BARD FINAL REPORT US-816-84

Please note that this report consists of two separate parts. The first portion of the report describes that work done by the United States Team and the second part of the report describes the work done in Israel.

ABSTRACT

The search for new genetic variation for use in basic studies of medfly biology leading to new genetic sexing and autocidal methods has been successful with a series of at least 10 potential new mutants identified. Moreover, one of the mutants, v-wing, has basically fulfilled the promise of this grant by leading to a new, effective genetic sexing system in the Mediterranean fruit fly. In addition to the goals of the original proposal we explored the techniques of genetic engineering to attempt to incorporate a conditional lethal gene onto the Y-chromosome of males by P-element mediated transformation. This attempt was not immediately successful for technical/biological reasons inherent in the differences between Drosophila and Ceratitis. We feel that the time spent in developing what is essentially a negative result was well spent since the future will certainly see the genetic engineering approach going into new areas for which we have helped lay the groundwork.

We view our major accomplishment as the discovery of a striking genetic mutant involving the wing shape of the medfly which has led to the development of a genetic sexing strain which offers us the opportunity to fully exploit the SIRM method for control or eradication of this worldwide pest species. In addition the white eye marker release stock and the large increase in basic genetic variation in the species will be of value to future research.

There were many "negative" results from this project which will also be of future use. These include mutagenesis and screening for XDH, ADH, and insecticide resistance alleles on a very large scale with only modest success.

OBJECTIVE

The prime goal of this project was the development of genetic sexing techniques to increase the effectiveness, efficiency, and acceptability of the sterile insect release method (SIRM, or SIT) for control of the Mediterranean fruit fly, Ceratitidis capitata (Wiedemann).

Rationale for Genetic Sexing.

The Mediterranean fruit fly is a major agricultural pest in temperate and sub-tropical regions worldwide, attacking over 150 varieties of cultivated fruit crops. It is found in Europe and the Mediterranean region, the Middle East, South Africa, South and Central America, Australia, Hawaii, and at times, California or elsewhere on the Continental U.S. Current control practice in most areas involves the use of bait sprays which contain an insecticide, usually malathion and are spread over wide areas.

The environmentally sound, pesticide free sterile insect release method (SIRM) would be the method of choice for controlling or eradicating the Mediterranean fruit fly if it were not for one major problem that greatly reduces the efficiency of the technique and another major problem which can cause farmers and home owners in the treated area to try to stop the "cure" since it is worse than the "disease."

A. Ovipositional "Stinging".

"A major consideration in contemplating the use of SIT for medfly control is the potential damage caused to the host commodities by the sterile females during the SIT release phase. Although these females are sterile and produce no offspring, they still attempt to oviposit in available host fruits, thus creating oviposition punctures which are prone to bacterial and fungal infections. Even without such infections the mere presence of oviposition punctures is often sufficient to drastically reduce the market value of such fruit and to render it unsuitable for export. For this reason, large scale employment of SIT is rarely considered by countries relying on fruit production for export. The employment of genetic sexing mechanisms prior to release would effectively eliminate this restriction on the use of SIT, and would make this means of pest control highly attractive to a number of the major fruit exporting countries. This, in turn, would create added interest, confidence and acceptance of SIT in the less affluent regions of the world. Not until methods become available to eliminate females from release consignments will SIT gain widespread acceptance for medfly control." [Busch-Petersen, J. Ripfel, A. Pyrek, and A. Kafu. 1988. Isolation and mass rearing of a pupal genetic sexing strain of the Mediterranean fruit fly, Ceratitidis capitata (Wied.). In, Modern Insect Control: Nuclear Techniques and Biotechnology. International Atomic Energy Agency, Vienna.]

B. Effectiveness and Efficiency.

"In addition, evidence from field cages has indicated that the release of only sterile males will increase the efficiency of the SIT by 50-100% owing to an elimination of the non-random mating commonly observed when releasing both sexes" [Busch-Petersen et al., Ibid.] referencing [Robinson, A. S., U. Cirio, G. H. S. Hooper, and M. Capparella. 1986. Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, Ceratitidis capitata. Entomol. Exp. Appl. 41:231-235].

One solution to all these problems is to develop an economical method of separating male and female flies through genetic methods i.e., genetic sexing. Moreover, a genetic sexing method would not only help us to avoid problems, but would add several distinct benefits to a SIRM program: 1) sterilization technologies can be fine tuned to obtain sterile males without regard for effects on females so that e.g., male competitiveness might be easier to improve; and 2) provide a genetic marker that could identify inadvertent releases of fertile males in SIRM programs and reduce opportunities for unexplained failures of SIRM programs.

Several laboratories are attempting to construct genetic sexing systems based on insecticide, or ethanol, or purine resistance, but these have proven elusive. They also all have the major drawback of requiring large amounts of toxic and/or expensive selective chemicals in the mass rearing system. Other attempts to produce genetic sexing systems use selection based on pupal color. These systems have one critical flaw; separation of as many as 100 million flies per day must be done by mechanical sorters at great expense and effort.

DESCRIPTION OF THE RESEARCH RESULTS

We can describe the research results over the course of this project under two broad categories; "negative" and "positive". Both types are of value although the positive accomplishments naturally are more immediately useful. The major accomplishment was the discovery of a structural wing mutant, the v-wing, which was subsequently developed into a viable genetic sexing system now undergoing further testing.

I. "NEGATIVE" RESULTS

a. Genetic engineering. Over the first two years of the grant much effort was spent on an attempt aimed at the construction of genetically engineered strains by means of the p-element mediated transformation system of Drosophila melanogaster. This transformation system involves the microinjection into embryos of DNA plasmids containing an antibiotic (neomycin) resistance gene and screening for antibiotic resistance in their progeny. The proposed protocol for p-element-mediated transformation involved the injection of a heat shock-neomycin resistance fusion developed to function as a dominant selective marker in the presence of the antibiotic, G418. This transformation vector is a defective p-element and is, therefore, unable to produce the transposase necessary for integration of the plasmid into the genome. The defective nature of the vector insures that after integration into the genome, the position of the plasmid will be

stable. Transposase activity is supplied by the co-injection of a helper plasmid which supplies the transposase activity but will not itself integrate. In Drosophila, this hsn gene fusion provides resistance to the highly toxic G418 at a level 2 to 3-fold higher than that required to kill susceptible larvae. The first step in determining the feasibility of using this system in the Mediterranean fruit fly was screening for sensitivity to G418. A complete dose-response curve was developed, with egg to pupal survivorship measured. Probit analysis of the dose-response data for the Mediterranean fruit fly indicates that the 95% LD occurs at a dose of 0.5 mg/ml of G418. A plan was developed to select for transformants at 1 mg/ml or twice the sensitivity level, paralleling the Drosophila system.

To date, approximately 5,000 embryos have been injected with almost 600 surviving the procedure, 85% of these survivors were recovered and established as G₀ lines for screening. 25 G418 resistant lines have been detected but molecular confirmation of transformation is lacking. After over 5000 eggs had been injected there was no evidence for an active transposase required for p-element incorporation in this species. Alternate methods using "nicked" or linear DNA for injection were tried with no success. Our results have been similar to several others in Europe and the U.S. and the initial optimism for this technique has subsided into the recognition that a great deal of basic molecular genetic research will be needed before practical results can be expected with the tephritid fruit flies.

b. Chemical, Insecticide and Antibiotic resistance [conditional lethality].

1. Xanthine dehydrogenase (XDH) During the first year particular effort was placed in finding mutations affecting the xanthine dehydrogenase system were made and some success was had. The 15 potential new mutants found were tested for XDH activity and purine sensitivity and purine sensitivity and enzyme levels in these new mutants were measured.

Purine sensitivity and enzyme levels in new mutants.

<u>Mutant</u>	<u>XDH(u/min)^a</u>	<u>XDH(spec. act.)^b</u>	<u>Purine sens.^c</u>
wild-type	10.2	281	87.0
Rosy	6.1	170	70.0
9	5.4	137	42.0
25	4.7	112	30.0
38	4.4	113	23.0
78	3.6	108	22.0
20	3.3	111	n.a.
37	2.9	70	38.0
118	3.3	88	22.0

^aFlourescent units per minute.

^bSpecific activity per mg of protein.

^cPercentage of pupation after 21 days.

2. We carried out extensive testing of antibiotics [including; kanamycin, spectinomycin, neomycin, chloramphenicol, gentamycin, demecolcine,

streptomycin, ampicillin, and hygromycin B] in Hawaii; with Israel concentrating on screening for insecticide resistance. To date after determining LD₅₀ for egg to adult survival for these compounds and then screening 5-6 million larvae we have found no increased resistance in our populations.

3. Alcohol dehydrogenase (ADH). During the course of the grant period it was decided to expand the search for conditional lethals for use in genetic sexing beyond the rosy-purine system to include other potential candidate methods. The availability of a screening method for ADH null alleles utilizing 1-pentyn-3-ol, pentynol, formed the basis for a large scale selection scheme. Individuals producing ADH metabolize pentynol to a toxic ketone and die in the larval stage. Individuals which are ADH null do not metabolize the chemical and develop normally. Therefore a series of large mass screenings was begun to look for specific mutants at the alcohol dehydrogenase locus (or loci). Screening involved millions of eggs (F₄ from EMS or formaldehyde mutagenized flies) on media containing the unsaturated alcohol, pentynol. This selection was carried out over a period of six months and involved the mass screening of approximately 4,000,000 individuals. A line was established which survives doses of 0.6% pentynol in the media (wild-type stocks are completely killed by 0.2% pentynol. Pupae assayed spectrophotometrically for ADH activity from this line have approximately 15% the enzyme level of wildtype stock on a per mg of protein basis. Possible null and reduced activity alleles were also analyzed electrophoretically on polyacrylamide and isoelectric focusing gels. However, the line has shown no sensitivity to ethanol applied in various ways to the larval medium. It is not clear why the stock should show increased resistance to pentynol, less enzyme activity, and yet no increase in ethanol sensitivity.

II. "POSITIVE" RESULTS

a. Genetic marker of released flies. A mutant phenotype was discovered in which the compound eye contains no pigment and only a light blue reflective/refractive color is present. This phenotype was studied and found to be due to an autosomal recessive gene. The important practical point about this mutation is that flies retain an easily [i. e., "naked eye"] identifiable white eye color even after death. This marker has the potential of saving large amounts of time and labor now spent in marking released flies during SIRM programs with fluorescent dyes and then identifying them under microscopes and UV light.

b. Basic genetic variation.

As is usual in genetic screening programs only a small (25% or less) of observed phenotypic variation is due to "well behaved" genes. This means that the phenotype is based on a highly penetrant genotype with good to excellent expressivity. We isolated a large number, mostly eye color and bristle abnormalities that did not meet these criteria. However, several highly valuable alleles have been found during this grant which are already of value in several aspects of the genetic work. The two most valuable, v-wing and white eye are described elsewhere in this report. There are 11 other eye color variants which are quite subtle and hard to screen and map, however the group of "good" (i. e., easy to screen, good penetrance and expressivity) include a deep red eye color

with flattened facets (flat red); shortened antennae approximately 40% of wild type (stubby antennae); female antennae covered with dark hairs, males with dark hairs on first segment (dark antennae); irregular facet pattern; reduced/missing compound eye; missing wing vein. All these mutants have been determined to be based on autosomal, recessive alleles; red eye, *r* remains the only sex-linked gene discovered in this species.

c. Genetic sexing stock [v-wing mutant].

The v wing stock was produced from a single female fly found in routine screening of laboratory stocks in 1988. After a true breeding stock was produced, crosses revealed that this apparently spontaneous mutant is an autosomal recessive located on the same chromosome as *ap* and *dc*. Homozygous v wing flies reared at room temperature (20-23°C) show a wide range of phenotypic expressions from 1-2% flies with near wild type wings to 30-40% with only stubby remnants; the remaining 60-70% have various degrees of wing damage and cannot fly. At 30°C 100% of the flies have only tiny stubs of the wings remaining and are totally unable to fly.

Due to fiscal and personnel constraints the development of a genetic sexing stock using the v-wing strain was handed over to a separate and concurrent research development project. This led to a stock of medflies which produces only v wing⁺ males, i.e. normal wing flies that can fly and disperse into the environment, and v wing females which, when reared at room temperature line are all v wing [and 98% flightless], but when reared at 30°C are 100% stubby wings and are totally unable to fly. This means that when reared for mass release the sterile males will be able to disperse immediately after adult emergence into the environment where they can be fully effective for the SIRM program. The females upon eclosion will be unable to disperse beyond the release sites and so will not be able to either mate with the released males nor to damage the fruits on the trees. This leads to an essentially complete removal of the deleterious effects of mass releasing female flies. This v-wing based stock can be incorporated into current rearing programs around the world without significant modifications to the facilities. Moreover, since sex ratios in this strain range from 60:40 males:females to 90:10 depending on rearing temperature which reduces the rearing costs at all stages. The main remaining problems with this stock is that it leads to a relatively late stage of separation with extra cost of handling pupae [this is partially offset by the male biased sex ratios described above] and it is untested under mass rearing conditions.

DESCRIPTION OF COOPERATION

Close contact with Dr Rossler is maintained through exchange of results and we were able to extensively discuss, review, and coordinate the US and Hawaii components of the project. Plans were developed for the exchange of mutant stocks, coordinated testing and screening for insecticide resistance genes, and the direction of future genetic research for our project. The exchange of genetic stocks has proven in the past to be one of the most fruitful areas of our program.

MAIN ACHIEVEMENTS

The search for new genetic variation for use in basic studies of medfly biology leading to new genetic sexing and autocidal methods has been successful with a series of at least 10 potential new mutants identified. We view our major accomplishment as the discovery of the v-wing mutant which was later developed into a genetic sexing stock. In addition the white eye marker release stock and the large increase in basic genetic variation in the species will be of value to future research. One way in which the research diverged from the original proposal was in the incorporation of techniques of genetic engineering. The goal of a conditional lethal gene incorporated onto the Y-chromosome of males by P-element mediated transformation is on hold for technical/biological reasons. We feel that the time spent in developing what is essentially a negative result was well spent since the future may well see the genetic engineering approach going into new areas for which we have helped lay the groundwork. Other "negative" results will also be of future use and include mutagenesis and screening for XDH, ADH, and insecticide resistance alleles on a very large scale with only modest success.

List of Publications Related to this BARD grant.

Saul, S. H. 1985. Diagnosing the heterogametic sex in the Mediterranean fruit fly (Diptera: Tephritidae): the first sex-linked gene. *Ann. Entomol. Soc. Am.* 78:198-200.

Saul, S. H. 1986. A personal view of applied biological research. *Proc. Hawaiian Entomol. Soc.* 27:137-140.

Saul, S. H., S. Y. T. Tam, and D. O. McInnis. 1988. Relationship between sperm competition and copulation duration in the Mediterranean fruit fly (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 81:498-502.

J. H. Postlethwait, S. H. Saul, and J. A. Postlethwait. 1987. The antibacterial immune response of the medfly, Ceratitis capitata. *J. Insect. Physiol.* 34:91-96.

J. H. Postlethwait, S. H. Saul, M. Robertson, D. Grace, K. Abrams, and E. Brandenburg. 1988. Mechanisms of immunity in Drosophila and Mediterranean fruit flies. In, American Chemical Society Symposium Series, Biotechnology in Crop Protection. ACS Publications, Washington, D.C.

Saul, S. H. 1986. Genetics of the Mediterranean fruit fly (Ceratitis capitata (Wiedemann)). *Agricultural Zoology Reviews.* 1:73-108.

Saul, S. H. 1987. Biotechnology and fruit flies. In, 2nd International Symposium on Fruit Flies, Sept., 1986, Crete, Greece. IAEA/FAO. Elsevier.

Saul, S. H., and S. D. McCombs. 1988. Developing genetic and biotechnological methods for controlling tropical fruit flies. In *Proceedings of the 11th International Congress for Plant Protection*, Oct. 1987, Manila, Philippines.

Saul, S. H. 1989, submitted to *J. of Heredity*. A genetic sexing system to improve the sterile insect technique against the Mediterranean fruit fly.

GENETIC SEXING OF THE MEDITERRANEAN FRUITFLY, Ceratitis capitata.

BARD PROJECT US-816-84 - FINAL REPORT

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Studies carried out in israel

INTRODUCTION.

The Sterile-Insect Releases Method (SIRM) had been used in the last 2 decades to control or eradicate the Mediterranean fruit fly (medfly) in limited test projects in central America, Hawaii and European countries and also in country-wide eradication programs of the pest in California, Mexico and other central American countries. It's application has been however limited by the inability to eliminate the females in the factory-reared flies prior to release. Elimination of the females is important for various reasons. Firstly, the ability of the released males to locate and mate with field (native) females will be greatly enhanced by the absence of the vast number of released (and sterilized) females. Secondly, the release of large numbers (50% of the released batches) of females, will result in culling of fruit due to attempts of these females to lay eggs and puncture the fruit in the process. This renders almost impossible the use of SIRM in countries which produce and export fresh fruit. The Mediterranean basin falls into that category.

Methods of "genetic Sexing" have been developed for the medfly in the past (Robinson & Rosslr, 1987; Rosslr, 1980, 1982a,b). They were based on sex-limited color pupae dimorphism and the separation of sexes by mechano-optical pupal sorting. With this method flies will have to be reared up to the pupal stage, and than 50% of the production (females) will be sorted out and discarded. Therefore, a "sex killing" system which operates on an early life-stage of the flies (eggs or first instar larvae) and eliminates it early in the

production process will be a more economically solution to the problem (Anonymous, 1986; Robinson et al, 1986).

In the research proposal of the present study we planned to accomplish the following objectives:

1. To screen for resistant traits using chemicals which are not currently used, or will not be used in the future, for the control of the medfly in the field and study the mode of inheritance of these genes.
2. To use the appropriate resistant traits and construct a "genetic sexing" strain of the medfly, which could be utilized for all-male releases in SIRM for the control or eradication of that pest.
3. To enhance our knowledge of the formal genetics of the medfly, by isolating, studying and mapping morphological mutants.

The final report includes the following:

1. A record of our attempts to screen for chemical resistance in the medfly.
2. An in-depth account of our studies with the insecticides Cyromazine^R and Avermectin^R, including studies on the mode of inheritance of their resistance in the medfly.
3. A description of the morphological mutants, isolated and studied in our laboratory, and the current status of the chromosomal maps of these mutnats.

1.SCREENING INSECTICIDES FOR MEDFLY RESISTANCE

We have screened a total of 11 chemicals as possible candidates for "genetic sexing" of the medfly. A rapid exposure methods of eggs and third instar larvae was developed to enable the construction of a dose-mortality curve for each chemical. We were searching for chemicals that affect the survival of eggs or larvae, those being the most feasible candidates for use in a rearing factory on a large scale.

Materials and methods.

A rapid system for exposing eggs and 3rd instar larvae. (Fig. 1)

Eggs or third instar larvae were exposed to given concentrations of the various chemicals tested for a predetermined period (mostly 30 minutes). They were then rinsed thoroughly with water for 60 minutes and placed for hatching or feeding on the regular larval diet. A simple device for rapid exposure was developed by us. Eggs or larvae were placed in a plastic vial with a screen bottom. The vial was immersed in a plastic cup which contained the chemical tested. Air was introduced into the vial through a plastic hose, and the eggs or larvae were constantly supplied with air and the chemical solution constantly stirred during the exposure period. The vial with the eggs or larvae was then transferred into another plastic cup which was immersed in a water bath. water was run through the vial for a period of 1 hour so that the residues of the chemical were completely

removed.

Chemicals used in the screening tests.

1. Alpha-tomatine, range of 100 - 200 ppm in the larval diet.
2. Avermectin, 1 - 1000 ppm. Exposure of eggs and larvae.
3. Cyromazine, 0.1 - 10 ppm in the larval diet.
4. DDT 25% (EC), 10 - 1000 ppm. Exposure of eggs and larvae.
5. Cyhalothrin 25% (EC), Karate^R. Exposure of eggs and larvae.
6. Lindane 20% (EC), 10 - 1000 ppm. Exposure of eggs and larvae.
7. Malathion 80% (EC), 10 - 1000 ppm. Exposure of eggs and larvae.
8. Morestan 25% (). Exposure of eggs and larvae.
9. Propoxur 20% (EC). Exposure of eggs and larvae.
10. Tedion 8% (WP). Exposure of eggs and larvae.
11. Pyriproxifen 10% (EC), (Tiger^R). Exposure of eggs and larvae.

Results

1. Alpha tomatine is a natural product found in Solanaceous fruits (tomatoes) prior to colorbreak. It was found to be toxic to medfly larvae at rates of 25-200 ppm (Chan & Tam, 1985). It was therefore included in our studies (Table 1). Our results showed no effect of the product on the survival of medfly larvae.

Table 1. Effect of alpha tomatine, incorporated in the larval diet, on survival of eggs and larvae of the Mediterranean fruit fly.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
		<u>Eggs 0</u>	<u>hours old</u>		
0	868	95.3	11.4 (10-13)	94.4	90.9
100	804	96.8	11.9 (10-13)	91.3	88.3
150	816	91.9	12.5 (10-15)	79.6	73.2
200	828	96.1	12.6 (11-14)	81.8	78.6
250	848	96.0	13.5 (11-16)	73.3	70.4
		<u>Eggs 48</u>	<u>hours old</u>		
0	837	96.3	12.0 (11-14)	88.1	84.8
100	819	95.0	11.9 (11-14)	91.6	87.1
150	819	96.1	12.8 (11-15)	54.5*	52.4
200	812	88.5	12.9 (11-15)	92.0	81.5
250	810	96.4	13.3 (11-15)	80.2	77.3

* One rep. out of 4 - no pupae ?!

2. Avermectin. The data will be presented in conjunction with the in-depth study of that product at a later stage in the current report.

3. Cyromazine. The data will be presented in conjunction with the in-depth study of that product at a later stage in the current report.

4. DDT. DDT was used in the past for the control of the medfly in Israel. The product was than replaced by Methoxychlor and later by Malathion. We tested the effect of DDT on eggs and third instar larvae of the medfly by dipping eggs for 1/2 hour in DDT suspension of varying concentrations (Tables 2 and 3). We found no effect on the

survival of medfly pre-imaginal stages at concentrations up to 1000 ppm. Only the immersion of eggs for 24 hours in 1000 ppm DDT did result in considerable mortality, but so were also the controls (Table 4).

Table 2. Effect of DDT, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in DDT suspension of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	816	82.2		75.4	62.0
10	836	82.4		77.9	64.2
100	827	80.9		70.0	56.6
1000	843	80.0		75.5	60.4
<u>Eggs 24 hours old</u>					
0	920	89.2		92.1	82.2
10	823	88.7		91.8	81.4
100	831	89.0		91.6	81.6
1000	826	89.1		88.3	78.7
<u>Eggs 48 hours old</u>					
0	821	59.7		68.0	40.3
10	880	63.2		65.8	41.6
100	850	62.0		67.2	41.6
1000	817	61.0		37.2	37.2
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	231	100		87.4	87.4
10	214	100		89.3	89.3
100	238	100		87.4	87.4
1000	222	100		82.4	82.4

Table 3. Effect of DDT, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in DDT suspension of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	865	77.5		87.0	67.4
1000	906	76.4		89.2	68.1
<u>Eggs 24 hours old</u>					
0	816	76.8		92.5	71.1
1000	959	77.3		94.9	73.3
<u>Eggs 48 hours old</u>					
0	819	78.0		92.2	71.9
1000	937	77.2		92.9	71.7
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	220	99.5		92.2	91.8
1000	251	98.8		94.8	93.6

Table 4. Effect of DDT, on survival the Mediterranean fruit fly. Immersion of eggs in 1000 ppm DDT suspension for 24 hours.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
0	819	41.1	14.0 (13-17)	62.6	25.8
1000	813	10.7	14.0 (13-15)	30.6	3.2

5. Cyhalothrin (Karate^R). The Synthetic pyrethroid compound was also tested by exposing eggs and third instar larvae in the suspension of the insecticide of varying concentrations for 30 minutes (Table 5). The exposure of eggs had little effect on the mortality of larvae and adult emergence. However, concentrations of 100 ppm and higher were lethal to third instar larvae.

Table 5. Effect of Cyhalothrin on survival of the Mediterranean fruit fly. Immersion of eggs and third instar larvae in Cyhalothrin suspension of varying concentrations for a period of 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	806	92.7	12.0 (9-14)	87.3	80.9
10	815	78.7	14.5 (11-17)	22.3	17.5
100	840	89.5	14.7 (11-17)	77.8	20.5
1000	832	85.2	14.3 (11-17)	80.5	30.3
<u>Eggs 24 hours old</u>					
0	815	73.3	10.4 (11-16)	85.1	62.3
10	1036	78.6	16.0 (11-19)	22.4	17.6
100	929	82.7	14.7 (11-19)	50.0	41.3
1000	880	80.6	14.2 (11-19)	59.9	48.3
<u>Eggs 48 hours old</u>					
0	946	66.0	13.9 (12-17)	78.7	51.9
10	816	66.2	15.3 (13-19)	58.0	38.4
100	823	67.0	15.5 (13-19)	53.5	35.8
1000	824	71.4	14.9 (13-19)	50.0	35.7
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	299	99.7		90.6	90.3
10	335	50.4		94.7	47.8
100	242	9.1		100.0	10.7
1000	272	0.0		0.0	0.0

6. Lindane. Lindane is a veteran product, and has little use in agriculture. We tested the product with the same standard procedure and found no effect on the survival of medfly eggs or larvae, except when third instar larvae were exposed to 1000 ppm for 30 minutes. (Table 6). Immersion of 24 hour old eggs in Lindane suspension produced results similar to those of DDT with the same technique (Table 7).

Table 6. Effect of Lindane on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in Lindane suspensions of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	917	88.1		74.9	66.0
10	896	87.3		77.2	67.4
100	815	88.5		81.6	72.1
1000	874	88.0		77.5	68.2
<u>Eggs 24 hours old</u>					
0	906	72.1		74.0	53.3
10	813	69.7		73.4	51.2
100	931	73.3		67.7	49.6
1000	914	74.0		70.7	52.3
<u>Eggs 48 hours old</u>					
0	837	76.1		71.3	54.2
10	848	76.3		79.9	61.0
100	859	75.9		67.5	51.2
1000	949	75.9		67.7	51.4
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	221	100		77.8	77.8
10	218	100		74.8	74.8
100	202	98.5		70.9	69.8
1000	227	92.5		0.0	0.0

Table 7. Effect of Lindane, on survival of the Mediterranean fruit fly. Immersion of eggs in Lindane suspension of for 24 hours.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 24 hours old</u>					
0	819	41.1	14.0 (13-17)	62.6*	25.8
1000	814	Could not count		1.6**	1.6

* of hatch. ** of eggs.

7. Malathion. Although malathion is still used extensively for the control of medfly adults in Israel and many other countries, we

incorporated it in our studies. Neither the exposure of eggs and third instar larvae to malathion up to 1000 ppm (Table 8) nor a longer exposure period (Table 9) had any effect on the mortality of the pre-imaginal stages of the medfly.

Table 8. Effect of Malathion, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in Malathion suspension of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<hr/>					
		<u>Eggs 0</u>	<u>hours old</u>		
0	834	87.6		83.6	73.3
10	840	87.4		78.1	68.2
100	884	88.0		82.1	72.3
1000	889	88.4		84.0	74.2
<hr/>					
		<u>Eggs 24</u>	<u>hours old</u>		
0	829	73.6		78.5	57.8
10	839	73.4		78.4	57.6
100	820	73.8		81.2	59.9
1000	819	74.8		73.2	54.8
<hr/>					
		<u>Eggs 48</u>	<u>hours old</u>		
0	812	84.4		49.6	41.9
10	815	80.9		49.8	40.2
100	805	82.6		51.3	42.4
1000	820	83.9		45.9	38.5
<hr/>					
	<u>Larvae</u>	<u>% pupa</u>	<u>Larvae III</u>	<u>% adult</u>	
0	161	99.4		68.1	67.7
10	258	98.1		79.1	77.5
100	247	100		73.3	73.3
1000	266	99.6		72.8	72.6

Table 9. Effect of Malathion, on survival the Mediterranean fruit fly. Immersion of eggs in 1000 ppm malathion suspension for 24 hours.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
		<u>Eggs 24</u>	<u>hours old</u>		
0	819	41.1	14.0 (13-17)	62.6	25.8
1000	808	41.5	13.9 (13-17)	64.1	25.9

8. Morestan. Morestan is actually a fungicide and not an insecticide. It was incorporated here together with tedion, to find out whether such compound might have any effect on the medfly under laboratory condition and hence be used later as sexing agents. Here too, the product had no effect on the mortality of the medfly in the exposure methods used for that study (Table 10).

9. Propoxur. That product was used successfully by Seawright and co-workers to develop a sexing system in a mosquito species (Anopheles albimanus) (Seawright et al., 1978). We carried out four tests with the product. 1) Exposure of eggs and third instar larvae to concentrations up to 500 ppm (Table 11). 2) Exposure of eggs and third instar larvae to concentrations of up to 2000 ppm (Table 12 and 13). 3) Exposure of 24 hours old eggs to 2000 ppm propoxur for periods up to 4 hours (Table 14). 4) Exposure of eggs to 2000 ppm propoxur for 24 hours (Table 15). The only evident effect was with 2000 ppm propoxur on third instar larvae and on eggs at a 24 hours exposure periods.

Table 10. Effect of Morestan, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in Morestan suspension of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<hr/>					
		<u>Eggs 0</u>	<u>hours old</u>		
0	821	72.5	13.6 (12-17)	83.5	60.5
100	868	48.4	13.8 (12-17)	67.4	32.6
1000	903	46.7	14.6 (12-17)	40.8	19.0
5000	876	41.4	14.4 (12-17)	50.7	21.0
<hr/>					
		<u>Eggs 24</u>	<u>hours old</u>		
0	878	63.8	14.6 (12-16)	70.4	44.9
100	895	63.2	15.0 (12-17)	65.1	41.1
1000	862	58.6	15.0 (12-17)	66.3	38.9
5000	817	54.7	14.9 (12-17)	59.7	32.7
<hr/>					
		<u>Eggs 48</u>	<u>hours old</u>		
0	850	53.3	16.0 (14-19)	60.9	32.5
100	811	43.4	16.2 (14-19)	62.5	27.1
1000	808	43.9	16.0 (14-19)	60.6	26.6
5000	830	41.3	16.0 (14-19)	57.1	23.6
<hr/>					
	<u>Larvae</u>	<u>% pupa</u>	<u>Larvae III</u>	<u>% adult</u>	
0	260	100.0		96.2	96.2
100	334	100.0		91.3	91.3
1000	295	100.0		98.0	93.8
5000	322	100.0		93.8	93.8

Table 11. Effect of Propoxur, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in propoxur suspension of varying concentrations (up to 500 ppm) for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
		<u>Eggs 0</u>	<u>hours old</u>		
0	1007	94.8		94.3	89.5
10	1041	96.6		96.1	93.0
100	967	96.6		94.8	91.5
500	1006	95.9		95.6	91.7
		<u>Eggs 24 hours old</u>			
0	825	77.5		85.8	66.4
10	914	80.2		86.5	69.4
100	914	80.4		83.7	67.3
500	827	77.3		84.8	65.5
		<u>Eggs 48 hours old</u>			
0	896	66.5		77.0	51.2
10	805	73.0		76.9	56.1
100	808	71.3		75.9	54.1
500	822	70.6		79.3	56.0
		<u>Larvae III</u>			
	Larvae		% pupa	% adult	
0	220		100	95.0	95.0
10	218		100	96.8	96.8
100	212		100	98.6	98.6
500	240		100	95.8	95.8

Table 12. Effect of Propoxur, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in Propoxur suspension of varying concentrations (up to 2000 ppm) for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	867	87.0		88.7	77.2
500	816	82.0		89.1	73.0
1000	821	83.3		89.2	74.3
2000	827	79.9		85.2	68.1
<u>Eggs 24 hours old</u>					
0	847	81.1		82.0	66.5
500	815	80.1		79.5	63.7
1000	827	78.4		79.6	62.4
2000	851	78.0		75.5	58.9
<u>Eggs 48 hours old</u>					
0	830	78.3		78.6	61.6
500	832	76.6		83.2	63.7
1000	870	76.6		83.2	63.7
2000	832	78.4		79.8	62.5
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	201	99.5		94.5	94.0
500	234	98.7		83.1	82.1
1000	239	73.2		85.7	62.8
2000	290	22.1		81.3	17.9

Table 13. Effect of Propoxur, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of exposed eggs or larvae in 2000 ppm Propoxur suspension for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
			<u>Eggs 0 hours old</u>		
0	865	77.5		87.0	67.4
2000	864	73.3		89.3	65.4
			<u>Eggs 24 hours old</u>		
0	816	76.8		92.5	71.1
2000	893	78.7		85.0	66.9
			<u>Eggs 48 hours old</u>		
0	819	78.0		92.2	71.9
2000	889	74.6		94.4	70.4
			<u>Larvae III</u>		
0	Larvae	% pupa		% adult	
	220	99.5		92.2	91.8
2000	266	85.0		88.9	75.6

Table 14. Effect of Propoxur on survival of the Mediterranean fruit fly. Immersion of 24 hours old eggs in 2000 ppm Propoxur for periods up to 4 hours.

Period	Eggs	% hatch	Dev. period	% pupa	% surviv.
1/2 h	821	89.3		82.7	73.8
2 h	823	87.6		81.8	71.7
4 h	808	85.6		91.8	78.6

Table 15. Effect of Propoxur, on survival the Mediterranean fruit fly. Immersion of eggs (24 hours old) in 2000 ppm Propoxur for 24 hours.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
0	819	41.1	14.1 (13-17)	62.6	25.8
2000	869	0		0	0

9. Tedion. No effect on the survival of the medfly was observed (Table 16).

Table 16. Effect of Tedion, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in Tedion suspension of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	831	87.6	12.2 (11-14)	51.0	44.6
10	843	89.3	12.0 (11-14)	44.2	39.5
100	889	89.4	12.0 (11-14)	51.0	45.7
1000	895	89.7	12.2 (11-14)	44.0	39.4
<u>Eggs 24 hours old</u>					
0	889	58.5	13.3 (12-15)	42.3	24.7
10	911	60.7	13.8 (12-15)	35.8	21.7
100	895	61.0	13.7 (12-15)	45.1	27.5
1000	831	61.4	13.2 (12-15)	38.2	23.5
<u>Eggs 48 hours old</u>					
0	910	53.8	13.4 (12-15)	43.7	23.5
10	949	54.8	13.5 (12-15)	51.9	28.5
100	902	51.6	13.3 (12-15)	49.5	25.5
1000	801	53.3	13.5 (12-15)	40.7	21.7
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	286	100.0		96.9	96.9
10	286	100.0		94.1	94.1
100	390	100.0		96.4	96.4
1000	338	100.0		91.0	91.0

11. Pyriproxifen. Tiger^R is a new Insect Growth Regulator produced by Sumitomo Ltd., Japan. This is a stomach poison with extreme effect on various insect pests at rather low concentrations. We tested the product with the standard exposure procedures (Table 17) with concentrations of up to 100 ppm and found almost no effect of the product on the survival of the medfly eggs or larvae.

Table 17. Effect of Tiger, on survival of eggs and third instar larvae of the Mediterranean fruit fly. Immersion of eggs or larvae in Tiger suspension of varying concentrations for 30 minutes.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
<u>Eggs 0 hours old</u>					
0	921	90.9	11.9 (10-14)	87.7	79.7
1	825	91.6	12.0 (10-14)	87.6	80.2
10	883	90.6	12.3 (11-14)	61.4	55.6
100	871	91.7	12.2 (11-14)	80.9	74.2
<u>Eggs 24 hours old</u>					
0	837	88.8	13.2 (12-16)	86.5	76.8
1	897	88.7	13.3 (12-16)	76.4	67.8
10	945	86.3	13.2 (12-16)	85.5	73.9
100	885	87.6	13.0 (12-16)	91.0	79.2
<u>Eggs 48 hours old</u>					
0	837	94.1	13.3 (12-16)	86.3	81.2
1	823	93.2	13.4 (12-16)	87.2	81.3
10	824	93.2	13.9 (12-16)	71.3	66.5
100	815	93.3	13.4 (12-16)	86.8	81.0
<u>Larvae III</u>					
	Larvae	% pupa		% adult	
0	226	100.0		88.1	88.1
1	251	100.0		89.6	89.6
10	258	100.0		84.1	84.1
100	284	100.0		56.7	56.7

2a. SELECTION FOR RESISTANCE TO CYROMAZINE IN THE MEDITERRANEAN FRUITFLY.

Introduction.

Cyromazine is a triazine compound with the chemical formula 2-cyclopropylamino- 4,6-diamino-5-triazine. It belongs to the s-triazine group which shows Insect Growth regulation (IGR) activity and some chemosterilant effects. The compound Cyromazine (Neforex^R, Larvadex^R) is a stomach poison, was found to be extremely active in the laboratory and in the field against a variety of dipterous larvae, (El-Oshar et al., 1985; Friedel & McDonnel, 1985) and has a low mammalian toxicity. These characteristics made it a desirable candidate for use in "genetic sexing" of the medfly, if selection for resistance was achieved.

Materials and methods.

1. Dose-mortality response. We used the 50% WP preparation of the compound in the larval diet. Preliminary tests were carried out with concentrations of 0.1, 1 and 10 ppm. We then tested concentrations between 1 and 10 ppm, and finally narrowed down the test to the intervals between 1 and 4 ppm cyromazine in the larval diet (at a 1 ppm step).

2. Selection for resistance on cyromazine containing diet. Cyromazine was incorporated in the larval diet (Radu et al., 1975) at the concentration of 3 ppm. A massive number of medfly eggs from our

laboratory culture (which was replenished by field collected material) were placed on top of the diet and the surviving pupae were used for further selection and propagation. The concentration of cyromazine was raised to 5 ppm in the 7th selection cycle (7 generations) , to 10 ppm in the 10th cycle and to 20 ppm at the 14th cycle at which it remains to the present. Further increase was not possible. For almost each exposure cycle we compared the mortality data (with Abbott's corrected figures) of the selected line with our regular laboratory reared line. We also recorded the effect of the cyromazine diet on the duration of the pre-imaginal development period of the exposed flies.

3. Mode of inheritance. Twice during the selection process we attempted to study the mode of inheritance of cyromazine "resistance". That was done by crosses within the parental "resistant" and "susceptible" lines and backcrosses of the F1 hybrids to the parental lines. These crosses and backcrosses were carried out for two consecutive generations in the 9th and 19th selected generations with 5 ppm and 20 ppm cyromazine as discriminating doses respectively.

4. Linkage studies. We also attempted to link the suspected "cyromazine resistance" site to a particular chromosome. The "resistant" line was crossed with various mapped morphological markers which were available at our laboratory. These studies failed and will not be reported here.

Results

1. Dose-mortality response. Eggs hatched normally and the first instar larvae which crawled on the paper were not affected by the material. The mortality of the later instars was low at the "1 ppm diet" but reached 100% in the "10 ppm diet" (Table 18). Further tests with intermediate concentrations of cyromazine in the larval diet placed the desirable discriminating dose for selection at 3 ppm. (Tables 19 & 20). Cyromazine caused also delayed mortality up to the emergence of adults from pupae. Some of the pupae surviving the cyromazine treatment were elongated and did not eclose.

Table 18. Effect of cyromazine (in the larval diet) on survival of eggs and larvae of the Mediterranean fruit fly.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
Exposure of larvae I + II in starter diet					
0	853	84.4	10.8 (10-13)	70.6	59.6
0.1	821	82.5	10.7 (9-13)	76.7	63.2
1	815	81.3	10.4 (9-13)	79.8	64.9
10	844	83.2		0.3	0.2
Exposure during the whole larval stage					
0	965	81.8	10.9 (10-13)	90.2	73.8
0.1	918	81.2	10.9 (9-13)	85.6	69.5
1	807	81.7	10.7 (9-13)	89.5	73.1
10	805	85.5		0.0	0.0

Table 19. Effect of cyromazine (in the larval diet) on survival of eggs and larvae of the Mediterranean fruit fly.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
0	851	89.7		82.4	73.9
4	876	92.6		0.0	0.0
7	838	87.8		0.0	0.0
10	817	90.8		0.0	0.0

Table 20. Effect of cyromazine (in the larval diet) on survival of eggs and larvae of the Mediterranean fruit fly.

ppm	Eggs	% hatch	Dev. period	% pupa	% surviv.
0	832	95.7		86.8	77.6
1	857	95.9		95.6	87.9
2	947	95.2		62.5	50.1
3	823	95.0		5.4	3.2
4	904	95.4		0.0	0.0

2. Selection for resistance on cyromazine containing diet. In June 1989 we have been rearing 48 generations of medflies on Cyromazine containing diet. During that period we observed an increase of resistance to cyromazine from 3 ppm at the initial stage to 20 ppm presently (Table 21). From the fourth generation we compared almost in every generation the effect of cyromazine on the selected line to it's effect on the regular (unselected) laboratory-reared line (Table 21). The development period of the medfly lines exposed to cyromazine was not affected by the product (Table 22).

We have attempted several times to raise the dose of cyromazine above 20 ppm and failed; as none survived the concentrations of 30 ppm or more. Thus, it seemed that the plateau was reached at 20 ppm cyromazine.

Table 21. Selection for cyromazine resistance in the Mediterranean fruitfly.
Abbott's corrected percent survival (of previous stage).

Gener.	date	ppm :	selected line :				Unselected line :		
			% hatch	% pupae	% adults	% total	% hatch	% pupae	% adults
1	06-May-86	3 :	99.3	6.2	73.9	4.6 :			
3	01-Jul-86	3 :	99.7	48.5					
		0 :							
4	25-Jul-86	3 :	104.0	95.4	100.4	95.7 :	101.0	29.3	59.4 :
		0 :							
6	09-Sep-86	3 :	112.8	55.0	92.8	51.1 :	118.0	50.6	53.6 :
		0 :							
7	03-Oct-86	5 :	98.1	16.6	70.0	11.6 :			
		0 :							
8	27-Oct-86	5 :	102.7	75.0	90.3	67.7 :	99.9	0.0	
		0 :							
9	17-Nov-86	5 :	99.7	95.6			103.5	0.0	
		0 :							
10	10-Dec-86	5 :	103.4	94.1	99.3	93.4 :	98.1	0.1	0.0 :
		0 :							
11	05-Jan-87	5 :	101.9	88.7	96.1	85.2 :	99.3	0.0	
		0 :							
11	07-Jan-87	10 :	98.4	27.4	41.1	11.2 :	100.5	0.0	
		0 :							
12	02-Feb-87	10 :	100.2	54.4	72.0	39.2 :	98.0	0.0	
		0 :							
13	25-Feb-87	10 :	102.2	74.6			99.7	0.0	
		0 :							
14	20-Mar-87	10 :	98.6	91.6	88.4	81.0 :	100.5	0.0	
		0 :							
14	30-Mar-87	20 :	100.6	52.3	50.8	26.6 :	97.7	0.0	
		0 :							
15	23-Apr-87	20 :	101.6	56.1	58.6	32.9 :	100.1	0.0	
		0 :							
16	14-May-87	20 :	96.2	51.0	80.8	41.2 :	101.8	0.0	
		0 :							
17	08-Jun-87	20 :	98.9	67.9	76.3	51.8 :	101.5	0.0	
		0 :							
18	30-Jun-87	20 :	105.1	73.4	88.1	64.7 :	99.7	0.0	
		0 :							
19	22-Jul-87	20 :	97.7	78.4	82.1	64.4 :	99.0	0.0	
		0 :							
20	17-Aug-87	20 :	104.9	70.8	85.3	60.4 :	98.3	0.0	
		0 :							
21	03-Sep-87	20 :	102.4	75.5	89.0	67.2 :	101.6	0.0	
		0 :							
48	12-Jun-89	20 :							
		50 :							

Table 22. Pre-imaginal development periods in the cyromazine selection process.

generation	cyromazine incorporated in diet			line	controls - no cyromazine in diet		
	diet ppm	average	range		diet ppm	average	range
1	3	11.48	11-13	cyr-line 3pp	0	12.49	11-14
3	3	11.79	10-14	cyr-line 3pp	0	12.45	11-15
4	3	10.29	10-12	cyr-line 3pp	0	11.11	10-13
6	3	10.33	9-13	cyr-line 3pp	0	9.81	9-13
7	5	9.22	9-10	cyr-line 5pp	0	9.09	9-10
8	5	9.76	9-11	cyr-line 5pp	0	9.68	9-11
9	5	9.18	9-11	cyr-line 5pp	0	9.07	9-11
10	10	10.13	10-11	cyr-line 10p	0	9.44	9-11
11	10	9.63	9-12	cyr-line 10p	0	9.64	9-12
12	10	9.51	9-12	cyr-line 10p	0	9.40	9-12
13	10	10.05	9-14	cyr-line 10p	0	9.61	9-14
14	10	11.29	10-13	cyr-line 10p	0	10.23	10-13
14	20	10.42	9-12	cyr-line 20p	0	10.65	9-12
15	20	10.38	10-13	cyr-line 20p	0	10.18	10-13
16	20	10.56	10-12	cyr-line 20p	0	10.14	10-12
17	20	10.05	9-12	cyr-line 20p	0	9.63	9-12
18	20	10.23	9-13	cyr-line 20p	0	10.04	9-13
19	20	9.75	9-12	cyr-line 20p	0	9.93	9-12
21	20	10.35	9-13	cyr-line 20p	0	10.38	9-13

3. Mode of inheritance. A hybrid line (cyromazine X wild type) was established in the 9th generation and the following crosses were carried out with the F1 hybrid and the parental lines to study the possible mode of inheritance of cyromazine resistance:

1. Cyromazine line X Cyromazine line
2. Cyromazine line X F1 hybrids
3. F1 hybrids X Cyromazine line
4. Wild type X Cyromazine lines
5. Wild type X F1 hybrids
6. wild type X wild type

All these crosses and backcrosses were carried out reciprocally, but the data of the reciprocal crosses were pooled, as no differences were observed.

Table 23. Mode of inheritance of cyromazine resistance in the Mediterranean fruit fly. Crosses and backcrosses in the 9th & 10th filial generations. (Discriminating dose 5 ppm).

Cross type	% survival (Abbott's Correct.)		
	Larvae	Adults	Total
Cyr X Cyr	94.1	92.5	90.1
Cyr X F1	37.8	79.3	29.5
F1 X F1	20.8	66.5	16.4
WT X Cyr	36.4	75.7	27.0
WT X F1	2.1	26.3	0.6
WT X WT	0.1	0.0	0.0

Cyr = Cyromazine selected line, WT = Wild-type (non-selected) line.
F1 = hybrids between WT and Cyr.

The offspring of the crosses were reared on a 5 ppm cyromazine diet, and the data were corrected using the Abbott's formula (Table 23). The results indicated that cyromazine resistance did exist and

that it was genetically controlled. Survival on cyromazine diet declined gradually with the "dilution" of the "cyromazine resistance" genes. Thus, when cyromazine resistant flies were inbred, survival reached 90% as compared to complete mortality of the wild-type line. Survival declined gradually from the backcross of the F1 hybrid with the cyromazine-resistant parents through the cross of cyromazine-resistant flies with wild-type flies, through the backcross of the F1 hybrid with the wild-type parents, through the cross of the F1 hybrids and finally through the backcross of F1 hybrids with the wild-type parent. The later cross resulted in almost total mortality, similar to that of the wild-type line. It seemed therefore that cyromazine resistance was not due to a major dominant gene, but rather a quantitative trait, based probably on a low number of additive genes.

Similar crosses were carried out in the 19th filial generation of the selected strain (Table 24).

Table 24. Mode of inheritance of cyromazine resistance in the Mediterranean fruit fly. Crosses and backcrosses in the 19th & 20th filial generations. (Discriminating dose 20 ppm).

Cross type	% survival (Abbott's Correct.)		
	Larvae	Adults	Total
Cyr X Cyr	65.2	83.6	63.0
Cyr X F1	26.7	76.4	20.6
WT X Cyr	1.0	0.0	0.0
WT X F1	0.0	0.0	0.0
WT X WT	0.0	0.0	0.0

Cyr = Cyromazine selected line, WT = Wild-type non-selected
F1 = hybrid between WT and Cyr.

Only the crosses within the Cyromazine selected line and between the cyromazine selected line and the F1 hybrids yielded any surviving offspring. The cross between the wild-type and cyromazine selected lines yielded no offspring similar to the cross within the wild-type line). Here too (as in Table 23) the data suggest that cyromazine selection is not governed by a major gene and is obviously not dominant. Thus, at-present that line could not be further used for the construction of a "genetic sexing" line in the medfly, although the possibility for positive selection for chemical resistance was proven in these experiments.

2b. AVERMECTIN^R

Avermectin ($C_{48}H_{72}O_{14} + C_{47}H_{70}O_{14}$) is a macro-lactone streptomycete-derived insecticide, discovered and developed by Merck, Sharp & Dome Res. Lab. (Schmidt, 1983). The commercial preparation is an emulsifiable concentrate containing 1.8% a.i.

Preliminary tests in our laboratory failed to reveal any effect of avermectin on medfly larvae, when incorporated in the larval diet. Two tests exposing freshly laid eggs, 0-3 hours old, to avermectin and subsequent rearing of the larvae on regular larval diet (Table 25) placed the LC_{50} around 5ppm avermectin (a.i.) and the LC_{90} close to 50 ppm. avermectin reduced egg hatch and caused also the delayed mortality of larvae. We also found that varying the duration of exposure between 0.5 and 4 hrs., had little effect on the toxicity of avermectin and used therefore a 50 ppm concentration and a 2 hours

exposure period for the above tests and subsequent selection program. Selection began in December 1985, and was carried on for 14 generations on 50 ppm avermectin. From the 15th generation on, the concentration of avermectin was raised to 100 ppm.

Table 25. Dose-mortality response of the Mediterranean fruit fly pre-pupal stages to avermectin (exposure of eggs).

Avermectin ppm a.i.	% survival (Abbott's corr.)			
	pupae/eggs		pupae/1st instar larvae	
	Test I	Test II	Test I	Test II
1	71.9	93.9	78.3	90.5
5	46.9	38.8	64.0	54.1
10	39.7	29.3	54.6	42.2
50	2.7	0.9	8.1	3.0
100	0.2	2.0	1.5	10.2

The line seemed to respond very quickly to the selection pressure. Egg hatch reached ca. 90% in the second generation, 1st larvae survival reached ca. 80% after 5 generations. The results with the second and third instar larvae were rather erratic, yet reaching 90% (of the hatched eggs) after 14 generations of selection.

Although selection seemed to proceed in the right direction we had to abandon further studies with avermectin, as we obtained erratic response to avermectin with the wild-type line during that period. Whereas 50 ppm avermectin resulted in 6.9% survival of wild-type larvae (pupation/hatch) at the initiation of our studies, it resulted in 20.7% survival 9 generations later in the same line without selection (as compared to 77.7% in the avermectin line) and

in 47.9% survival 14 generations later (as compared to 81.9% in the avermectin line). It seemed that we had either a problem with the exposure method (which remained the same throughout that period), or that we encountered inconsistency in the avermectin used (we used more than one batch of avermectin), or that breeding or unexpected genetic changes in the wild-type line caused these results. Raising the concentration of avermectin to 100 ppm or even 200 ppm did not change that trend and resulted in even more puzzling data. (At 100 ppm, wild-type line survival - 22.4%, avermectin line survival - 64.3%; at 200 ppm, wild-type line survival - 45.3%, avermectin line survival - 80.6%).

3. MORPHOLOGICAL MUTANTS IN THE MEDITERRANEAN FRUIT FLY.

Until 1984 we isolated the following morphological mutants: ap, B, dc, or, wp, h, Cy, dp and bo. Most of them were thoroughly studied and assigned to the arbitrary linkage groups A - E, (Saul & Rossler, 1984). The mutants were later assigned to chromosomes in cooperation with laboratories in Patras, Greece and Pavia, Italy. Chromosomes follow the numbering by Radu, Rossler & Koltin, (1976).

During the period 1984 - 1988 we isolated 14 additional morphological mutants. Eight were studied and some appeared in publications. The others are still investigated (Table 26). The established mutant strains (gr, ru, ro, Sr, Sp, sb, ew, bl, db) will be described here. The others will only be mentioned by name.

Eye shape and color mutants.

garnet eye color (gr)

Adults with the garnet eye color were found in January 1984 in a laboratory strain of the medfly. The color matches Plate 11, E8 in "Methuen Handbook of Color" (Kornerup & Wancher, 1967). The trait is autosomal, linked to bo (Linkage group E) and recessive. The trait was described by Rossler & Rosenthal (1988).

ruby eye color (ru)

A single female with ruby colored eyes was found in March 1987 in a laboratory strain of the medfly. The eyes were actually

lacking the bluish reflection, as was later observed in crosses with other available eye color mutants. The name "ruby" is therefore temporary as the name "reflectionless", which seems more apt, is already allocated to a mutant found by Carante in France (Carante, 1982). It is possible that these two mutants are identical. Ruby is autosomal, recessive and in linkage group B, (chromosome 5).

rough eye (ro)

Three females with rough eyes were found in November 1986 in the laboratory strain of the medfly. The ommatidia which are usually regular and of even size were uneven in size, and disarrayed, giving the eye a rough appearance. The trait is autosomal, recessive and in linkage group B (chromosome 5).

Initially, the line performed poorly, and was almost lost due to very low egg hatch. The egg hatch of sibmating within the line was 20.8% and when the rough-eyed females were crossed to wild-type males the egg hatch dropped to 0.27%. The rough-eyed males readily inseminated the wild-type females resulting in 87.4% egg hatch. This problem disappeared eventually and the line is maintained in our lab. with satisfactory egg hatch and reproduction.

dark-brown eye (db)

One female with dark-brown eyes was found in September 1988 in line-69 (a genetic sexing line with brown-pupae males carrying an autosomal-Y translocation and dark-pupae females). The eye color

matches Plate 8-F7 in "Methuen handbook of Color" (Kornerup & Wancher, 1967). The trait is autosomal, recessive and located on chromosome 4.

Abdominal mutants

Sergeant (Sr)

Two females, with 3 intersegmental dorso-lateral grey lines on the abdomen instead of the normal 2 lines, were found in November 1986 in the laboratory strain. The trait is autosomal, dominant and in linkage group A (chromosome 4). Wild-type flies are still produced in the line despite repeated attempts to produce a true-breeding line through selection and numerous single-pair crosses. The currently maintained line derives from a single-pair cross between Sr adults in June 1987 which produced 26 Sr and 1 wild-type progeny. The F2 of the cross yielded 189 Sr and 48 wild-type flies. Rearing and continuous selection of the sr phenotypes resulted in a line with 90% Sr flies.

Table 26. Morphological mutants of the Mediterranean fruitfly, maintained in Rheovot, Israel.

Symbol	Name	Mode	Linkage group	Chromosome	Source
<hr/>					
<u>Group A. Studied and published</u>					
ap	apricot eye	r	A	4	3
B	Bar eye	D	A	4	4
dc	double SFO				
	bristle	r	A	4	3
or	orange eye	r	B	5	2
wp	white pupa	r	B	5	1
Cy	Curly wings	D	B	5	4
dp	dark pupa	r	C	3	3
bo	brown orange				
	eye	r	E		4
gr	garnet eye	r	E		4
 <u>Group B. Studied and not published yet</u>					
Sp	Spotty abdomen	D	A	4	
Sr	Sergeant	D	A	4	
h	harpoon SFO				
	bristle	r	B	5	
ro	rough eye	r	B	5	
ru	ruby eye	r	B	5	
ew	eroded wings	r	C	3	
sb	stout bristles	r	A	4	
db	dark-brown eye	r	A	4	
bl	"blond" body	r	B	5	
 <u>Group C. Isolated - still under study</u>					
Pe	bulgy eyes	D	?	?	
??	eyes wider than the <u>Bar</u> mutant, but not wild-type				
tg	thoracic grove	?	?	?	
we	white-eye	r	?	?	

SFO = Superior fronto-orbital bristle (males' "cera")

Sources.

1. Rossler, 1979; 2. Rossler, 1982d; 3. Rossler & Koltin, 1975; 4. Rossler & Rosenthal, 1988.

Spotty (Sp)

A male with two dorso-lateral melanic spots on the first abdominal segment was found in July 1986 in the laboratory strain. A true-breeding line was established within 11 generations. The trait is autosomal, dominant and in linkage group A (chromosome 4). Although the phenotype is fully penetrant in the line, it seems to be less so in the heterozygous state, or in crosses with other mutant strains. The spots become either small or pale and difficult to distinguish.

Various mutants

eroded wings (ew)

A single male with eroded wings was found in September 1987 in the laboratory reared culture. The proximal portion is missing including large parts of the cells between the first radial vein and the third+fourth median vein. The missing portion reaches the proximity of the radial-median cross-vein, and the i-m cross-vein is completely missing. The trait is autosomal, recessive and in linkage group C (Chromosome 3).

stout bristles (sb)

Medfly adults, with short and stout bristles on the head and thorax, were found among the progeny produced by the "branched"

mutants. Reproduction was initially very poor, although a full complement of eggs was found in the the females' ovaries and the males had viable and motile sperm. Reproduction turned normal eventually and a true-breeding culture was obtained in April 1988. The trait seems to be autosomal and recessive and is still studied.

"blond" (bl) body

Adults lacking the black body pigment were found in october 1988 in the "ruby" line. Instead of the black pigment they carried a rust-colored pigment. The trait is autosomal recessive and located on chromosome 5.

Recombination in males and females of the Mediterranean fruit fly.

The level of genetic recombination in males and females of the Mediterranean fruit fly (map units) of that species is presented in table 27. During the study period we accumulated data on the genetic recombination in males and females in 28 pairs of loci. There seemed to be no dependence between the males' and females' recombination levels. We also noted that most of the loci in our study had rather high recombination levels in females. In the 28 pairs studied the average recombination levels in females was $32.44\% \pm 16.32$.

Only two chromosomes with multiple markers were available for comparison studies. Six out of 9 pairs showed male recombination. In chromosome 5 (66.6%) and the average recombination level in that

chromosome reached 37.47 ± 12.37 . Ten out of 17 pairs showed male recombination in chromosome 4 (58.8%) and the average recombination level in females reached 31.66 ± 17.94 .

High recombination levels in males involved loci with dominant mutations such as Cy on chromosome 5 and Sr and Sp on chromosome 4. The highest levels, as well as the lowest ratios of female/male recombination levels were obtained with the Sr locus. In 4 out of 5 cases (with Sp, lt, B and db) the levels in males almost matched those in females.

Genetic recombination in males was mainly studied in repulsion heterozygotes. Only the dc-ap and or-wp pairs were studied both in repulsion and coupling. It seemed that coupling reduced somewhat the recombination levels both in females and males. The level of recombination in males increased in the presence of a translocation on the same chromosome (from 0.14% to 0.32%), but not as much as the presence of translocation on another chromosome (from 0.14% to 1.98% in coupling and from 0.96% to 4.00% in repulsion), (Table 28).

In conclusion, recombination in medfly males is common, is not caused by chromosomal aberrations and is not restricted to a particular chromosome or a particular locus, although certain loci show higher levels of recombination in males.

The effect, of the occurrence of genetic recombination in males, on the integrity of a "genetic sexing" strain for SIT, has been discussed extensively in the past (Rossler, 1982a,c,d; 1985).

TABLE 27. Recombination in males and females of the Mediterranean fruit fly.

Loci pairs	% recombination		
	Males	Females	Ratio Females/Males
<hr/>			
		<u>Chromosome 3</u>	
ew - dp	0.14	21.57	155
<hr/>			
		<u>Chromosome 4</u>	
ap - B	0	0.53	
ap - sk/dc	0.96	18.80	19.85
ap - Sr	0	45.10	
ap - Sp	0	40.16	
ap - sb	0.54	9.02	16.70
lt - B	0	47.90	
lt - sk/dc	0	42.22	
lt - Sr	48.47	49.88	1.03
lt - Sp	2.30	40.16	17.46
lt - sb	0.19	46.40	244.21
B - sk/dc	0	10.38	
B - Sr	0.11	0.18	1.64
B - Sp	0	45.30	
sk/dc - Sr	0.15	32.33	215.53
sk/dc - Sp	3.70	43.10	11.65
Sr - Sp	15.80	17.80	1.13
Sr - db	49.20	49.10	1.00
<hr/>			
		<u>Chromosome 5</u>	
or - Cy	12.10	25.50	2.11
or - wp	1.05	48.60	46.29
or - ru	0	34.60	
Cy - wp	8.10	43.30	5.35
Cy - ro	5.93	43.40	7.32
Cy - ru	10.91	11.99	1.10
wp - ro	0	34.9	
wp - ru	0.60	50.96	84.93
ru - bl	0	44.01	
<hr/>			
		<u>Chromosome 6</u>	
bo - gr	3.80	11.10	2.92

Table 28. Effect of the linkage configuration (coupling/repulsion) and the presence of chromosomal translocation on the recombination level in males and females of the Mediterranean fruitfly.

Genotype	Loci pair	% recombination	
		In males	In females
ap +/+ dc	ap dc	0.96	21.60
+ +/ap dc	ap dc	0.14	18.80
+ or/wp +	wp or	1.05	51.22
+ +/wp or	wp or	0.40	45.89
T(Y:dp ⁺);ap +/+ dc	ap dc	4.00	--
T(Y:dp ⁺);+ +/ap dc	ap dc	1.98	--
T(Y:ap ⁺ dc ⁺)/ap dc	ap dc	0.30	--

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REFERENCES.

- ANONYMOUS, 1986. Report on Research Co-ordination Meeting on the Development of Sexing Mechanism in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Joint FAO/IAEA Div., Colymbari, Crete 16-21 September 1986. 134.
- CARANTE, J.P., 1982. Genetics of the Mediterranean fruit fly, Ceratitis capitata (Diptera: Tephritidae), a 'reflectionless' eye mutant. *Annals Entomol. Soc. Amer.*:613.
- CHAN, H. T. Jr., TAM, S. Y. T., 1985. Toxicity of alfa-tomatine to larvae of the Mediterranean fruitfly (Diptera: Tephritidae). *Jour. Econ. Entomol.* 78:305.
- EL-OSMAR, M. A., MOTOYAMA, N., HUGHES, P. B., DAUTERMAN, W. C., 1985. Studies on cyromazine in the house fly, Musca domestica (Diptera: Muscidae), *J. Econ. Entomol.* 78:1203.
- FREIDEL, T., MCDONNELL, P. A., 1985. Cyromazine inhibits reproduction and larval development of the Australian sheep blow fly (Diptera: Calliphoridae), *J. Econ. Entomol.* 78:368.
- KORNERUP, A., WANCHER, J.H., 1967. *Methuen Handbook of Colour*, (2nd Ed.). Methuen & Co. New York.
- RADU, M., ROSSLER, Y. KOLTIN, Y., 1975. The chromosomes of the Mediterranean fruit fly, Ceratitis capitata (Wied.): Karyotype and chromosomal organization. *Cytologia* 40:823.
- ROBINSON, A. S., RIVA, M. E., ZAPATER, M., 1986. Genetic sexing in the Mediterranean fruit fly, Ceratitis capitata, using alcohol dehydrogenase locus, *Theo. Appl. Gen.* 72:455.

- ROBINSON, A. S., ROSSLER, Y., 1987. Report of the Consultants' Meeting on the Development of Sexing Mechanism in the Mediterranean Fruit Fly, Ceratitis capitata through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Joint FAO/IAEA Div. (1-5 June, 1987, Vienna, Austria), 6pp.
- ROSSLER, Y., 1979. The genetics of the Mediterranean fruit fly,: A "white pupa" mutant. Annals Entomol. Soc. Amer. 72: 583.
- ROSSLER, Y., 1980. "69-apricot" a synthetic strain of the Mediterranean fruit fly, Ceratitis capitata (Dip: Tephritidae) with sex-limited pupal color and eye color marker. Entomophaga 25:275.
- ROSSLER, Y., 1982a. "Genetic sexing of insects by phenotypic characteristics, with special reference to the Mediterranean fruit fly.", Sterile Insect Technique and Radiation in Insect Control (Proc. Symp. Neuherberg, 1981), IAEA, Vienna, 291pp.
- ROSSLER, Y., 1982b. "Genetic sexing of insects by phenotypic characteristics with special reference to the Mediterranean fruit fly", Sterile Insect Technique and Radiation in Insect Control, FAO/IAEA STI/PUB/595:291.
- ROSSLER, Y., 1982c. Genetic recombination in males of the Mediterranean fruit fly, and its relation to automated sexing methods. Annals Entomol. Soc. Amer. 75:28.
- ROSSLER, Y., 1982d. Recombination in males and females of the Mediterranean fruit fly (Diptera: Tephritidae) with and without chromosomal aberrations. Annals Entomol. Soc. Amer. 75:619.

- ROSSLER, Y. 1985. Effect of genetic recombination in males of the Mediterranean fruit fly (Diptera: Tephritidae) on the integrity of "genetic sexing" strains produced for sterile-insect-releases. *Annals Entomol. Soc. Amer.* 78:265.
- ROSSLER, Y., KOLTIN, Y., 1976. The genetics of the Mediterranean fruit fly, Ceratitidis capitata: Three morphological mutations. *Annals Entomol. Soc. Amer.* 69:604.
- ROSSLER, Y., ROSENTHAL, H., 1988. Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): Eye color, eye shape and wing mutations. *Annals Entomol. Soc. Amer.* 81:350.
- SAUL, S.H., ROSSLER, Y., 1984. Genetic markers of the autosomal linkage groups of the Mediterranean fruit fly, Ceratitidis capitata (Diptera: Tephritidae). *Annals Entomol. Soc. Amer.* 77: 323.
- SCHMIDT, C. D., 1983. Activity of Avermectin against selected insects in aging manure, *Environ. Entomol.* 12:455.
- SEAWRIGHT, J. A., KAISER, P. E., DAME, D. A., LOFGREN, C. S. 1978. Genetic method for the preferential elimination of the females of Anopheles albimanus. *Science*, 200:1303.

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- ROSSLER, Y., ROSENTHAL, H., 1988. Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): Eye color, eye shape and wing mutations. *Annals Environ. Entomol.* 81:350-355.
- ROSSLER, Y., 1986 (abst.). Developing resistant Mediterranean fruit fly strains for "genetic sexing" in sterile insect release programs. *Proceedings IIInd International Symp. on Fruit Flies* (Crete, Colymbary)
- ROSSLER, Y., 1988. Selection for resistance in the Mediterranean fruit fly for genetic sexing in sterile insect technique programmes. pp. 228-240 in: *Proc. Symp. FAO/IAEA Vienna, 1987.* IAEA Publ. STI/PUB/763.

FIGURE 1A. Device for the exposure of eggs and larvae to chemicals.

General view.

FIGURE 1B. Device for the exposure of eggs and larvae to chemicals.
The plastic cup with the chemical tested for rinsing
water.

FIGURE 1C. Device for the exposure of eggs and larvae to chemicals.

The container for eggs or larvae, placed inside the plastic cup (Fig. 1B).