Genetic Sexing of the Mediterranean Fruit Fly

PROCEEDINGS OF THE
FINAL RESEARCH CO-ORDINATION MEETING
COLYMBARI, CRETE, GREECE, 3–7 SEPTEMBER 1988
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES
IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1990

GENETIC SEXING
OF THE MEDITERRANEAN FRUIT FLY

PANEL PROCEEDINGS SERIES

GENETIC SEXING OF THE MEDITERRANEAN FRUIT FLY

PROCEEDINGS OF THE
FINAL RESEARCH CO-ORDINATION MEETING
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE
AND HELD IN
COLYMBARI, CRETE, 3–7 SEPTEMBER 1988

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1990

GENETIC SEXING OF THE MEDITERRANEAN FRUIT FLY
IAEA, VIENNA, 1990
STI/PUB/828
ISBN 92-0-111190-8
ISSN 0074-1876

Printed by the IAEA in Austria
February 1990

FOREWORD

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is one of the most damaging of pests to fruit and vegetables throughout the world. It attacks more than 200 plant species, causing enormous losses. Furthermore, it is a quarantine pest and thus countries in which the insect become established cannot export susceptible hosts into many countries where that pest does not exist. The current control method is limited to an insecticide bait spray which must be applied from two to eight times per year, depending upon climatic conditions, sequence of hosts, etc.

The medfly has been eradicated in Mexico and part of Guatemala, and several small spot infestations have been eradicated in the United States of America, by means of the sterile insect technique (SIT). This technology requires mass rearing of huge numbers of the pest, irradiation sterilization and release into the medfly infested areas.

In the early 1980s, it was recognized by the FAO and the IAEA that a genetic sexing method for the medfly would greatly improve the efficacy of the medfly SIT and reduce its costs. Efficacy would be increased because only males would be released, thus eliminating sortative mating between laboratory reared males and females, as well as biasing the ratio in favour of males to females, in which most of the males would be sterile. Cost reduction would be achieved through the reduced number of mass reared insects required, assuming that the females could be killed in the egg or neonate larval stage. Furthermore, a considerable reduction in factory space and personnel required for mass rearing would be achieved. Another advantage would be that sterile stings (sterile females attempting to lay eggs in fruit) would be eliminated and thus the SIT could be used in control programmes as well as eradication programmes. At the present time, countries with a well developed fruit production industry are reluctant to use the SIT because the sterile stings reduce the quality of the crop and thus its value.

These Proceedings summarize the research and development findings of the Agency's co-operators in the co-ordinated research programme to develop a genetic sexing method for the medfly. Great progress has been made in many aspects of medfly genetics, including the development of a number of genetic sexing strains. However, none of these meets all the requirements for the ideal genetic sexing strain of the medfly, and therefore research and development need to be continued.

INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is rated as one of the world's most important agricultural insect pests. During the present century this insect has spread from its native habitats in Africa and the Mediterranean region to most of the tropical and subtropical regions of the world. Its host range of more than 200 species of fruit and vegetables covers almost 100 economically important plants, including the *Citrus* and *Prunus* species, figs, coffee, pears, etc.

Since its foundation in 1964, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has been actively involved in the development and application of the sterile insect technique (SIT) for the control or eradication of the medfly, through its co-ordinated research programmes (CRPs), through technical co-operation and through research and development at the FAO/IAEA Agricultural Laboratories at Seibersdorf, Austria. This involvement has concentrated mainly on the development of mass rearing technology, the quality control of mass reared medflies, and the transfer of this technology and know-how to countries involved in area wide control or eradication of the medfly. In 1983, a five year CRP on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and Other Genetic Measures was initiated with the ultimate aim of producing genetic mechanisms in the medfly that would allow the conditional elimination of females early in the mass rearing process. Participants in this programme included virtually all laboratories concerned with medfly genetics, some laboratories involved in genetic sexing of other pest insects, and the FAO/IAEA Agricultural Laboratories at Seibersdorf. The concluding Research Co-ordination Meeting (RCM) of this CRP was held in Colymbari, Crete, Greece, on 3–7 September 1988. The contributions made at that meeting and reported here thus constitute the latest status of medfly genetics and medfly genetic sexing research, and cover the genetics, cytogenetic and population genetics of this important pest insect.

When the present CRP was initiated, very little was known about the genetics of the medfly, and the genetic mutant map was limited to six to eight autosomal recessive and one dominant mutant. A concerted effort was made to isolate additional mutants, both through inbreeding of laboratory and wild strains and through induction with mutagens. A total of 51 morphological and biochemical mutants have now been isolated and allocated to the six linkage groups, including one X linked gene, and twelve, three, fourteen, thirteen and eight genes on linkage groups 2, 3, 4, 5 and 6 respectively. Five of these mutants code for dominant morphological characters, 28 are biochemical variants, two are sex ratio distorters, one is a lethal, while the

rest code for recessive morphological characters. A further 40 mutant and biochemical characters have been observed, but it still remains to confirm these and to allocate them to their respective linkage groups.

A total of 17 chromosomal translocations have been isolated. Eleven of these involve the Y chromosome and one to three autosomes, while six involve reciprocal translocation between two autosomes. The latter rearrangements all suppress female recombination in a proportion of chromosome 4 and are being used in the isolation of temperature-sensitive recessive lethal factors. Several of the translocations were also used in establishing the correlation between linkage groups and chromosome numbers. This has now been completed for the first five linkage groups, while direct evidence has yet to be obtained for the correlation of linkage group 6 with chromosome 6.

The meiotic and mitotic chromosomes in the medfly are extremely short and dense and, consequently, are of limited value in determining the position of breakpoints involved in chromosomal rearrangements. In 1986, Dr. D. Bedo, Commonwealth Scientific and Industrial Research Organization (CSIRO), with a technical contract funded by the IAEA, isolated polytene chromosomes from trichogen cells and salivary glands of the medfly. These are now being actively investigated by participants of the present CRP. Subsequent comparisons of the banding patterns of polytene chromosomes from these two types of cells revealed little or no correlation. Also, although the sex chromosomes are not present in the salivary gland cells, they are detected as heterochromatic bodies with a large nucleolus in the trichogen cells. Thus, a characterization of the polytene chromosomes of both types of cells is necessary and is in progress.

Biochemical characterization of the medfly has led to a wealth of information on the spatial and temporal distribution of medfly population. Although this species displays rather low levels of genetic polymorphism as compared, for example, to *Drosophila* species, both seasonal and geographical changes have been observed. Biochemical analysis has also proved useful in establishing the effectiveness of SIT eradication programmes. A programme on restriction fragment length polymorphism (RFLP) has recently been initiated. Such a study should provide additional information on the population structure of the medfly and may provide much needed data on sources of reinfestation following SIT programmes in both isolated and non-isolated areas.

As a result of the CRP, a consultants meeting was held in 1985 to evaluate the application of genetic engineering and recombinant DNA technology in the development of genetic sexing mechanisms in the medfly. Following this meeting, which was highly enthusiastic and encouraging, a technical contract was awarded to Dr. C. Savakis, Institute of Molecular Biology and Biotechnology (IMBB), Crete, Greece, to investigate possible mechanisms of gene transformation in the medfly. Although transformation with the *Drosophila* p element was unsuccessful, preliminary data were sufficiently encouraging for the IMBB to continue this research

independently, albeit in close collaboration with the FAO/IAEA through a research agreement. Transformation is now being investigated using other transposable elements. In addition, several medfly genes have been cloned and are now being hybridized, by participants of the CRP, to medfly polytene chromosomes in order to determine the exact chromosomal positions of these genes and to confirm the last linkage group/chromosome correlation.

Several genetic sexing strains have been developed in the medfly during the course of the CRP. These include several strains incorporating pupal colour dimorphisms, as well as sensitivity to purine and secondary alcohols. Two of the pupal colour strains have been tested under mass rearing conditions: one proved stable, while the second broke down rapidly on several occasions. The stable strain was subsequently tested for mating competitiveness in field cages and proved highly successful. It also proved efficient in SIT field release programmes on Procida Island, Italy. Potential separation procedures under mass rearing conditions are now being investigated in a strain incorporating female sensitivity to allyl-alcohol.

Throughout the duration of the CRP, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, through the Entomology Unit at the FAO/IAEA Agricultural Laboratories, Seibersdorf, has been actively involved in research and development of genetic sexing mechanisms in the medfly. Procedures have been established here for the induction of mutations through chemical mutagenesis, and a series of female recombination strains have been developed and analysed. Both parameters are essential ingredients in the induction and isolation of novel genes for medfly genetic sexing and are at present being utilized at several co-operating laboratories. Several temperature sensitive lethal factors have been induced although none proved viable, probably owing to the high frequency of concurrently induced normal lethals. Corrective measures have been taken to alleviate this problem and the programme is being continued. A pupal colour genetic sexing strain was also developed at Seibersdorf. This strain was tested for stability and quality under mass rearing conditions (up to 3.6 million flies per week) and proved to be both stable and of high quality. A recent field cage evaluation of this strain indicated high mating competitiveness when competing with a standard wild type strain.

Early experience with unstable genetic sexing strains indicated a high potential for genetic instability. Factors contributing to such instability are being actively investigated at the FAO/IAEA Agricultural Laboratories at Seibersdorf in an attempt to allow countermeasures to be taken. This investigation includes direct experimentation as well as computer modelling. In addition, the laboratory is involved in the mass rearing and assessment of genetic sexing strains developed by CRP participants.

Substantial progress has thus been made towards the construction of medfly genetic sexing strains with conditional lethals, although the final goal of developing an early acting mechanism has yet to be reached. Sex sorting by pupal colour has

reached the practical stage, and an apparently stable genetic sexing strain has been mass reared and tested for stability and quality at the FAO/IAEA Agricultural Laboratories, Seibersdorf. Practical field testing of this strain is now being planned. In addition, extensive genetic linkage maps of morphological mutants and biochemical markers have been produced, critical information on sex determination and sex distortion has been obtained, a vast amount of data on the population genetics of this species has been assembled, and polytene chromosomes have been isolated and studied. The CRP also aroused an active interest in the utilization of recombinant DNA technology for the alteration or improvement of medfly strains, and basic information relating to this aspect of medfly genetics is rapidly accumulating.

The present RCM, preceded by the European Molecular Biology Organization (EMBO) Workshop on Molecular Biology of *Drosophila* (26 Aug.–2 Sep. 1988) and a Workshop on Genetic Engineering and Genetic Sexing in Insects of Economic Importance (3–5 Sep. 1988), was held concomitantly with an International Workshop on Molecular Biology and Molecular Genetics of Lepidoptera (5–10 Sep. 1988). All four meetings were organized by the scientific staff of the Institute of Molecular Biology and Biotechnology, University of Crete, headed by Prof. F.C. Kafatos. Scientists from all three workshops participated actively in the RCM and made presentations on basic aspects of molecular biology and genetic engineering of both medfly and other insects of economic importance, as well as on the relevance of this technology to pest control. The interactions and discussions among scientists of all four meetings proved highly stimulating, as is reflected in the recommendations arising from this RCM.

In addition to the contributions presented at the RCM and the recommendations made by the participants, this publication contains, as an Annex, the third update of the Joint FAO/IAEA Division's Medfly Genetics Information Circular. It is hoped that this compilation of opinions and data will stimulate further research into the applicability of both basic genetics and molecular biology to applied aspects of insect pest control.

Part I

GENETICS, CYTOGENETICS AND POPULATION GENETICS

POPULATION GENETICS OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

A. KOURTI, M. LOUKAS
Department of Genetics,
Agricultural University of Athens,
Athens, Greece

A.P. ECONOMOPOULOS
FAO/IAEA Entomology Unit,
IAEA Seibersdorf Laboratory,
Vienna

Abstract

POPULATION GENETICS OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

The genetic structure of 15 wild populations of Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), sampled from different geographical areas and different host fruit tree species, has been studied. Each population was analysed for 25 enzyme systems detected electrophoretically. All the Mediterranean populations proved to be highly monomorphic ($\bar{H} = 0.053$) whereas those from South Africa and Réunion were highly heteromorphic ($\bar{H} = 0.234$ and 0.153 respectively). As the urea denaturation method was used, no hidden genetic variability caused by the usual electrophoretic conditions was detected. The most probable explanation of the low genetic variability observed in the introduced populations of the medfly seems to be the historical reasons, namely the time elapsed since colonization and the number of individuals of the founder population. Experiments in the field and in the laboratory failed to detect any pattern of preference for oviposition sites in the medfly populations. More specifically, the differences in allele frequencies for the polymorphic loci among these populations do not seem to be correlated either with the taxonomic status of the host fruit or with the size of the fruit. It is concluded that the medfly as a fine grained species may utilize many alternative food resources without an apparent action of selection (or at least one that is detectable by the methods used). By estimating the genetic distances between the populations and/or by using the allozymes as genetic markers the route(s) of dispersion of the fly from its geographic centre of origin, which is placed in Africa, could be roughly traced. Finally, no systematic changes in allele frequencies were observed in populations reared on artificial substrate.

1. INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most important agricultural pests. It is considered [1] to have originated from tropical Africa, from where it spread to north and south

Africa, invaded Spain and subsequently spread into the European Mediterranean countries and the Middle East. It appeared in Hawaii, Costa Rica, South America, spread north through Central America and finally into southern Mexico. There are 253 fruit trees, nut trees and vegetables recorded as medfly hosts. Of the 253 hosts, 40 are considered "heavily or generally infested".

A wealth of information has been published regarding the biology, physiology, behaviour and ecology of the insect. However, given its importance, it is still poorly studied from the standpoint of population genetics.

Here we report our findings from a research programme on the genetic structure of 15 natural populations of medfly supported financially by the Joint FAO/IAEA Division from 1984 to 1988. Our aims are:

(a) To define (i) the amount of genetic variation maintained in natural populations and (ii) the differences between geographically distinct populations. The surprisingly low genetic variation retained by these populations led to the examination of several hypotheses in order to answer the question: "How is it possible for a polyphagous species such as the medfly to maintain so little variation?" We have attempted to detect probable hidden genetic variability caused by the usual electrophoretic conditions by utilizing the urea denaturation techniques.

(b) To study the probable scenario of dispersion of the fly from its geographical centre of origin by using the allozymes as genetic markers and by estimating the genetic distances between the populations.

(c) To detect interpopulation genetic variation associated with the taxonomic status or the size of the host fruits. Possible detection of different races within the same species [2, 3] is of great importance because it may call for a revision of the control strategies employed and therefore increase the effectiveness of the method for controlling the fly.

(d) To study possible genetic changes induced in artificially reared populations of medfly. Such knowledge may be of great importance in monitoring the effectiveness of a mass rearing programme and in assessing the quality of the released flies [4–6].

2. MATERIALS AND METHODS

2.1. Populations

Fifteen wild (ten Mediterranean) and three laboratory populations were studied. The three laboratory colonies were examined every generation starting from generation 0 (founding population). About 200 pupae collected from the founder sample of the three populations were used to obtain gene frequency estimates for the

founder population. From then on, samples of 100–200 individuals were removed from every colony in successive generations and used for electrophoresis. This continued until the fourth or fifth generation. The laboratory rearing of the medfly was on the larval diet used for *Dacus oleae* [7]. The adults were fed a diet consisting of yeast hydrolysate and sucrose at 1:4.

2.2. Enzymes studied

The following 25 enzyme loci were scored for electrophoretically detectable variation: peptidase-1 (PEP-1); peptidase-2 (PEP-2); peptidase-3 (PEP-3); tetrazolium oxidase (TO); glutamate-oxaloacetate transaminase (GOT-1 and GOT-2); diaphorase (DIAPH-1 and DIAPH-2); alcohol dehydrogenase (ADH); adenylate kinase (AK); glucose-6-phosphate dehydrogenase (G-6-DH); 6-phosphogluconate dehydrogenase (6-PGD); mannose-phosphate isomerase (MPI); isocitric dehydrogenase (IDH); octanol dehydrogenase (ODH); hexokinase (Hk-1 and Hk-3); fumarase (FUM); leucine aminopeptidase (LAP); esterase (EST); malate dehydrogenase (MDH); phosphoexose isomerase (PHI); phosphoglucomutase (PGM); malic enzyme (ME); and α-glycerophosphate dehydrogenase (α-GPD).

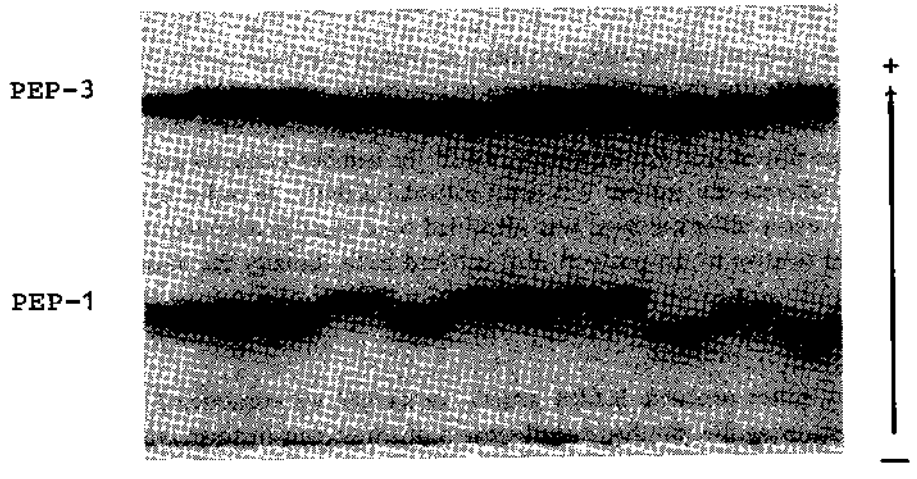


FIG. 1. Zymogram of PEP-3 and PEP-1; phenotypes from left to right.
PEP-3: 1.00/1.00, 1.06/1.00, 1.06/1.00, 1.00/1.00, 1.00/1.00, 1.00/1.00, 1.06/1.00, 1.06/1.00, 1.00/1.00, 1.00/1.00, 1.06/1.00.
PEP-1: 1.26/1.00, 1.26/1.00, 1.26/1.00, 1.26/1.26, 1.26/1.00, 1.26/1.26, 1.26/1.26, 1.26/1.26, 1.00/1.00, 1.26/1.00, 1.00/1.00.

The enzymes LAP and EST were detected in the pupal stage and all the others in the adult stage. For all systems we used the horizontal starch gel electrophoresis (Fig. 1). For the first eight systems, the electrode buffer was made of 0.18M tris, 0.1M boric acid and 0.004M EDTA.2Na at pH8.6. The gel buffer consisted of one part electrode buffer and three parts water. For the next ten systems, the electrode buffer was made of 0.25M tris, pH8.5, and the gel buffer consisted of one part electrode buffer and four parts water. For LAP and EST enzyme systems, the electrode buffer consisted of 0.19M boric acid and 0.05M LiOH (pH8.2); the gel buffer consisted of nine parts of a buffer made of 0.05M tris, 0.007M citric acid (pH8.6) and one part of a buffer made of 0.76M boric acid and 0.2M LiOH (pH8.2). Finally, for the last five systems, the electrode buffer consisted of 0.1M tris, 0.1M maleic acid, 0.01M EDTA.2Na, and 0.001M $MgCl_2$, brought to pH7.4 with 4N NaOH, and the gel buffer consisted of one part electrode buffer and nine parts water (except for the enzyme PHI for which a dilution 1:6.6 was used). Staining recipes for all the enzymes can be found in Ref. [8], except for the following:

— GOT, for which α-ketoglutaric acid, pyridoxal 5-phosphate, L-aspartic acid, and Fast Blue BB salt were diluted in a 0.1M sodium phosphate buffer (final pH7.5);
— AK, for which glucose, $MgCl_2$, NADP, Hk, ADP, G-6-PD, NBT and PMS were diluted in 0.1M tris (pH8.0);
— MPI, for which mannose-6-phosphate, $MgCl_2$, NADP, G-6-PD, PHI, NBT and PMS were diluted in 0.1M tris (pH8.5);
— PEP-2, for which L-phenylalanyl-L-proline was used as substrate.

Loci with similar in vitro enzymatic acitivity were distinguished by being given a number denoting the position, relative to the origin, occupied by the most common allele of each locus in such a way that loci closer to the origin have a smaller number than more distant loci. For the enzyme GOT, the number 1 denotes anodal enzymatic activity whereas the number 2 denotes cathodal activity. In order to name sites of activity, every allozyme band was identified by a number denoting the position of the band relative to the position of a standard band (usually the most common).

2.3. Urea denaturation method

To detect probable hidden genetic variability we constructed 20 'isogenic' strains by using the 'brother–sister' system of single-pair matings (since in medfly a balanced strain as in *Drosophila* has not yet been constructed). All the adults of the crosses in the first generation came from a natural population from Crete. Urea denaturation was performed before electrophoresis. Five females of each strain were homogenized in 3 mL of urea solution. The extract was placed in an incubator at 37°C. The molarity of urea solutions used, the denaturation time in minutes and the staining time after electrophoresis are indicated in Table I. We have used three

TABLE I. TECHNICAL DETAILS OF UREA DENATURATION METHOD

Marker locus	Molarities of urea solutions	Denaturation time (min)	Staining time (min)
ODH	3, 4, 5, 6	12	120
ME	2, 3, 4, 5, 6, 7	10	30
PEP-1	14, 15, 16, 17	25	30

enzyme systems: two monomorphic (ODH and ME) and one highly polymorphic (PEP-1) in all populations studied. The molarities of urea solutions were chosen after preliminary trials to give clear cut results. These molarities proved not to differ very much from those used in *Drosophila subobscura* [9] for the same enzyme systems. For each enzyme the first lower molarity of urea solution gives nearly normal reaction. As the molarity increases, the reaction become progressively fainter while at the higher molarity of each case the enzymatic reaction is totally inhibited.

2.4. Host acceptance patterns for oviposition sites

2.4.1. Experiments with natural populations

Two pairs of populations coming from two different localities in Crete were analysed for 23 enzyme loci (all the previous loci except EST and LAP). The first pair of populations (Crete-I and Crete-II) derived from infested grapefruit (Crete-I) and oranges (Crete-II) collected in May 1986 from two adjacent orchards. The second pair derived from infested grapefruit (Crete-III) and peaches (Crete-IV) collected in April 1986 and July 1986, respectively, from two adjacent orchards. The mean diameters of the infested grapefruit (production of the previous year maintained on the trees), oranges and peaches from which the populations were collected were 11.7 ± 0.9 cm, 6.3 ± 0.5 cm and 6.8 ± 0.6 cm, respectively.

2.4.2. Experiments in the laboratory

Two medfly strains were established: one homozygous for allele PEP-$1^{1.26}$ and the other homozygous for allele PEP-$1^{1.00}$. The first strain came from flies that emerged from grapefruit and the second from flies emerging from peaches. The two

strains were maintained in the laboratory for two consecutive generations on grapefruit and peaches respectively. In the third generation, 300 pupae from each strain were carried to different cages containing artificial medium. Ten days after emergence 100 females of each strain (probably already inseminated) were transferred to two new cages (50 females in each cage), so that each new cage contained 100 females. The females of each cage oviposited on four grapefruit and four peaches placed in each cage. These fruits were replaced daily by new ones for ten consecutive days. Totals of 449 pupae from the peaches and 285 from the grapefruit were collected. All the adults that emerged (411 from peaches and 245 from grapefruit) were electrophorized for PEP-1.

TABLE II. CROSSES PERFORMED TO STUDY MENDELIAN SEGREGATIONS

Locus	Parents	Offspring		Expected ratio	χ^2	P
PEP-1	1.26/1.00 × 1.00/1.00	1.00/1.00	8	1:1	0.22	>0.50
		1.26/1.00	10			
	1.26/1.00 × 1.26/1.00	1.26/1.26	5	1:2:1	0.33	>0.80
		1.26/1.00	12			
		1.00/1.00	7			
PEP-3	1.06/1.00 × 1.00/1.00	1.06/1.00	7	1:1	0.33	>0.50
		1.00/1.00	5			
MPI	1.43/1.25 × 1.25/1.00	1.43/1.25	12	1:1:1:1	1.04	>0.70
		1.43/1.00	10			
		1.25/1.25	15			
		1.25/1.00	13			
	1.19/1.00 × 1.08/0.87	1.19/1.08	8	1:1:1:1	2.36	>0.50
		1.19/0.87	3			
		1.00/1.08	6			
		1.00/0.87	5			
	1.00/0.87 × 1.00/0.87	1.00/1.00	10	1:2:1	1.20	>0.50
		1.00/0.87	14			
		0.87/0.87	6			

3. RESULTS AND DISCUSSION

3.1. Allozyme variation in natural populations

Table II gives the results of the crosses performed to test Mendelian segregations of the allozyme variants of the polymorphic loci PEP-1, PEP-3 and MPI. In all cases the phenotypic proportions of the offspring do not deviate significantly from the expected ratios under Mendelian segregation.

Table III gives the allele frequencies of all loci at which at least two electrophoretic variants were observed. PEP-1, EST and secondarily PEP-3 and MPI loci proved to be polymorphic in all populations studied. MPI locus is extremely polymorphic in the South Africa population, displaying in a sample of 120 individuals as many as 14 alleles. A high number of alleles for MPI locus was also found in a population from Kenya (unpublished data).

Table IV shows the degree of genetic variation of each population expressed by the expected mean heterozygosity ($\bar{H}$), by the mean proportion of polymorphic loci (%P) and by the mean 'actual number' (n) and 'effective number' (n_e) of alleles per locus. $\bar{H}$ is the mean of H's over all loci (monomorphic and polymorphic) of each population. H measures the fraction of individuals expected to be heterozygous for the locus in question and is calculated from the equation $H = 1 - \Sigma p_i^2$, where p_i is the frequency of the i-th allele, and n_e is the mean of n_e's over all loci (monomorphic and polymorphic). The n_e of a particular locus is calculated from the equation $n_e = 1/\Sigma p_i^2$ [10]. The 'effective number' of alleles is usually more useful in population genetics than the 'actual number', since many of the alleles contribute very little to the genetic variance of a population (because they are represented only once or twice in the population).

The main feature of Table IV is the extremely low genetic variability of the introduced populations compared with the populations of Réunion and South Africa. This is in agreement with the results of Huettel et al. [11] and Morgante et al. [12]. Huettel et al. [11] found that in populations of medfly from Israel, Hawaii and Costa Rica the average heterozygosities were 7.1%, 3.4% and 3.6% and the mean proportions of polymorphic loci 17%, 13% and 9% respectively. Morgante et al. [12] reported that in Brazilian populations of medfly the mean degree of heterozygosity was 3%.

These observations lead to the following question: if allozyme variation has any role in the adaptation of the species to different host environments, how is it possible for a polyphagous species such as the medfly to retain so little variation?

Since the early works of Lewontin and Hubby [13], Harris [14], Lewontin [15], Selander and Young [16], it has been shown that 40% of the loci in a population are polymorphic and that 12–15% of the loci of an average individual are heterozygous in natural populations of plants and animals. The corresponding mean numbers for the Mediterranean populations of medfly are 15.0% and 5.3%.

TABLE III. ALLELE FREQUENCIES IN 15 WILD POPULATIONS OF MEDFLY
(further explanations in text)

Loci	Populations														
	Cyprus	Kala-mata	Chios	Attiki	Procida	Crete	Hawaii	Egypt-I	Egypt-II	Israel	Spain	Brazil	Guate-mala	Réunion	South Africa
MPI:															
1.43	—	—	—	—	—	—	—	—	—	—	—	—	—	0.024	0.025
1.39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.008
1.35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.017
1.25	—	—	—	—	—	—	—	—	—	—	—	—	—	0.032	0.042
1.19	—	—	—	—	—	—	—	—	—	—	—	0.025	—	0.024	0.042
1.12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.042
1.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.042
1.04	—	—	—	—	—	—	—	—	—	—	—	—	—	0.032	0.083
1.00	0.042	0.038	0.059	0.058	0.190	0.037	0.257	0.375	0.317	0.208	0.162	0.662	0.376	0.214	0.275
0.97	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.042
0.92	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.025
0.87	0.958	0.962	0.941	0.942	0.810	0.963	0.743	0.625	0.683	0.792	0.838	0.313	0.624	0.674	0.300
0.83	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.017
0.74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.042
N[a]	120	156	110	120	108	135	113	120	112	178	136	120	97	126	120
GOT-2:															
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.946	0.911
0.47	—	—	—	—	—	—	—	—	—	—	—	—	—	0.054	0.089
N	80	80	80	80	80	80	80	80	80	80	80	80	80	112	107

PGM:															
1.26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.004
1.13	—	—	—	—	—	—	—	—	—	—	—	—	—	0.036	0.087
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.981	0.932	1.000	1.000	1.000	1.000	0.928	0.788
0.89	—	—	—	—	—	—	—	0.019	0.068	—	—	—	—	0.036	0.117
0.79	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.004
N	80	80	80	80	80	80	80	77	140	80	80	80	80	110	120
ODH:															
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.900
0.92	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.100
N	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80
PHI:															
1.21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.042
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.871
0.79	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.087
N	80	80	80	80	80	80	80	77	140	80	80	80	80	110	120
Hk-1:															
1.10	—	—	—	0.006	—	—	—	—	—	—	—	—	—	0.013	0.429
1.00	1.000	1.000	1.000	0.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.994	0.700	0.500
0.90	—	—	—	—	—	—	—	—	—	—	—	—	0.006	0.287	0.071
N	80	80	80	80	80	80	80	80	80	80	80	80	80	115	120
G-6-PD:															
1.02	—	—	—	—	—	0.006	—	—	—	—	0.031	0.120	0.103	—	0.051
1.00	1.000	1.000	1.000	1.000	1.000	0.949	1.000	1.000	1.000	1.000	0.802	0.800	0.661	1.000	0.678
0.98	—	—	—	—	—	0.045	—	—	—	—	0.167	0.080	0.236	—	0.271
N	80	80	80	80	107	154	80	80	80	80	96	120	87	110	118

TABLE III. (cont.)

Loci	Populations														
	Cyprus	Kala-mata	Chios	Attiki	Procida	Crete	Hawaii	Egypt-I	Egypt-II	Israel	Spain	Brazil	Guate-mala	Réunion	South Africa
IDH:															
1.10	—	—	—	—	—	—	—	—	—	—	—	—	—	0.104	0.012
1.00	1.000	1.000	0.982	1.000	1.000	0.978	1.000	0.633	0.707	0.728	1.000	1.000	1.000	0.835	0.838
0.83	—	—	0.018	—	—	0.022	—	0.367	0.293	0.272	—	—	—	0.061	0.150
N	80	80	85	80	80	114	80	120	140	160	80	80	80	115	80
Hk-3:															
1.09	—	—	—	—	—	—	—	—	—	—	—	—	—	0.361	0.267
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.639	0.733
N	80	80	80	80	80	80	80	80	80	80	80	113	80	115	120
LAP:															
1.14	—	—	—	—			—			—				—	0.111
1.05	—	0.004	—	—			0.007			—				0.112	0.417
1.00	1.000	0.996	1.000	1.000			0.850			1.000				0.888	0.472
0.95	—	—	—	—			0.143			—				—	—
N	60	118	65	60			140			80				76	72
PEP-1:															
	1.38	—	—	—	—	—	—	0.042	—	—	—	—	—	—	—
1.26	0.483	0.351	0.191	0.450	0.325	0.621	0.056	0.535	0.489	0.442	0.524	0.519	0.727	0.652	0.683
1.00	0.517	0.649	0.809	0.550	0.675	0.379	0.944	0.465	0.511	0.558	0.476	0.481	0.273	0.348	0.275
N	177	171	282	404	151	161	206	101	140	189	155	134	119	112	142
PEP-2:															
1.41	—	—	—	—	—	—	—	—	—	—	—	—	—	0.018	0.021
1.32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.042

PEP-2: (cont.)															
1.22	—	—	—	—	—	0.125	—	0.043	0.031	—	—	0.027	0.030	0.357	0.218
1.00	1.000	1.000	1.000	1.000	1.000	0.875	1.000	0.957	0.969	1.000	1.000	0.973	0.970	0.625	0.600
0.92	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.056
0.68	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.063
N	177	171	282	404	151	168	206	81	80	189	118	130	150	112	142
PEP-3:															
1.09	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.007
1.06	0.023	0.076	0.064	0.121	0.172	0.089	0.628	0.005	0.011	—	0.278	0.047	—	0.902	0.796
1.00	0.977	0.924	0.936	0.879	0.828	0.911	0.372	0.995	0.989	1.000	0.722	0.953	1.000	0.098	0.190
0.93	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.007
N	177	171	282	387	151	168	206	101	140	189	158	138	119	112	142
6-PGD:															
1.17	—	—	—	0.008	—	—	—	—	—	—	—	—	—	—	0.013
1.00	1.000	1.000	1.000	0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.974
0.90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.013
N	80	80	80	80	80	80	80	80	80	80	80	80	80	110	118
EST:															
1.50	0.002	0.002	—	—			—			—				—	
1.25	0.025	0.052	—	—			—			0.019				—	
1.10	—	0.004	—	0.085			—			0.006				0.188	
1.04	—	—	—	—			—			—				0.006	
1.00	0.194	0.658	0.398	0.438			0.083			0.267				0.154	
Silent	0.779	0.284	0.602	0.480			0.917			0.708				0.652	
N	201	247	182	160			163			160				91	

[a] N is the number of individuals electrophorized for each particular enzyme.

TABLE IV. POPULATIONS STUDIED, SAMPLING AREAS, HOST FRUIT TREES AND GENETIC VARIATION EXPRESSED IN VARIOUS WAYS
(further explanations in text)

Population	Sampling area	Host fruit tree	$\bar{H}$	%P	n	n_e
Cyprus	Nicosia	Tangerines	0.039	8.0	1.24	1.07
Kalamata	Kalamata	Figs	0.046	12.0	1.32	1.08
Chios	Chios	Figs	0.042	16.0	1.20	1.07
Attiki	Votanikos	Oranges	0.057	16.0	1.28	1.11
Crete	Chania	Grapefruit	0.046	17.4	1.30	1.07
Procida	Procida	Figs	0.045	17.4	1.17	1.08
Israel	Bet Dagan	Apricots	0.066	16.0	1.24	1.11
Egypt-I	Kalubia Governorate	Apricots	0.068	13.0	1.26	1.13
Egypt-II	El-Fayoum	Apricots	0.068	17.4	1.26	1.12
Hawaii	Hawaii	Guavas	0.055	20.0	1.24	1.09
Spain	Puente nuevo	Peaches	0.065	17.0	1.22	1.11
Guatemala	—	Coffee	0.063	13.0	1.26	1.12
Brazil	—	Coffee	0.063	13.0	1.30	1.07
Réunion	Réunion	Coffee	0.153	44.0	1.84	1.27
S. Africa	Stellenbosch	Guavas	0.234	54.2	2.71	1.54

According to theory [17] and some experimental studies [18, 19] one would expect a positive correlation between the genetic variation of a species and the degree of its environmental diversity. However, comparison of the genetic variation levels between monophagous fruit fly species and the polyphagous medfly disagrees with the theory. *Dacus oleae, Rhagoletis pomonella, Rhagoletis completa* and *Delia antigua* are monophagous species infesting olive trees, species of the plant families *Rosaceae* and *Juglandaceae* (walnut trees) and cultivated *Allium* species (onions) respectively. Their respective degrees of mean heterozygosity were found to be 24.7% [6], 18.1% and 7.5% [3] and 9% (M. Harris, personal communication), which are consistently higher than the heterozygosity values reported for the introduced populations of medfly.

It could be argued, however, that the low genetic variation observed is due to a technical artefact: electrophoretic alleles ('electromorphs') group together many real alleles that cannot be differentiated by such a crude technique as simple gel electrophoresis. Recently, by employing a large number of techniques coupled with electrophoresis (thermostability, different gel concentrations and buffer systems, sensitivity to urea), a considerable amount of allelic variation has been revealed [9, 20–26].

As mentioned in Section 2, we tried to construct 'isogenic' strains of medfly by utilizing the 'brother–sister' system of matings. Only two offspring (one male and one virgin female) of each pair were used as the pair of the next generation. This procedure was followed for 14 successive generations. In this system of matings the heterozygosity of the t generation is given from the heterozygosity in the t-1 generation by the equation [10]:

$$H_t = 0.809\ H_{t-1}$$

or from the heterozygosity in generation 0, by the equation:

$$H_t = (0.809)^t\ H_0$$

By assuming $H_0 = 1$ and for $t = 14$,

$$H_{14} = 0.051$$

Therefore, each of the 20 strains is expected to retain 5% of its original heterozygosity.

All the 20 'isogenic' strains behaved in exactly the same way for each of the three enzyme systems studied. In other words, they did not display either quantitative or qualitative differences. Therefore, although we used only 20 strains and three enzyme systems, our results indicate that the low genetic variability detected in medfly populations by the usual electrophoretic method is a real one, or at least that a probable hidden genetic variation does not seem to contribute significantly either in increasing the number of alleles or in increasing the degree of heterozygosity.

On the other hand, the low levels of genetic variation of the populations of medfly may reflect the results of historical causes, i.e. the time elapsed since colonization and the number of flies that formed the founder population. It can be seen from Table IV that only the Réunion and South Africa populations maintain high levels of genetic variation: $\bar{H} = 0.153$ and 0.234 respectively.

Tropical west Africa is regarded as the centre of origin of the medfly. The biologically effective time of separation of species such as *Dacinae* and *Trypetinae* is usually assigned to the beginning of the Oligocene epoch, about 36 MYR. (See Ref. [27] for discussion of the geological setting; Ref. [28] for other examples of Old and

TABLE V. GENETIC IDENTITY VALUES, I (ABOVE THE DIAGONAL), AND GENETIC DISTANCES, D (BELOW THE DIAGONAL), BETWEEN 14 NATURAL POPULATIONS OF MEDFLY (GIVEN AS MULTIPLES OF 10^3)
(further explanations in text)

	Cyprus	Kalamata	Chios	Attiki	Procida	Crete	Israel	Egypt-I	Spain	Guatemala	Brazil	Hawaii	Réunion	S. Africa
1	—	999	996	1000	997	997	995	989	986	985	980	974	942	932
2	1±7	—	999	999	998	995	995	987	993	980	979	981	943	932
3	4±13	1±7	—	997	998	990	993	985	982	977	977	983	936	925
4	0	1±7	3±11	—	998	997	995	988	988	987	981	980	949	938
5	3±11	2±9	2±9	2±9	—	994	995	989	994	988	985	987	949	942
6	3±11	5±15	10±21	3±11	6±16	—	993	988	989	992	979	970	951	941
7	5±15	5±15	7±18	5±15	5±15	7±18	—	988	983	986	984	972	939	933
8	11±22	13±24	15±26	12±23	11±22	12±23	2±9	—	977	989	988	965	939	938
9	14±25	7±17	18±28	12±24	6±16	11±22	17±28	23±32	—	981	985	974	953	942
10	15±26	20±30	23±31	13±23	12±23	8±19	14±24	11±22	19±29	—	993	957	941	939
11	20±30	21±31	23±32	19±29	15±26	21±31	16±27	12±23	15±26	7±18	—	966	938	943
12	26±34	19±29	17±27	20±30	13±24	30±37	28±35	36±40	26±34	43±44	35±39	—	961	948
13	60±52	59±51	66±55	52±48	52±48	50±47	63±53	63±53	48±46	60±52	64±54	40±42	—	979
14	70±52	70±56	78±59	64±54	60±52	61±52	69±56	64±54	59±52	62±53	59±51	53±49	21±31	—

New World groups that diverged at this time because of the drifting apart of Africa and South America; and see also Ref. [29].) The high degree of heterozygosity of the South Africa population in combination with the high number of alleles, many of which are rare, is additional evidence in support of this hypothesis. The low genetic variability observed in the wild populations of medfly might therefore have arisen from the casual introduction of a small number of individuals (the founder effect [30]), which later spread to different geographical areas.

It has been proposed [1] that the medfly first invaded Spain in 1842 and subsequently spread into France, Italy, Greece and the Middle East. There is, of course, no way to determine the amount of genetic variation contained in the founder population, simply because there is no unified theory for predicting colonization success at the species level [31]. The allele frequency distribution in the native populations is U shaped, which appears to be typical of distributions of populations assumed to have maintained large population sizes in the recent past. The distributions of the derived populations are effectively bimodal, with almost complete absence of rare and low frequency alleles, and peaks in the 0.40–0.60 and 0.90–1.00 frequency classes. Such distributions have been predicted for populations that have gone through a genetic bottleneck [32–34]. This is exactly the pattern found in medfly from Kenya and South Africa (the probable source areas) and from the Mediterranean countries, Guatemala, Costa Rica and Hawaii [11], and also in the present study.

Table V gives the genetic identity values, I, and the genetic distances, D [35], between all the pairwise comparisons of 14 populations of medfly based on 23 loci. The values of I can be classified as three groups. One group includes combinations of any two populations except those of Réunion and South Africa (there are 66 such values). The second group includes all the pairs 'any population (except South Africa)–Réunion' and 'any population (except Réunion)–South Africa' (there are 24 such values). The third group consists of a simple value, the index of identity of the pair 'South Africa–Réunion'. The non-parametric two sample rank test [36] for this pattern gives a standard normal deviate $U_P = -7.2$. The two tailed probability that a value as large as this or larger is due to chance is approximately equal to zero. Therefore, there are statistically significant differences in the genetic structure between the introduced and the native populations of medfly. From the distance matrix of Table V the dendrogram of Fig. 2 was constructed, according to the unweighted pair group mean analysis (UPGMA) method [37]. Although a small number of loci contribute to the differentiation of the D values between populations (only the polymorphic loci of the introduced populations), the most significant features of the dendrogram depicted in Fig. 2 are the following:

(a) There is a clear cut separation of the introduced and the source populations.
(b) The population of Réunion belongs to the same lineage as the population of South Africa, indicating that this population derived from a different route of dispersion than, for example, the Mediterranean populations.

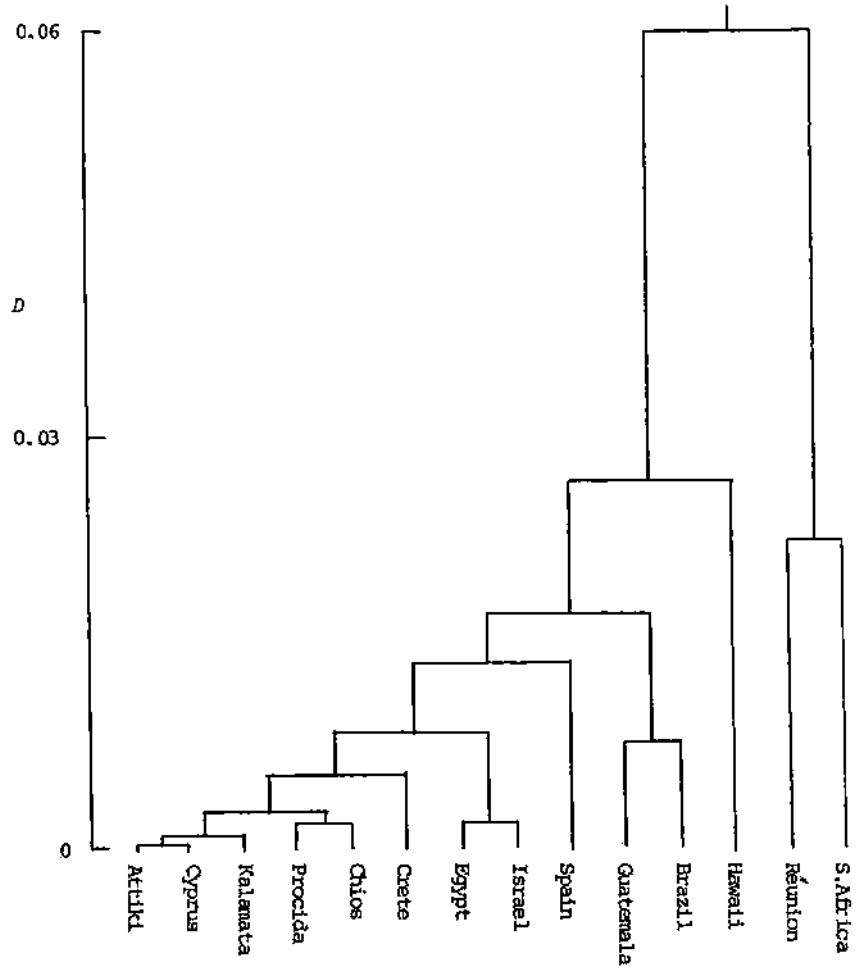


FIG. 2. Dendrogram of 14 natural populations of medfly constructed from the distance matrix shown in Table V.

(c) The lineage leading to the introduced populations (except the Hawaii population) splits into two lineages. One leads to the populations of Guatemala and Brazil and the other to the Mediterranean populations.

(d) The lineage leading to the Mediterranean populations splits again into two. One lineage leads to the population of Spain and the other to the rest of the Mediterranean populations. The latter splits further into two sublineages: that of the populations Israel and Egypt and that of the populations Attiki, Cyprus, Kalamata, Procida, Chios and Crete.

TABLE VI. ALLELE FREQUENCIES AT THREE ENZYME LOCI IN THE MEDITERRANEAN POPULATIONS OF MEDFLY AND IN THE POPULATIONS FROM SOUTH AFRICA, GUATEMALA AND BRAZIL

Loci	Guatemala	Brazil	Spain	S. Africa	Egypt	Med. populations[a]
IDH:						
1.10	—	—	—	0.012	—	—
1.00	1.000	1.000	1.000	0.838	0.670	0.955
0.83	—	—	—	0.150	0.330	0.045
G-6-PD:						
1.02	0.103	0.120	0.031	0.051	—	0.001
1.00	0.661	0.800	0.802	0.678	1.000	0.993
0.98	0.236	0.080	0.167	0.271	—	0.006
PGM:						
1.26	—	—	—	0.004	—	—
1.13	—	—	—	0.087	—	—
1.00	1.000	1.000	1.000	0.788	0.956	1.000
0.89	—	—	—	0.117	0.044	—
0.79	—	—	—	0.004	—	—

[a] Except the populations from Spain and Egypt.

The dendrogram of Fig. 2, based on quantitative data, seems to be in agreement with the proposal of Hagen et al. [1] concerning the dispersion of the fly from its geographical centre of origin. On the other hand, the qualitative data (using the alleles as genetic markers to trace the routes of dispersion of the fly) support such a hypothesis in some cases but not in others. Table VI shows the allele frequencies at three loci in the populations from South Africa, Guatemala and Brazil (the last two supposedly 'introduced' from Spain), as well as in the Mediterranean populations. We observe that while the type of alleles and their frequencies at G-6-PD locus support the hypothesis of Hagen et al. [1], the allele distribution of the IDH and PGM loci support another route of dispersion of the fly, from Africa to the Mediterranean countries, not through the Iberian peninsula but through the Nile valley to Egypt. Of course, to answer this question, many more populations from different countries would need to be electrophoretically analysed.

TABLE VII. ALLELE FREQUENCIES IN FOUR WILD POPULATIONS OF MEDFLY FROM CRETE

Loci	Populations			
	Crete-I (grapefruit)	Crete-II (oranges)	Crete-III (grapefruit)	Crete-IV (peaches)
PEP-1:				
1.26	0.621	0.559	0.675	0.662
1.00	0.379	0.441	0.325	0.338
N[a]	161	170	143	136
PEP-2:				
1.22	0.125	0.087	0.136	0.129
1.00	0.875	0.913	0.864	0.871
N	168	126	143	136
PEP-3:				
1.06	0.089	0.117	0.087	0.125
1.00	0.911	0.883	0.917	0.875
N	168	158	143	136
MPI:				
1.00	0.037	0.048	0.084	0.085
0.87	0.963	0.952	0.916	0.915
N	135	168	143	136
G-6-PD:				
1.02	0.006	0.006	0.045	0.018
1.00	0.949	0.969	0.927	0.942
0.98	0.045	0.025	0.028	0.040
N	154	160	143	136
IDH:				
1.00	0.978	0.997	1.000	1.000
0.83	0.022	0.003	—	—
N	114	149	85	81
$\bar{H}$	0.046	0.044	0.049	0.050

[a] N is the number of individuals electrophorized for each particular enzyme.

3.2. Host acceptance patterns for oviposition sites

Prokopy et al. [38] reported that some, if not most, of the observed interpopulational variation in host acceptance pattern of *Rhagoletis pomonella* is attributed to genetic differences between populations of that species. Jaenike and Grimaldi [39] found substantial genetic variation for host preference among strains of the polyphagous *Drosophila tripunctata* species. Prokopy et al. [40] reported significant interpopulational differences among medfly females for oviposition sites. Their data suggest that the size of the fruit and not its taxonomic status has a strong influence on the acceptance pattern of each population and that at least a portion of the interpopulation variation had a genetic basis.

Although the striking similarities in the genetic structure of the populations of medfly infesting different host fruits is in apparent contradiction to a host acceptance pattern associated with genetic differences, it can be argued that the genetic changes for host preference, if they do exist, may be too small to be detected by the usual population samples, and/or they could be detected only if samples from different kinds of fruit tree species, cultivated in the same area and collected at the same time, were studied. To study this possibility we analysed 23 enzyme loci in two pairs of populations coming from two different localities in Crete. The populations of each pair came from adjacent orchards but from different fruit trees (see Section 2) with significantly different fruit sizes.

Table VII gives the allele frequencies of all loci of which at least two variants were observed and the expected mean heterozygosity ($\bar{H}$) of each population. On the basis of all 23 loci studied (polymorphic and monomorphic) the genetic identity values [35] in all pairwise comparisons of the populations studied were estimated to

TABLE VIII. OFFSPRING OF SINGLE PAIR CROSSES BETWEEN INDIVIDUALS COMING FROM TWO DIFFERENT HOST FRUITS

Single pairs	No. of single pairs	Mean No. of adults/pair
♀ grapefruit × ♂ grapefruit	20	83±15
♀ orange × ♂ orange	20	91±13
♀ grapefruit × ♂ orange	10	79±21
♀ orange × ♂ grapefruit	10	88±20

be 1.000 for the pairs 'Crete-I — Crete-II', 'Crete-I — Crete-III', 'Crete-I — Crete-IV', Crete-III — Crete-IV' and 0.999 for the pairs 'Crete-II — Crete-III', 'Crete-II — Crete-IV'. Thus, all populations displayed extremely high coincidence in their genetic structures, irrespective of the taxonomic status or the size of the host fruit.

Moreover, single pair crosses between individuals coming from grapefruit and oranges yielded a mean number of offspring (adults) which do not deviate significantly (by using the t distribution in all pairwise comparisons) (Table VIII). It follows that in artificial food the fertility of females coming from different fruits of different sizes is more or less the same irrespective of the fruit from which the male parent was derived.

Of course, the possibility remains that the host preference patterns for oviposition sites reported for the medfly [40] are due to relatively few changes or that they may not involve the genetic loci that we studied. For this reason we performed another type of experiment by utilizing two medfly strains homozygous for two different PEP-1 alleles (see Section 2) and coming from grapefruit and peaches. Among the 411 adults coming from peaches, 187 were found to be homozygous for allele PEP-$1^{1.26}$ and 224 homozygous for allele PEP-$1^{1.00}$. Among the 245 adults coming from grapefruit, 131 were found to be homozygous for allele PEP-$1^{1.26}$ and 114 homozygous for allele PEP-$1^{1.00}$. Although the females showed some tendency to oviposit on the fruit in which they were fed as larvae, the χ^2 homogeneity tests (to test the deviation from the 1:1 ratio) were not statistically significant ($0.05 < P < 0.10$ in the first case and $0.20 < P < 0.30$ in the second).

These results, which are independent of the larval viability in the different host fruits, failed to detect any pattern of preference for oviposition sites in the medfly. It therefore seems that medfly may experience the different host plants as a fine grained environment. It is possible that the different host fruits do not represent different environments, because they probably do not differ in the chemical compounds which provide the substrates for the enzymes studied. The fact that the same kind of fruit is not available throughout the year forces the medfly to shift from host to host. In other words, what appears to be a heterogenous environment could be a fine grained one for the medfly species. Since the fine grained species use many alternative food resources, they require functional flexibility [41]. Thus, selection would increase the frequency of the allele which, averaged over environments, provides the highest fitness.

3.3. Genetic changes in artificially reared colonies

Introduction of insects from the wild into the laboratory may result in changes in the physiology, behaviour and reproductive biology of the insect. The net result of these changes is to improve the adaptation of the population to laboratory conditions. Very often, such insects are used for basic biological studies or for control of

the species. It is therefore very important to be at least familiar with laboratory induced changes and the factors that may cause them. Bush and Neck [4] showed that the mass reared populations of screw-worm differed very significantly in their genetic make-up from their wild counterparts. Particularly dramatic changes were noted in the frequency of the allelic forms of the enzyme α-GPD, which were found to be directly related to differences in field performance of the screw-worm flies [42]. Zouros et al. [5] and Loukas et al. [6] reported dramatic changes in the allele frequencies of the loci 6-PGD and ADH in populations of *Dacus oleae* following artificial rearing. Moreover, they showed that artificial rearing of the olive fruit fly causes profound changes throughout the genome, particularly at those loci which are highly polymorphic in natural populations.

To study the genetic changes induced in the laboratory, we have monitored the changes in allele frequencies of the polymorphic loci PEP-1, PEP-3 and EST in three populations of medfly during the first four or five generations of artificial rearing. Table IX gives the allele frequencies at the loci surveyed in the founding generation and the subsequent generations of artificial rearing. Two statistics are useful in interpreting the results shown in Table IX: the standard error (SE) of the allele frequency and the χ^2 test of homogeneity. Approximate 95% confidence intervals can be obtained by taking two SEs round the frequency estimate. When the confidence limits do not overlap, the null hypothesis that the two allele frequencies in the founding (generation 0) and in the last generation are not different can be rejected at $\alpha = 0.05$. This is the case for PEP-1 in Chios ($\chi^2 = 22.4$, $P < 0.001$) and Cyprus ($\chi^2 = 6.7$, $P < 0.01$) populations, for PEP-3 in Chios population ($\chi^2 = 5.1$, $P < 0.05$) and for EST in Cyprus population ($\chi^2 = 32.2$, $P < 0.001$). One explanation for these results is that the variation at these loci is selectively neutral. Under this hypothesis we could expect that, through the process of random drift, the colonies will eventually be fixed for the more frequent allele (with a probability equal to its frequency). This hypothesis is supported by the fact that the changes in allele frequencies mentioned before are not statistically significant or of the same direction in all populations. Of course a neutrality hypothesis cannot be easily tested without knowledge of the effective size of the colonies. The fact that the very rare alleles of EST locus continue to be present even after five generations of artificial rearing seems to support some form of balancing selection which prevents the extinction of rare alleles. However, the changes observed, if they are not selectively neutral, do not seem to affect severely (or to be connected to) the differences in field performance of the fly. The eradication of medfly from southern Mexico by releasing mass reared sterilized flies [43] supports such an assertion.

TABLE IX. ALLELE FREQUENCY CHANGES AT THE PEP-1, PEP-3 AND EST LOCI OF MEDFLY UNDER ARTIFICIAL REARING

(*the allele frequencies of the wild populations correspond to T = 0*)

Colony	Lab. generations	PEP-1		PEP-3		Sample size
		1.26	1.00	1.06	1.00	
Chios	0	0.180±0.021	0.820±0.021	0.067±0.014	0.933±0.014	164
	1	0.148±0.019	0.852±0.019	0.085±0.015	0.915±0.015	182
	2	0.149±0.019	0.851±0.019	0.103±0.016	0.897±0.016	185
	3	0.045±0.015	0.955±0.015	0.040±0.014	0.960±0.014	101
	4	0.054±0.014	0.946±0.014	0.029±0.010	0.971±0.010	140
Cyprus	0	0.438±0.025	0.517±0.025	0.023±0.008	0.977±0.008	177
	1	0.466±0.026	0.534±0.026	0.017±0.007	0.983±0.007	178
	2	0.409±.0032	0.591±0.032	0.009±0.006	0.991±0.006	116
	3	0.398±0.027	0.602±0.027	0.000	1.000	161
	4	0.345±0.025	0.655±0.025	0.000	1.000	184
Attiki	0	0.410±0.029	0.590±0.029	0.112±0.018	0.882±0.018	145
	1	0.398±0.044	0.602±0.044	0.159±0.033	0.841±0.033	62
	2	0.402±0.033	0.598±0.033	0.089±0.019	0.911±0.019	112
	3	0.455±0.024	0.545±0.024	0.141±0.017	0.859±0.017	220
	4	0.446±0.029	0.554±0.029	0.104±0.018	0.896±0.018	149
	5	0.443±0.029	0.557±0.029	0.152±0.021	0.848±0.021	148

Colony	Lab. generations	EST						Sample size
		1.50	1.25	1.10	1.04	1.00	Silent	
Chios	0	—	—	—	—	0.398±0.026	0.602±0.026	182
	1	—	—	—	—	0.338±0.025	0.662±0.025	180
	2	—	—	—	—	0.328±0.025	0.672±0.025	177
	3	—	—	—	—	0.378±0.025	0.622±0.025	186
	4	—	—	0.016±0.007	—	0.327±0.026	0.657±0.026	162
Cyprus	0	0.002±0.002	0.025±0.008	—	—	0.194±0.020	0.779±0.021	201
	1	0.002±0.002	0.006±0.004	—	—	0.140±0.016	0.850±0.016	242
	2	—	0.018±0.006	—	—	0.100±0.014	0.882±0.015	220
	3	—	0.030±0.010	—	—	0.070±0.014	0.900±0.017	160
	4	—	0.025±0.008	—	—	0.048±0.011	0.927±0.014	180
Attiki	0	—	—	0.085±0.016	—	0.435±0.028	0.480±0.028	160
	1	0.003±0.003	—	0.038±0.011	—	0.435±0.028	0.524±0.028	160
	2	0.004±0.004	—	0.068±0.016	0.008±0.006	0.393±0.033	0.527±0.032	122
	3	0.003±0.003	—	0.070±0.014	—	0.401±0.027	0.526±0.027	171
	4	0.003±0.003	—	0.055±0.013	—	0.423±0.028	0.519±0.028	155
	5	0.003±0.003	—	0.033±0.010	—	0.442±0.027	0.522±0.028	164

4. CONCLUSIONS

(1) All the introduced natural populations of medfly displayed an exceptionally low degree of genetic heterogeneity. This finding is better explained by historical reasons.

(2) The most probable route of dispersion of the fly from its geographical centre of origin seems to be through the Iberian peninsula, although a route through the Nile valley cannot be excluded.

(3) The artifical rearing of the medfly does not seem to be associated with an apparent selection or with genetic changes as dramatic as those reported for other insects.

(4) Patterns of preference for oviposition sites associated either with the taxonomic status of the fruit or with the size of the fruit were not detected among the medfly females.

REFERENCES

[1] HAGEN, K.S., WILLIAM, W.W., TASSAN, R.L., Mediterranean fruit fly: the worst may be yet to come (Report of Progress in Research Mar.–Apr. 1981), Calif. Agric. **35** (1981) 5–7.

[2] RICHARDSON, R.H., ELLISON, J.R., AVERHOFF, W.W., Autocidal control of screwworms in North America, Science **215** (1982) 361–370.

[3] BERLOCHER, S.H., BUSH, G.L., An electrophoretic analysis of *Rhagoletis* (Diptera: Tephritidae) phylogeny, Syst. Zool. **31** (1982) 136–155.

[4] BUSH, G.L., NECK, R.W., Ecological genetics of the screwworm fly *Cochliomyia hominivorax* (Diptera: Tephritidae) and its bearing on the quality control of mass-reared insects, Environ. Entomol. **5** (1976) 821–826.

[5] ZOUROS, E., LOUKAS, M., ECONOMOPOULOS, A., MAZOMENOS, B., Selection at the alcohol dehydrogenase locus of the live fruit fly *Dacus oleae* under artificial rearing, Heredity **48** (1982) 169–185.

[6] LOUKAS, M., ECONOMOPOULOS, A.P., ZOUROS, E., VERGINI, Y., Genetic changes in artificially reared colonies of the olive fruit fly (Diptera: Tephritidae), Ann. Entomol. Soc. Am. **78** (1985) 159–166.

[7] TSITSIPIS, J.A., An improved method for the mass rearing of the olive fruit fly, *Dacus oleae* (Gnelin) (Diptera: Tephritidae), Z. Angew. Entomol. **83** (1977) 919–926.

[8] LOUKAS, M., KRIMBAS, C.B., Isozyme techniques in *D. subobscura*. DIS 55 (1980) 157–158.

[9] LOUKAS, M., VERGINI, Y., KRIMBAS, C.B., The genetics of *D. subobscura* populations — XVII. Further genic heterogeneity within electromorphs by urea denaturation and the effect of the increased genic variability on linkage disequilibrium studies, Genetics **97** (1981) 411–429.

[10] CROW, J.E., KIMURA, M., An Introduction to Population Genetics Theory, Harper and Row, New York and London (1970).

[11] HUETTEL, M.D., FUERST, P.A., MARUYAMA, T., CHAKRABORTY, R., Genetic effects of multiple population bottlenecks in the Mediterranean fruit fly *Ceratitis capitata*, Genetics **94** (1980) 47.

[12] MORGANTE, J.S., de SOUZA, H.M.L., de CONTI, E., CYTRYMOWICZ, M., Allozymic variability in an introduced fruit fly pest *Ceratitis capitata* (Wiedemann), 1824 (Diptera: Tephritidae), Rev. Brasil. Genet. **2** (1981) 183–191.

[13] LEWONTIN, R.C., HUBBY, J.L., A molecular approach to the study of genic heterozygosity in natural populations — II. Amount of variation and degree of heterozygosity in natural populations of *D. subobscura*, Genetics **54** (1966) 595–609.

[14] HARRIS, H., Enzyme polymorphisms in man, Proc. R. Soc. London **164** (1966) 298–310.

[15] LEWONTIN, R.C., An estimate of average heterozygosity in man, Am. J. Hum. Genet. **19** (1967) 681–685.

[16] SELANDER, R.K., YOUNG, S.Y., Protein polymorphism and genic heterozygosity in a wild population of the house mouse (*Mus musculus*), Genetics **63** (1969) 653–667.

[17] GILLESPIE, H.H., LANGLEY, C.H., A general model to account for enzyme variation in natural populations, Genetics **76** (1974) 837–848.

[18] POWELL, J.P., Genetic polymorphisms in varied environments, Science **174** (1971) 1035–1036.

[19] LEVINTON, J., Genetic variations in a gradient of environmental variability: marine Bivalvia (Mollusca), Science **180** (1973) 57–75.

[20] WRIGHT, T.R.F., MacINTYRE, R.J., Heat-stable and heat-labile esterase-6^F enzymes in *D. melanogaster* produced by different Est-6^F alleles, J. Elisha Mitchell Sci. Soc. **81** (1965) 17.

[21] SINGH, R.S., HUBBY, J.L., THROCKMORTON, L.H., The study of genic variation by electrophoresis and leat denaturation techniques at the octanol dehydrogenase locus in members of the *D. virilis* group, Genetics **80** (1975) 637–650.

[22] SINGH, R.S., LEWONTIN, R.C., FELTON, A.A., Genic heterogeneity within electrophoretic "alleles" of xanthine dehydrogenase in *D. pseudoobscura*, Genetics **84** (1976) 609–629.

[23] COYNE, J.A., Lack of genetic similarity between two sibling species as revealed by varied techniques, Genetics **84** (1976) 593–607.

[24] COYNE, J.A., FELTON, A.A., Genic heterogeneity at two alcohol dehydrogenase loci in *D. pseudoobscura* and *D. persimilis*, Genetics **87** (1977) 285–304.

[25] COYNE, J.A., FELTON, A.A., LEWONTIN, R.C., Extent of genetic variation at a highly polymorphic esterase locus in *D. pseudoobscura*, Proc. Natl. Acad. Sci. U.S.A. **75** (1978) 5090–5093.

[26] SINGH, R.S., Genetic heterogeneity within electrophoretic "alleles" and the pattern of variation among loci in *D. subobscura*, Genetics **93** (1979) 997–1018.

[27] TARLING, D.H., "The geological evolution of South America with special reference to the last 200 million years", Evolutionary Biology of the New World Monkeys and Continental Drift (CIOCHON, R.L., CHARELLI, A.B., Eds), Plenum Press, New York (1980).

[28] SARICH, V.M., CRONIN, J.E., "South American mammal molecular systematics, evolutionary clocks, and continental drift", ibid.

[29] BEVERLEY, S.M., WILSON, A.C., Molecular evolution in *Drosophila* and the higher Diptera — II. A time scale for fly evolution, J. Mol. Evol. **21** (1984) 1–13.

[30] MAYR, E., "Change of genetic environment and evolution", Evolution as a Process (HUXLEY, J., HARDY, A.C., FORD, E.B., Eds), George, Allen & Unwin, London (1954).

[31] LEVINS, R., WILSON, M., Ecological theory and pest management, Annu. Rev. Entomol. **25** (1980) 287–308.

[32] NET, M., MARUYAMA, T., CHAKRABORTY, R., The bottleneck effect and genetic variability in populations, Evolution **29** (1975) 1–10.

[33] BERLOCHER, S.H., The genetics of speciation in *Rhagoletis* (Diptera: Tephritidae), PhD Thesis, Univ. of Texas, Austin (1976).

[34] CHAKRABORTY, R., FUERST, P.A., NEI, M., Statistical studies on protein polymorphisms in natural populations — III. Distribution of allele frequencies and the number of alleles per locus, Genetics **94** (1980) 1039–1063.

[35] NEI, M., Genetic distance between populations, Am. Nat. **106** (1972) 283–292.

[36] BROWNLEE, K.A., "Statistical theory and methodology", Science and Engineering, 2nd edn, Wiley, New York and London (1967).

[37] SNEATH, P.H.A., SOKAL, R.R., Numerical Tachonomy, W.H. Freeman, San Francisco (1973).

[38] PROKOPY, R.J., AVERILL, A.L., COOLEY, S.S., ROITBERG, C.A., KALLET, C., "Variation in host acceptance pattern in apple maggot flies", Insect–Plant Relationships (Proc. 5th Int. Symp. Wageningen, 1982), Centrum voor Landbouwpublikaties en Landbouwdokumentaties, Wageningen (PUDOC) (1982).

[39] JAENIKE, J., GRIMALDI, D., Genetic variability for host preference within and among populations of *D. tripunctata*, Evolution **37** (1983) 1023–1033.

[40] PROKOPY, R.J., McDONALD, P.T., WONG, T.T.Y., Inter-population variation among *Ceratitis capitata* flies in host acceptance pattern, Entomol. Exp. Appl. **35** (1984) 65–69.

[41] VALENTINE, J.W., "Genetic strategies of adaptation", Molecular Evolution (AYALA, F.J., Ed.), Sinauer, Sunderland, MA (1976).

[42] WHITTEN, C.J., Use of the isozyme technique to assess the quality of mass-reared sterile screwworm flies, Ann. Entomol. Soc. Am. **73** (1980) 7–10.

[43] HENDRICHS, J., ORTIZ, G., LIEDO, P., SCHWARZ, A., "Six years of successful medfly program in Mexico and Guatemala", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Athens, 1982), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1983) 353–365.

GENETIC VARIATION IN WILD MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), FROM KENYA*

T.K. MUKIAMA, J.K. MURAYA
Department of Botany,
University of Nairobi,
Nairobi, Kenya

Abstract

GENETIC VARIATION IN WILD MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), FROM KENYA.

The electrophoretic variation of eight enzymes — β-hydroxy acid dehydrogenase (β-HAD); 6-phosphogluconate dehydrogenase (6-PGD); phosphoglucomutase (PGM); aldehyde oxidase (AO); malate dehydrogenase (MDH); alcohol dehydrogenase (ADH); α-glycerophosphate dehydrogenase (α-GPDH); and glutamate oxaloacetate transaminase (GOT) — was studied in Kenyan strains of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). The medflies were collected from ripe coffee berries in eight localities in the country. The berries were kept in paper cartons lined with sawdust in the insectary, and pupae were routinely recovered over a period of 14 days. Adults emerged in cages within the next 14 days, upon which they were used for gel electrophoresis. The *Mdh* locus was monomorphic, while only two alleles per locus were detected at the *Ao*, *Adh*, *α-Gpdh* and *Got* loci. Four different alleles were identified at the *Pgm* locus, six at the *6-Pgd* locus, and seven at the *β-Had* locus. In addition, null *β-Had* and *6-Pgd* heterozygotes were present in specimens from Kabete and Machakos. The mean heterozygosities for the two adequately sampled localities, Kabete and Machakos, were 0.143 and 0.100 respectively. The allele frequencies at the various loci were in agreement with Hardy-Weinberg expectations, except the *β-Had* locus in the Kabete population, where a significant deviation was observed.

1. INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is recognized as a major pest of cultivated fruit crops in Central and South America, Europe, North Africa and the Middle East. Investigations on *C. capitata* in the East African region have mainly dealt with its association with coffee, but no significant adverse effects on the quality of the crop have been demonstrated by the work of Gibson [1], Abasa [2] and Waikwa [3].

* This work forms part of a Joint FAO/IAEA research programme on the development of genetic sexing mechanisms in *C. capitata* (Contract No. 3615/R3/RB).

Owing to the seasonal nature of many fruits, the medfly may be forced to shift from one host to another and vice versa. Moreover, its wide geographical distribution indicates a considerable degree of environmental plasticity. Implicitly, correspondingly wide genetic variation may be expected at the genome level.

The purpose of this study was to investigate the electrophoretic variation of eight enzymes, as an indication of genetic diversity in these wild populations. The enzymes were β-hydroxyacid dehydrogenase (β-HAD), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM), aldehyde oxidase (AO), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), α-glycerophosphate dehydrogenase (α-GPDH), and glutamate oxaloacetate transaminase (GOT). The study was initiated in 1984 and continued until 1988.

2. MATERIALS AND METHODS

The medflies used in this study originated from sampling sites in (a) Nairobi, Kiambu, Machakos, Mery, Embu, and Muranga districts, all within the central highlands east of the Rift Valley; (b) Taita Taveta district in the foothills of Mount Kilimanjaro; and (c) Kakamega and Bungoma districts in western Kenya. *Arabica* coffee is a major cash crop in all these agricultural districts except those in the western part of the country where *robusta* coffee is better suited.

Ripe coffee berries were picked and transferred to the laboratory, where they were kept in paper cartons lined with sawdust. These were inspected daily for the presence of pupae. This routine check was maintained for two weeks, or until the fruit deteriorated to the extent that it was unlikely to contain larvae. Pupae were transferred into cages, where adults emerged within 10–14 days. The imagos were then immediately used for electrophoresis.

β-HAD, 6-PGD and PGM were screened by horizontal starch gel electrophoresis using the basic techniques described by Ayala et al. [4]. AO, MDH, ADH, α-GPDH and GOT were run on a vertical polyacrylamide gel system described by Hames and Rickwood [5]. All enzymes were stained, using the procedures of Steiner and Joslyn [6].

Electromorphs separated in starch gels were scored by the method described by Mukiama [7], while those separated in polyacrylamide gels were scored according to the method of Ayala et al. [4].

3. RESULTS AND DISCUSSION

Tables I and II show the allele frequencies at the tested loci in different localities. Table III shows the heterozygosities per locus in the populations studied. Comprehensive data were obtained from Kabete and Machakos. Table IV shows the

TABLE I. ALLELE FREQUENCIES AT THE *β-Had*, *Pgm* AND *6-Pgd* LOCI IN WILD *C. capitata* FROM KENYA

		Locality		
		Kabete	Meru	Machakos
β-Had:	n	962	84	282
Allele:	1	0.809	0.821	0.933
	F	0.003	0.024	0.021
	F_2	0.099	0.107	0.021
	F_3	0.005	0	0
	S	0.007	0	0.007
	S_2	0.025	0.036	0.004
	S_3	0	0.012	0
	Null	0.026	0	0.007
	Others	0.026	0	0.007
Pgm:	n	830	26	362
Allele:	1	0.758	1.00	0.903
	F	0.128	0	0.044
	S	0.108	0	0.050
	S_2	0.006	0	0.003
6-Pgd:	n	430	56	470
Allele:	I	0.947	0.946	0.919
	F	0.012	0	0.015
	S	0.005	0.036	0.002
	S_2	0	0	0.004
	F_2	0.006	0.018	0.004
	F_3	0.002	0	0
	Null	0.014	0	0.028
	Others	0.014	0	0.028

TABLE II. ALLELE FREQUENCIES AT THE *Ao*, *Adh*, *α-Gpdh* AND *Got* LOCI IN WILD *C. capitata* FROM KENYA

		Locality						
		Kabete	Machakos	Embu	Muranga	Taita	Kakamega	Bungoma
Ao:	n	85	65	0	0	0	0	0
Allele:	100	0.998	0.989	—	—	—	—	—
	110	0.002	0.011	—	—	—	—	—
Adh:	n	65	64	—	—	—	8	6
Allele:	100	0.951	0.965	—	—	—	1.00	1.00
	110	0.049	0.035	—	—	—	0	0
α-Gpdh:	n	57	78	33	39	12	0	10
Allele:	100	0.969	0.959	1.00	1.00	1.00	—	1.00
	109	0.031	0.041	0	0	0	—	0
Got:	n	117	65	39	0	0	0	0
Allele:	100	0.995	0.965	0.917	—	—	—	—
	106	0.005	0.031	0.083	—	—	—	—

TABLE III. HETEROZYGOSITIES PER LOCUS AT THE LOCI STUDIED

Population	Locus							Mean
	β-Had	*Pgm*	*6-Pgd*	*Ao*	*Adh*	*α-Gpdh*	*Got*	
Kabete	0.332	0.396	0.103	0.004	0.093	0.060	0.010	0.143
Machakos	0.129	0.185	0.153	0.022	0.067	0.079	0.068	0.100
Embu	—	—	—	—	—	0	0.152	0.076
Muranga	—	—	—	—	—	0	—	0
Meru	0.313	—	0.104	—	—	—	—	0.208
Taita	—	—	—	—	—	0	—	0
Kakamega	—	—	—	—	0	—	—	0
Bungoma	—	—	—	—	0	0	—	0

TABLE IV. χ^2 VALUES OF GOODNESS OF FIT BETWEEN OBSERVED AND EXPECTED GENOTYPIC FREQUENCIES UNDER HARDY–WEINBERG PREDICTIONS (P = 0.05)

Locus	Locality	n	χ^2	DF[b]
β-Had	Kabete	481	31.69[a]	6
	Meru	42	0.97	4
	Machakos	141	2.91	5
Pgm	Kabete	415	7.03	3
	Meru	13	—	—
	Machakos	181	0.25	3
6-Pgd	Kabete	215	4.18	5
	Meru	28	0.31	2
	Machakos	235	7.71	5
α-Gpdh	Kabete	57	0.74	1
	Machakos	78	1.90	1
Got	Kabete	117	0.02	1
	Machakos	65	0.99	1
	Embu	39	1.98	1
Adh	Kabete	65	0.02	1
	Machakos	64	0.34	1
Ao	Kabete	85	1.28	1
	Machakos	65	0.02	1

[a] Significant deviations.
[b] DF = degrees of freedom.

statistical significance (P = 0.05) of observed deviations from those expected under the predictions of the Hardy–Weinberg equilibrium.

No variation was detected at the *Mdh* locus in all specimens tested. Two alleles each were identified at the *Ao, Adh, α-Gpdh* and *Got* loci. Without exception, the commonest of the two alleles was always present in very high frequency. Only this allele was identified in specimens from Muranga, Taita, Kakamega and Bungoma, but this is probably due to the small samples collected from these areas.

At least seven different alleles were identified at the *β-Had* locus. In addition, null heterozygotes were detected in up to 50 specimens from Kabete and four from Machakos.

At the *6-Pgd* locus, at least six different alleles were present. Null heterozygotes were similarly observed in 12 specimens from Kabete and 26 from Machakos.

Four different *Pgm* alleles were identified from the Machakos and Kabete populations. Only the commonest one, however, was observed in Meru.

In terms of conventional parameters of genetic variation, the mean heterozygosity, the proportion of polymorphic loci and the mean number of alleles per locus are 0.143, 0.375 and 3.000, respectively, for the Kabete population, and 0.100, 0.375 and 2.875, respectively, for the Machakos population. These figures clearly indicate that the two populations are very similar in terms of genetic variability. This may be expected because the two areas are quite similar in terms of geographical and climatic conditions and are less than 100 km apart.

Similar studies, more diversified over time and space, have been reported by a number of workers on the medfly [8–10]. In addition, these investigations have covered over 20 enzyme loci. The values of mean heterozygosity ($\bar{H}$) reported for samples of *C. capitata* from regions of Africa south of the Sahara were: Kenya, 0.164 [10]; South Africa, 0.177 [8]; and Réunion, 0.099 [8]. These findings compare favourably with our present results. The corresponding $\bar{H}$ values for localities in the Mediterranean area (except Sardinia) are considerably lower [8]. While it is not easy to account for these differences, it is most probable that ancestral populations (like those of Kenya and South Africa) have maintained a large size over time, thus retaining most of their genetic variability. On the other hand, introduced populations, like those of Réunion and most Mediterranean localities, may have lost their rare alleles owing to a combination of factors including founder effects, population bottlenecks and random genetic drift.

The only significant deviation under the assumptions of the Hardy–Weinberg equilibrium (Table IV) was at the *β-Had* locus in specimens from Kabete. This deviation offers an opportunity to examine that population further for evidence of gene flow, non-random mating, selection, and other factors which may tend to upset this theoretical expectation.

The observation of null heterozygotes may be difficult to ascertain at this stage owing to the lack of evidence from matings. It is also not clear whether these nulls originate from natural chromosome deletions or point mutations. However, calculations based on the results and theoretical considerations indicated a very high deficiency of null heterozygotes at the *β-Had* locus in the Kabete population. Similar deficiencies were also determined at both the *β-Had* and *6-Pgd* loci in both the Kabete and Machakos populations. No null homozygotes were observed. More investigations of these considerations are required.

4. CONCLUSIONS

A study of genetic variation at eight enzyme loci of *C. capitata* populations from two localities, Kabete and Machakos, has been completed. This survey was extended to cover six other localities in Kenya's coffee growing districts. Consideration of the conventional parameters showed this genetic variability to be among the highest reported so far for this species. With one exception, all the variant alleles scored were shown to be in Hardy–Weinberg equilibrium. Null heterozygotes were also detected at some loci. As a result of these findings, it is inferred that local strains of the medfly possess a very wide gene pool which may be exploited in future as a genetic resource for rare genes in any future genetic approaches to control.

REFERENCES

[1] GIBSON, A., Fruitfly damage in Kenya coffee and its possible effect on quality, Kenya Coff. **35** (1970) 260.

[2] ABASA, R.O., Observations on the seasonal emergence of fruitflies on a Kenya coffee estate and studies of the pest status of *Ceratitis capitata* Wied. in coffee, East African Agric. For. J. **37** (1973) 181.

[3] WAIKWA, J.W., Economic importance of *Ceratitis capitata* in Kenya, Kenya Coff. **44** (1979) 7.

[4] AYALA, F.J., POWELL, J.R., TRACEY, M.L., MOURAO, C.A., PERES-SALAS, S., Enzyme variability in *Drosophila willistoni* group — 4. Genic variation in natural populations of *Drosophila willistoni*, Genetics **70** (1972) 113.

[5] HAMES, B.D., RICKWOOD, D. (Eds), Gel Electrophoresis of Proteins: A Practical Approach, Oxford Univ. Press (1981) 290 pp.

[6] STEINER, W.W.M., JOSLYN, D.J., Electrophoresis techniques for the genetic study of mosquitoes, Mosq. News **39** (1979) 35.

[7] MUKIAMA, T.K., Genetic variation in wild *Anopheles arabiensis* Patton of Mwea Irrigation Scheme, Kenya, Insect Sci. Applic. **8** (1987) 245.

[8] KOURTI, A., LOUKAS, M., ECONOMOPOULOS, A., "Genetic studies in medfly populations", Report on Research Co-ordination Mtg on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Vienna, July 1985, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[9] MILANI, R., GASPERI, G., MALACRIDA, A., Recent progress and perspectives in the genetical studies on *Ceratitis capitata*, ibid.

[10] GASPERI, G., MALACRIDA, A.R., MILANI, R., "Protein variability and population genetics of *Ceratitis capitata*", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, E.P., Ed.), Elsevier, Amsterdam and New York (1987) 149–157.

SALIVARY GLAND POLYTENE CHROMOSOME MAPS IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.): CORRELATION BETWEEN LINKAGE GROUP AND CHROMOSOME*

A. ZACHAROPOULOU
Division of Genetics, Cell and Developmental Biology,
Department of Biology,
University of Patras,
Patras, Greece

Abstract

SALIVARY GLAND POLYTENE CHROMOSOME MAPS IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.): CORRELATION BETWEEN LINKAGE GROUP AND CHROMOSOME.

Salivary gland polytene chromosome maps of the five autosomes of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), are presented. Detailed mapping of the bands was achieved by dividing the entire polytene chromosome set into 100 sections, using prominent bands as markers. Each chromosome, irrespective of its size,was divided into 20 regions. Two loci, *dp* and *wp*, were mapped on the third and fifth chromosome respectively. The correlation was established between the polytene, the mitotic chromosomes and linkage groups in *C. capitata*. A comparison of polytene chromosomes from the salivary gland and male orbital bristle cells revealed interesting differences.

1. INTRODUCTION

The aim of the research reported here was to construct standard salivary gland chromosome maps in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), and to correlate the polytene chromosomes to mitotic elements and linkage groups. Such maps constitute the basis for genetic and cytogenetic studies in this important species. The mitotic karyotype of *C. capitata*, as has been described by several authors, consists of six pairs of chromosomes: one for the sex and five autosomes. The proposed nomenclature system [1] takes the sex chromosomes as the first pair and the autosomes as pairs 2 to 6 in descending order of their length.

In salivary gland nuclei, only five chromosomes are observed which correspond to the five autosomes. No banded sex chromosomes are found [2]. The same

* This work, which took one year from 15 Dec. 1987, forms part of a joint FAO/IAEA research programme on the development of genetic sexing mechanisms in *C. capitata* (Research Contract No. 4949/RB).

condition is true for the male orbital bristle cells [3, 4]. The orbital bristle polytene chromosomes and salivary gland chromosomes show significant differences in the banding pattern. The differences are so extreme that they seem to belong to two different species. This observation, together with the restriction of the orbital bristle to male pupae only, made it necessary to construct two reference maps, one for each polytene tissue.

In this report the detailed photographic maps of the salivary gland chromosomes in *C. capitata* are presented. Furthermore, the correlation between the mitotic chromosomes, linkage groups and polytene chromosomes is established by using several Y–autosome and autosome–autosome translocations. The differences in the banding pattern between the tissues in *C. capitata* are also discussed.

2. MATERIALS AND METHODS

2.1. Fly stocks

A mass reared population of *C. capitata*, with the standard gene arrangement, was used for the construction of polytene chromosomes. This population was maintained in our laboratory for more than twelve years. Several Y–autosome and autosome–autosome translocation strains were also used in order to find the homologies between the mitotic and polytene chromosomes. These strains are as follows:

Strain 127: The genotype is T(Y:dp^+/*dp*) males and *dp/dp* females. It breeds true for brown pupae males and dark pupae females [5].
Strain *wp23:* The genotype is T(Y:wp^+)/*wp* males and *wp/wp* females and breeds true for brown pupae males and white pupae females [6].
Strains 114 and 1: Both have a Y–2 translocation [7].
Strains 30/55 and 19: Both carry an autosome–autosome translocation [8].

2.2. Mitotic preparations

Air dried neuroblast preparations of third instar larvae, 5–6 days old, were used as described in Ref. [2]. The C banded mitotic chromosomes were prepared according to Bedo [9]. Mitotic preparations were observed with a Leitz Orthoplan microscope.

2.3. Polytene chromosome preparations

Salivary gland chromosome slides were prepared as described in Ref. [2]. The process is as follows: the salivary glands, after dissection in 45% glacial acetic acid, were first fixed in 3N HCL for about 5 min and then in lacto-acetic acid (1:2 lactic acid:60% acetic acid) for 5 min. They were then stained in lacto-acetic orcein for

20–30 min and finally squashed in lacto-acetic acid. The polytene chromosomes were examined under phase contrast.

2.4. Construction of composite polytene chromosomes

Well spread nuclei were selected and photographed. Pictures showing the best morphology for each region were selected and used to construct the chromosomes presented. The polytene genome was then divided into 100 sections and each polytene element was sectioned into 20 regions, taking into account the most prominent or distinctive bands. In each section, subsections were lettered using distinctive features wherever possible. Finally, the chromosomes were numbered from 2 to 6 according to their correlation to the mitotic chromosomes.

2.5. Correlation between the mitotic and polytene chromosomes

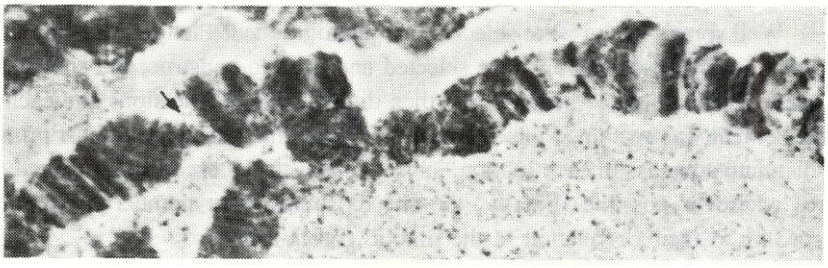
The examination of mitotic preparations in Y–autosome translocations revealed the autosome involved in the translocation, since the Y chromosome is darkly stained. In some instances C banded preparations were examined to confirm the translocation. Moreover, air dried mitotic preparations were examined derived from F_1 heterozygous larvae in autosome–autosome translocations.

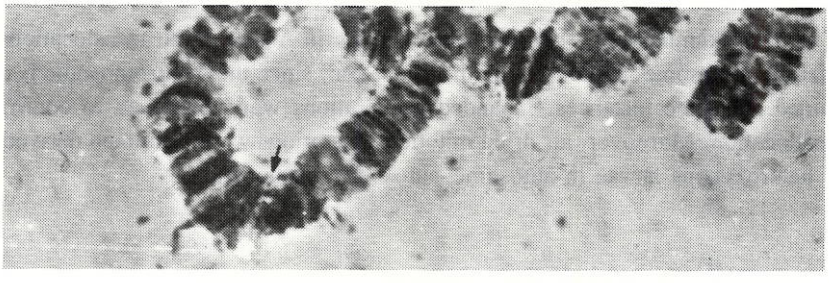
3. RESULTS AND DISCUSSION

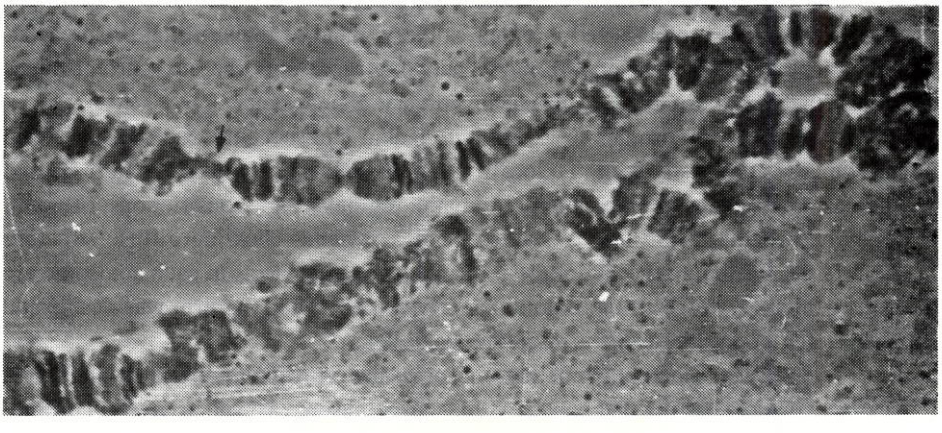
The polytenization in *C. capitata* is restricted to autosomes only. No banded sex chromosomes are observed in either salivary gland or male orbital bristle cells, while, in the latter, a heterochromatic network represents the sex chromosomes [2–4, 10]. Such a structure is absent in salivary gland nuclei.

Polytene chromosomes in *C. capitata* lack a chromocentre, so the site of the centromere is not easily seen. To localize the possible site of the centromere, I used some characteristics of the heterochromatic regions, e.g. constrictions or weak points and regions with heavily stained bands, or bands showing diffuse structure (Fig. 1). According to the site of the centromere, the two arms in each autosome are of unequal size, which fit the mitotic karyotype well. The long arm was designated as the left (L) and the short arm as the right (R).

The salivary gland polytene chromosomes are a difficult material owing to the extensive ectopic pairing that occurs within a chromosome as well as among chromosomes. Moreover, some chromosome regions show very poor banding, which adds to the difficulty of analysis. However, with the method proposed here, a sufficient number of well spread nuclei can be observed. In each polytene element, in addition to the tips, which are very characteristic, there are several distinct regions which constitute important diagnostic landmarks.







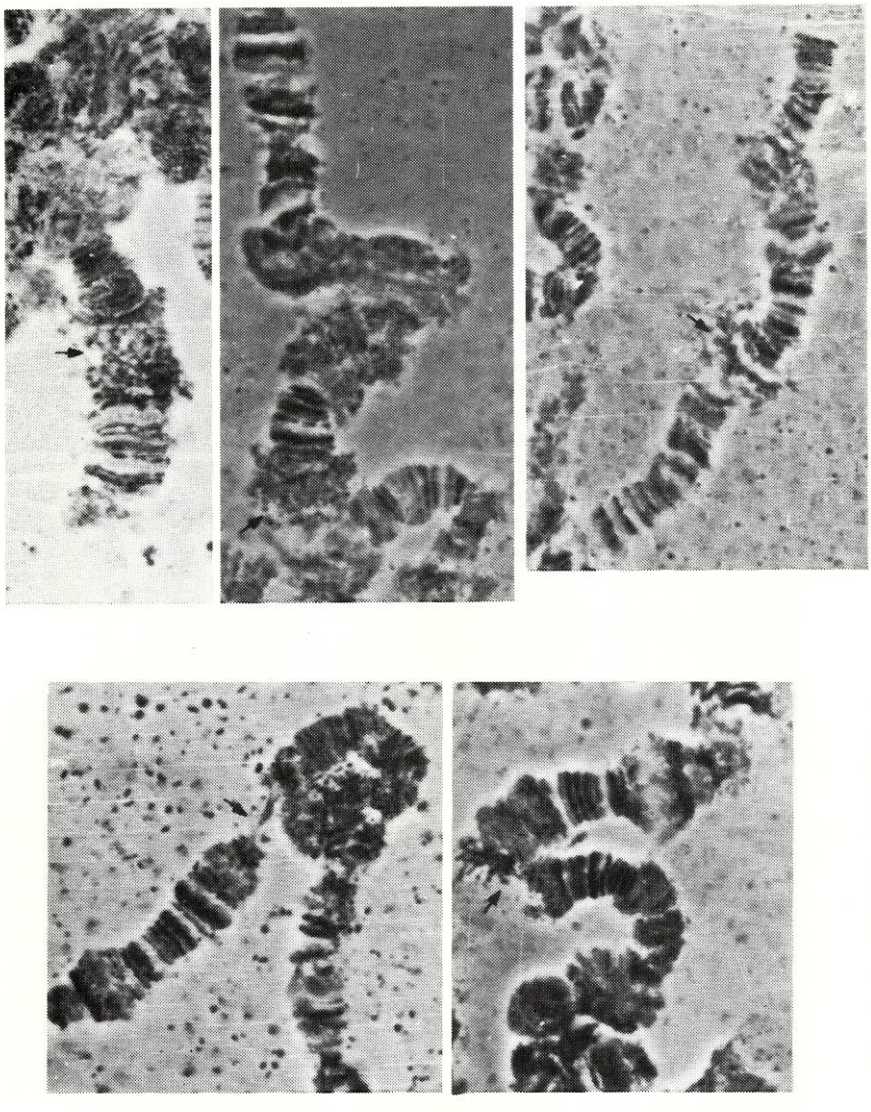


FIG. 1. Centromeric regions of the 5 salivary gland chromosomes in C. capitata. *Arrows indicate the site of centromere.*

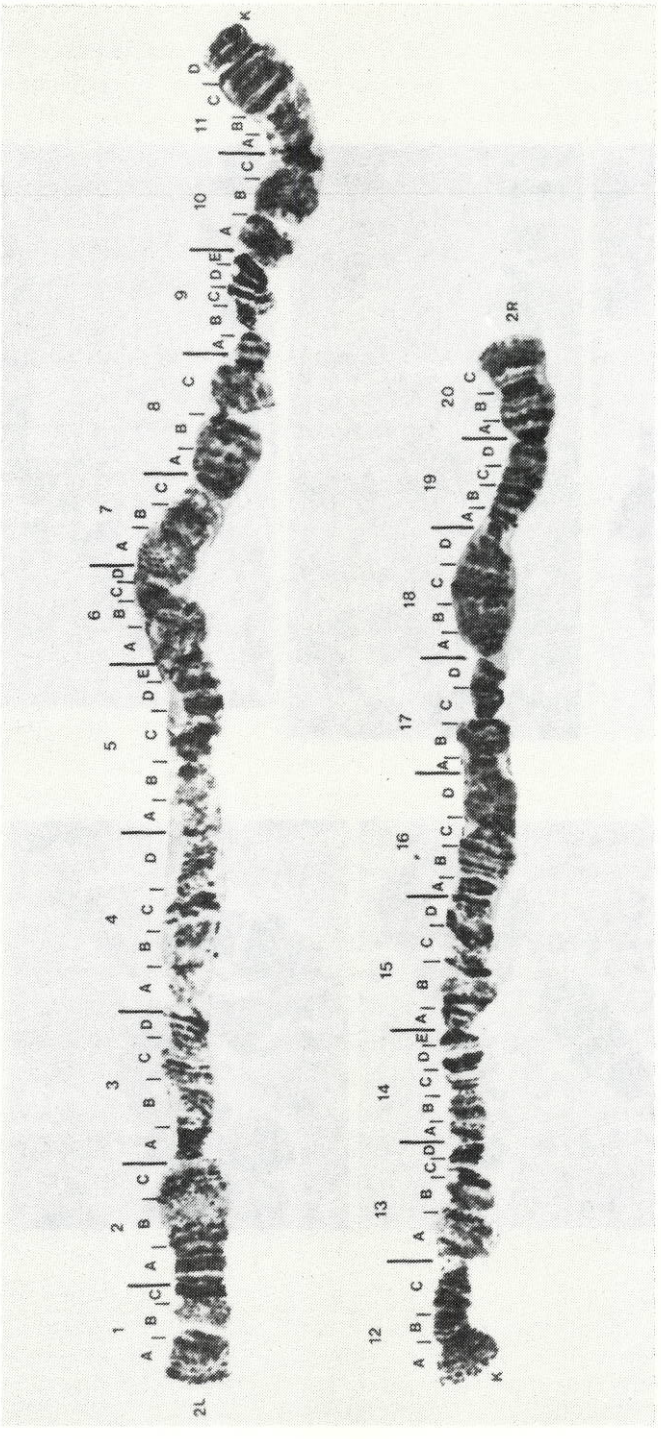


FIG. 2. Polytene chromosome 2, sections 1–20 (K = centromere).

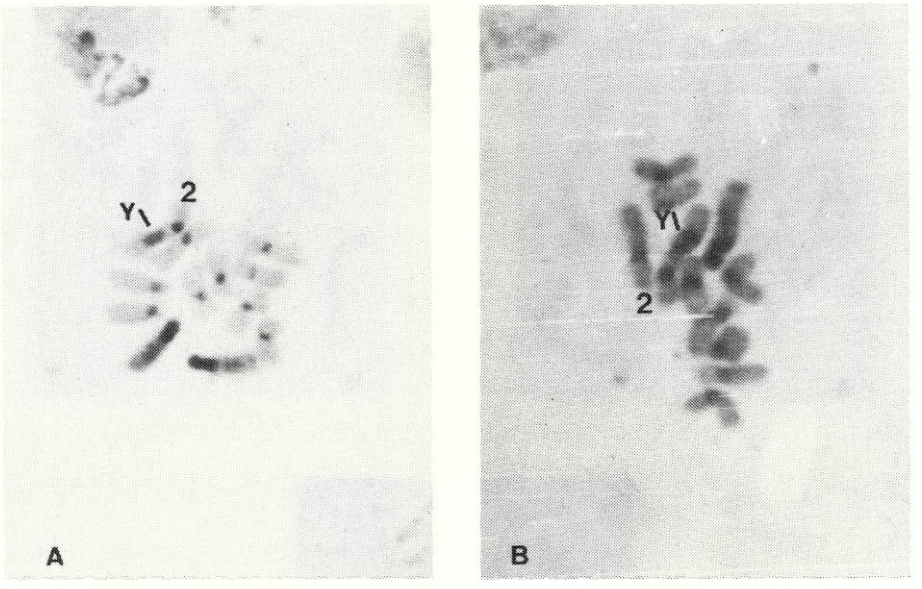


FIG. 3. C banded mitotic metaphases in male translocation lines: (A) strain 114, (B) strain 1.

3.1. Chromosome 2 (sections 1–20, Fig. 2)

This is the longest chromosome of the set. Characteristic of this element is the very poor banding appearance of the left arm, with the exception of sections 1–3, which makes it difficult to analyse. These difficulties are mainly due to the extensive ectopic pairing and a number of weak points that usually break during preparation of the slide. However, some regions that serve as diagnostic landmarks can be distinguished. The left free end is identified by its swollen tip 1A and the lightly stained region in 1B. The right arm has always a very good banding morphology with the more characteristic features, the usually fan-like region near the centromere 11B-C and the free end. The tip is fairly square or slightly triangular. A number of dark bands and constrictions in 20B, 20A and in the boundary of sections 19/20 and 19/18 are important diagnostic features for this arm.

The total length of this chromosome and the length of its arms leave little doubt that it corresponds to the chromosome 2 of the mitotic complement. C banded mitotic preparations in strains 114 and 1 (Fig. 3) showed that the Y chromosome was inserted in the long arm of chromosome 2 proximal to the centromere. Analysis of the polytene chromosomes of the same strains failed to identify the autosome bearing

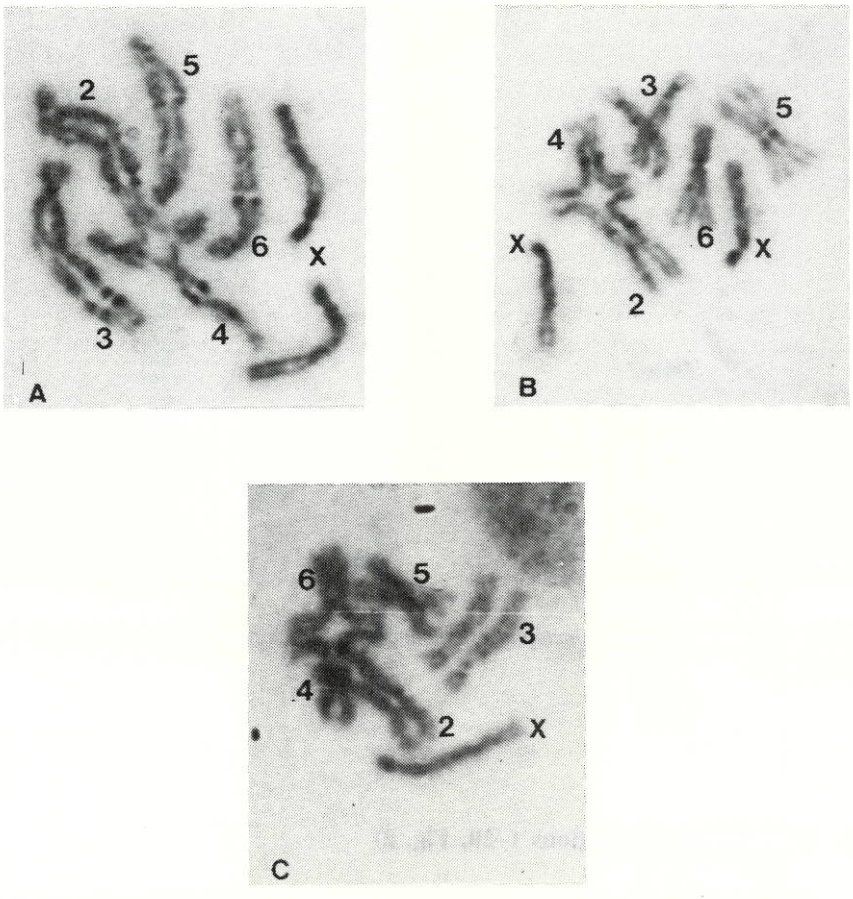


FIG. 4. (A) Mitotic prophase. (B) Early mitotic metaphase in heterozygous females from translocation strain 30/55, showing a chromosome 2–4 pairing cross. (C) Early mitotic metaphase in a male translocation heterozygote from strain 19 showing a chromosome 4–6 pairing cross.

the insertion. This may be due to the obscure morphology of the long arm in this chromosome. This was confirmed by analysis in the two translocation strains 30/55 and 19. Air dried mitotic preparations from heterozygous larvae are shown in Fig. 4. It is clear that both strains have chromosome 4 involved in the translocation. Moreover, the longest chromosome 2 has interchanged in the strain 30/55 with one of the smallest autosomes in the strain 19. Polytene chromosome analysis revealed that the chromosomes interchanged in both strains (Figs 5 and 6). The results establish that this polytene element corresponds to the longest chromosome 2.

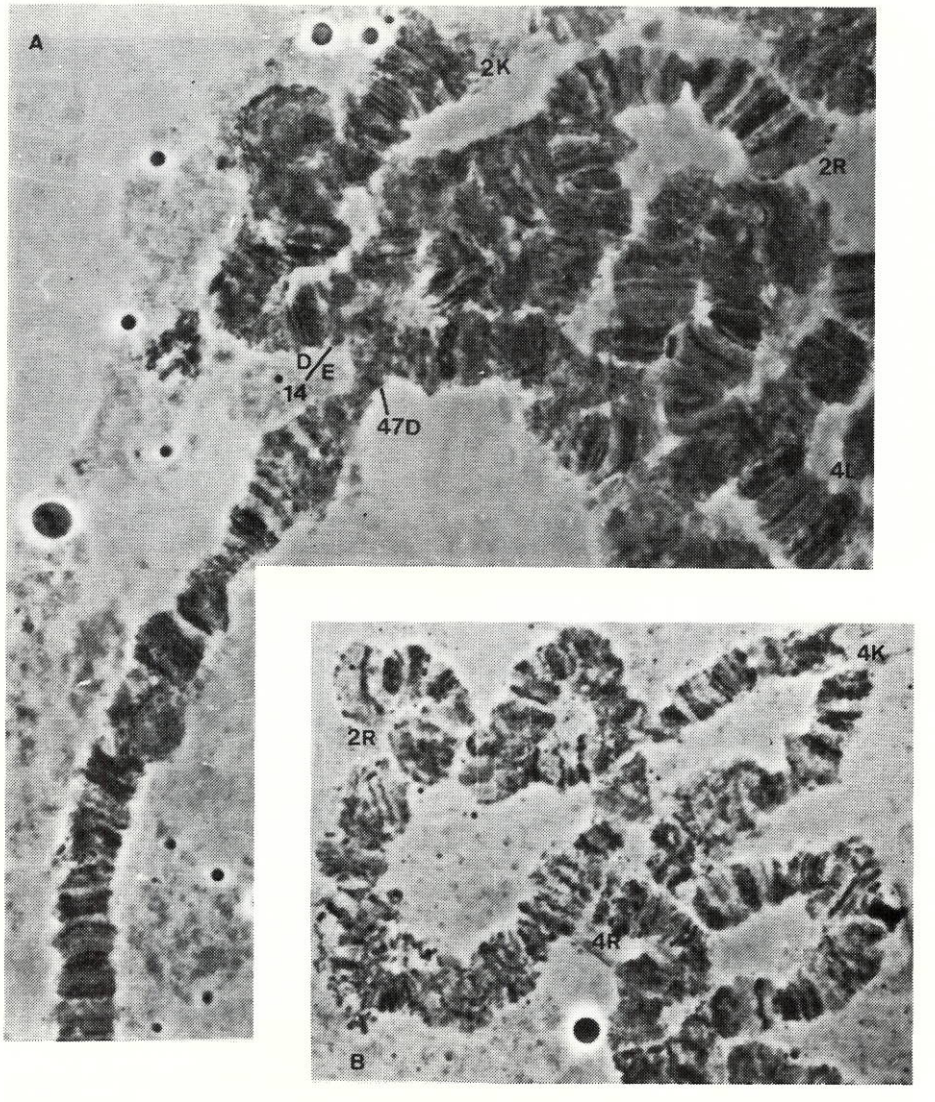


FIG. 5. Polytene chromosomes from translocation strain 30/55. (A) Heterozygote; (B) homozygote. Breakpoints in both chromosomes are indicated. 2K and 4K are centromeres.

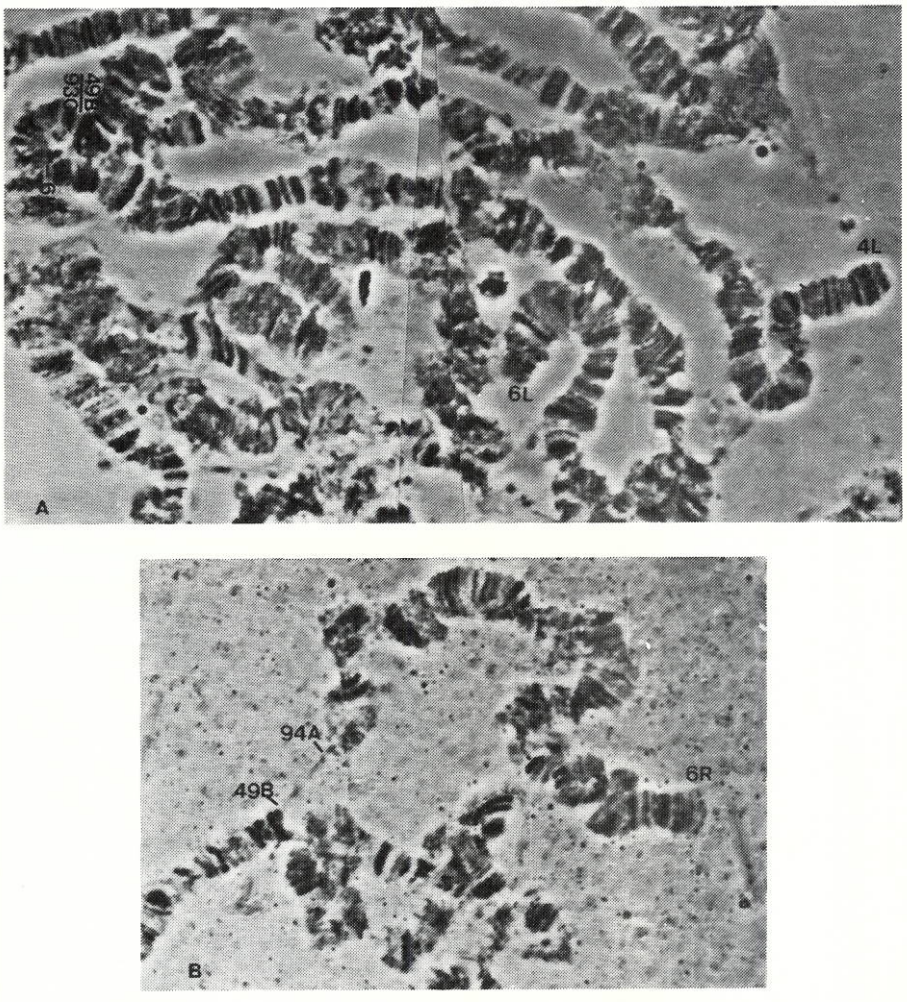


FIG. 6. Polytene chromosomes from translocation strain 19. (A) Homozygote; (B) heterozygote. Breakpoints in both chromosomes are indicated. 4K and 6K are centromeres.

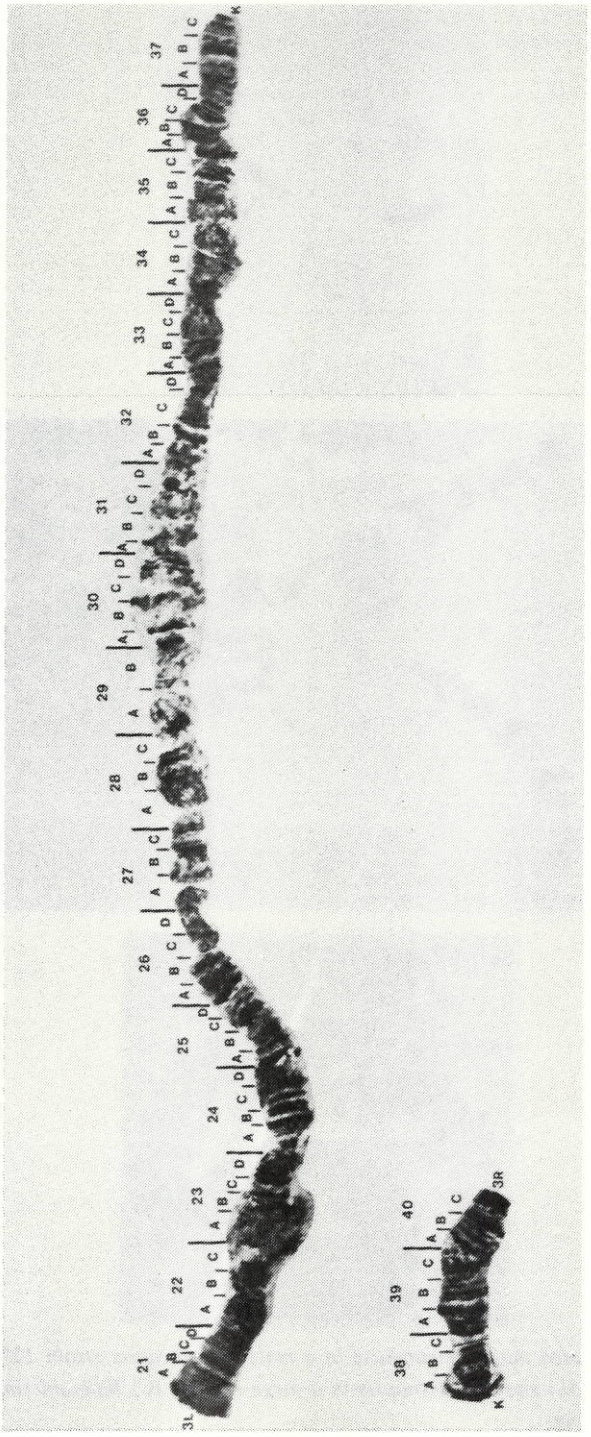


FIG. 7. Polytene chromosome 3 (sections 21–40) (K = centromere).

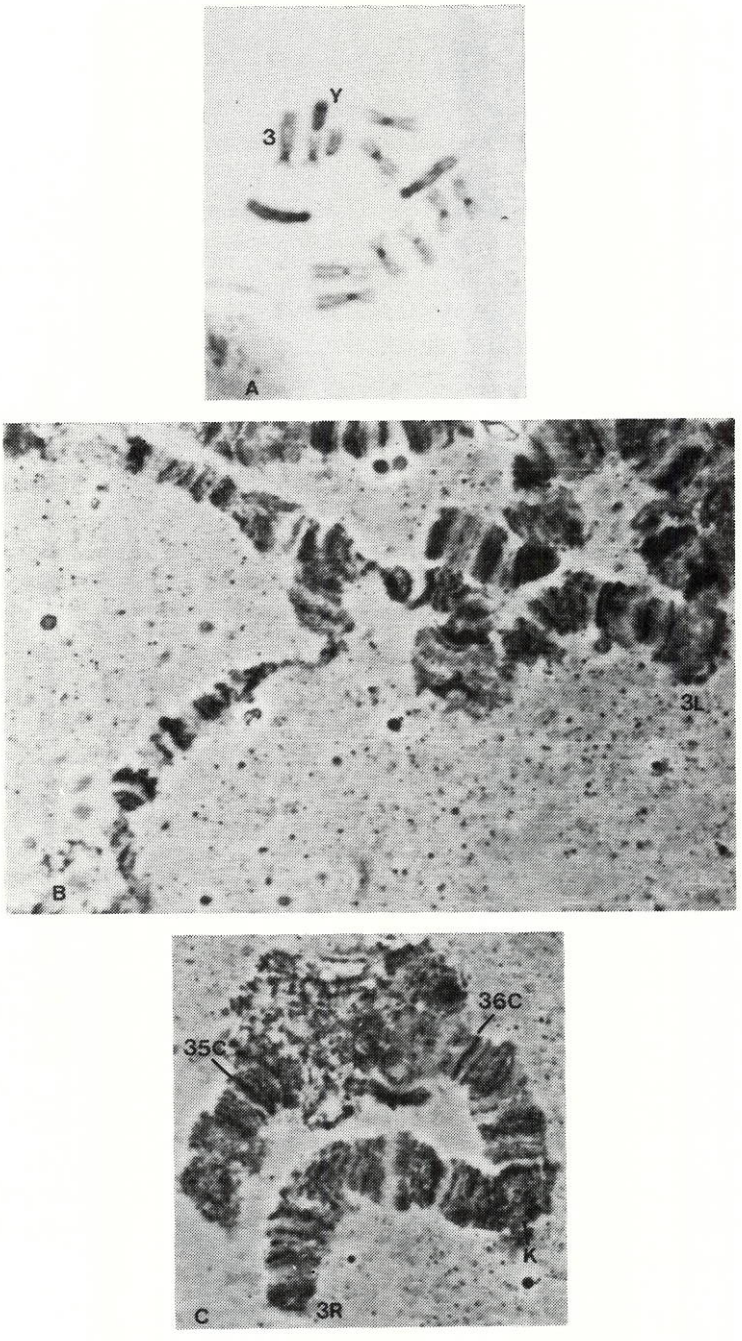


FIG. 8. (A) C banded mitotic metaphase in a male translocation strain 127. (B) Polytene chromosome arm (3L) showing asynapsis in a large region. (C) Balbiani rings in the same polytene chromosome.

The *Adh-1* locus has been localized on chromosome 2 [11]. This locus belongs to the same linkage group as the loci *Mpi, Est-6, Aox* and *Mdh-2*. Consequently this linkage group correlates with chromosome 2 and polytene element 1–20.

3.2. Chromosome 3 (sections 21–40, Fig. 7)

This polytene element is homologous to the third 'acrocentric' chromosome of the mitotic complement, as was revealed by the analysis of Y–autosome translocation in strain 127. Figure 8 shows the C banded mitotic chromosome as well as the polytene element that is very often observed in such an asynapsed condition. Chromosome 3 is easily recognized by the unique region 30–31,which always contains a pair of dark bands in 30A, and two successive expanded regions 30B-C and 31B-C. Some chromosome regions usually have a poor banding appearance, like sections 26E-29C and 33B-35B, making them somehow difficult to analyse. The 3L free end is characterized by its semi-fan shape, 21A, and two dark bands in 21B. Although a detailed study during development has not been made, some alternative patterns have been found; the most striking is the region 35C-36C, where two large puffs, showing a typical form of Balbiani rings, were observed (Fig. 8C).

The right arm has a good banding appearance with a number of puffs and dark bands along it. The 3R tip is recognizable by its squarish shape, consisting of a number of very close thin bands.

By using the Y–3 translocation in strain 127 it was possible to map the *dp* locus on chromosome 3. Thus, the C linkage group, as labelled by Saul and Rössler [12], corresponds to the third chromosome and mitotic element 21–40.

3.3. Chromosome 4 (sections 41–60, Fig. 9)

This element is the most distinct of the complement and is easily recognized by a number of characteristic features which act as diagnostic landmarks. As mentioned in Section 3.1 above (Figs 5 and 6), this element is well correlated to chromosome 4 of the mitotic karyotype since it is the translocated chromosome in both strains 30/55 and 19. The two tips are easily recognized. The end of 4L is identified by the dark band 41A, two thin bands 41B, and the heavily stained band in section 41D. The right free end is characterized by its fairly square shape with a number of thin bands and two bundles of bands in 60B and 60A. Two morphological genes, the *ap* and *dc*, have been mapped on chromosome 4 [8]. Thus the linkage group A [12] corresponds to the polytene element 41-60.

3.4. Chromosome 5 (sections 61–80, Fig. 10)

This chromosome corresponds to one of the two smallest autosomes 5 or 6. Air dried and C banded mitotic preparations in strain *wp23* (Fig. 11) showed that

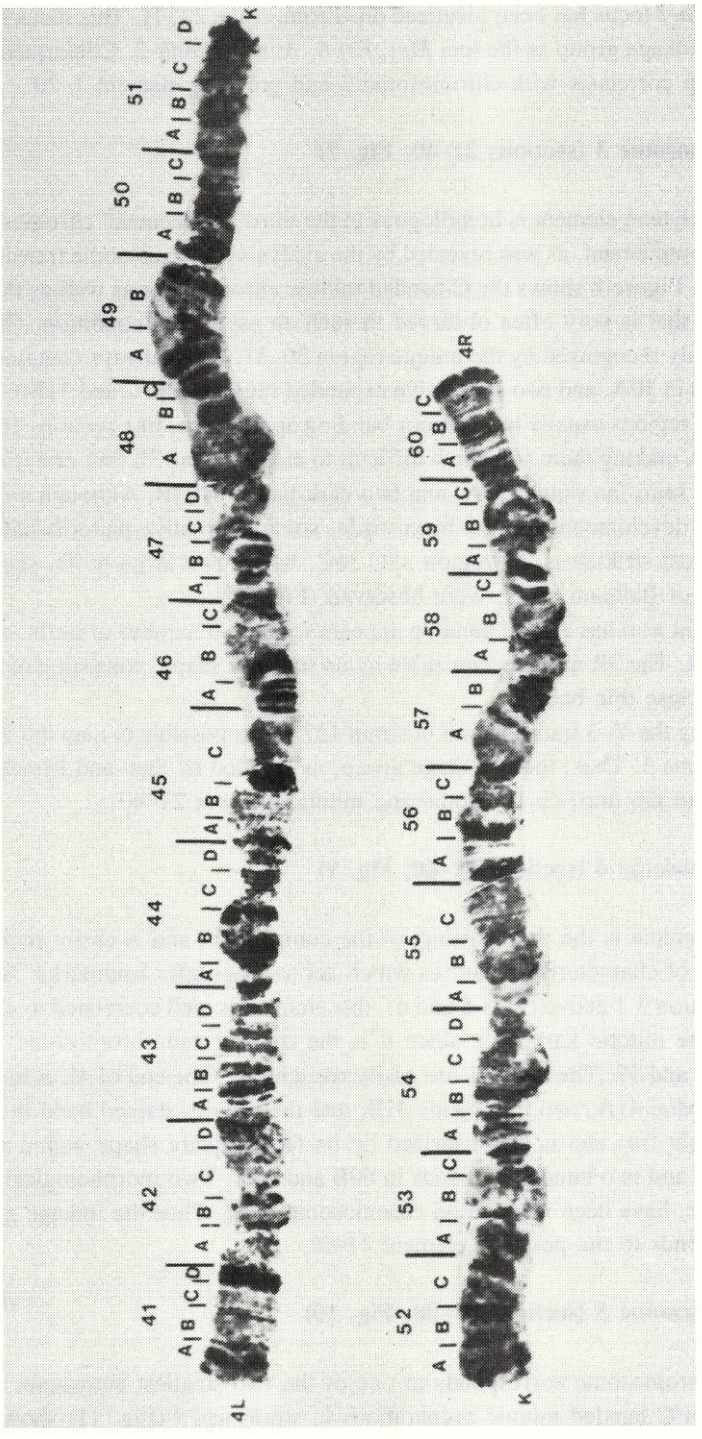


FIG. 9. Chromosome 4, sections 41–60 (K = centromere).

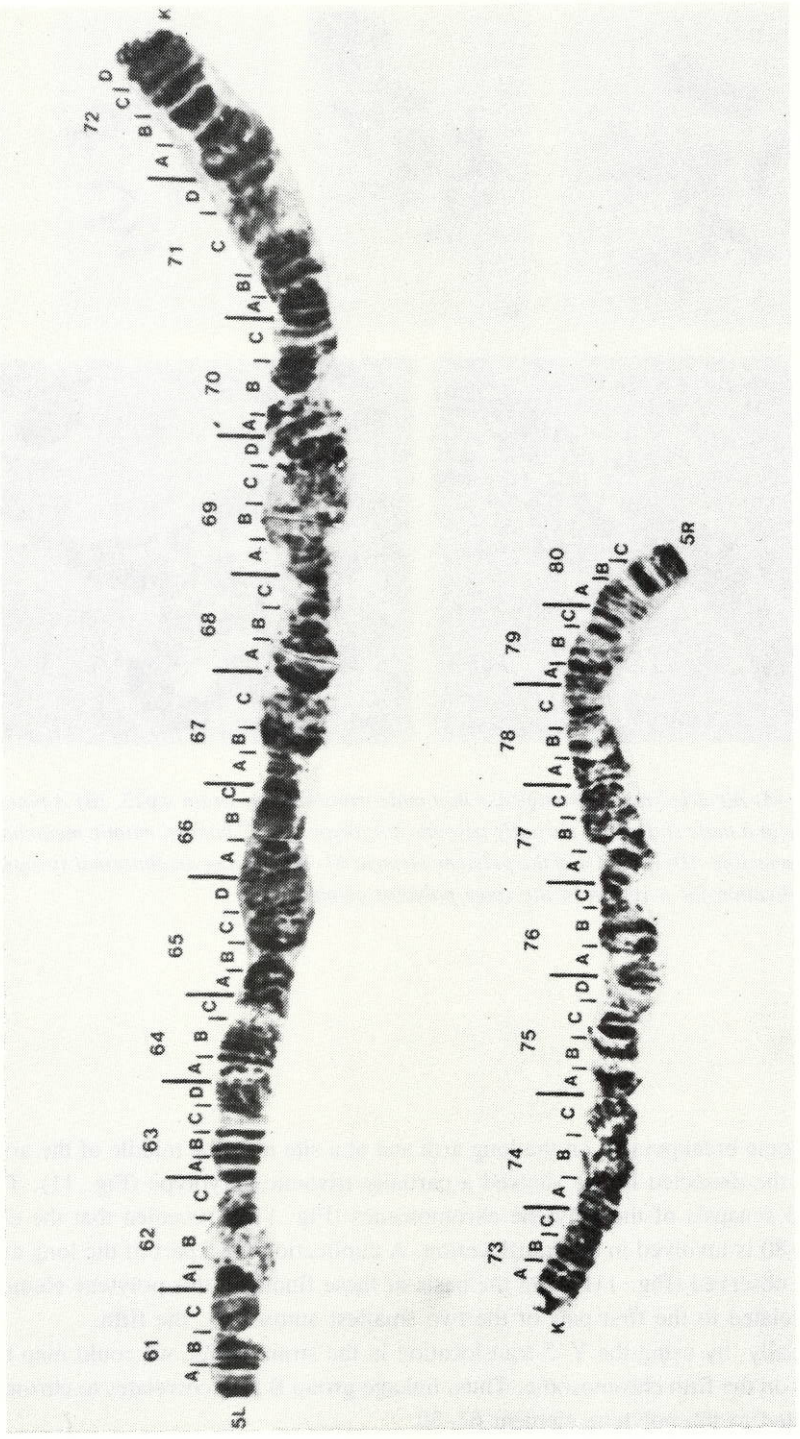


FIG. 10. Chromosome 5, sections 61–80 (K = centromere).

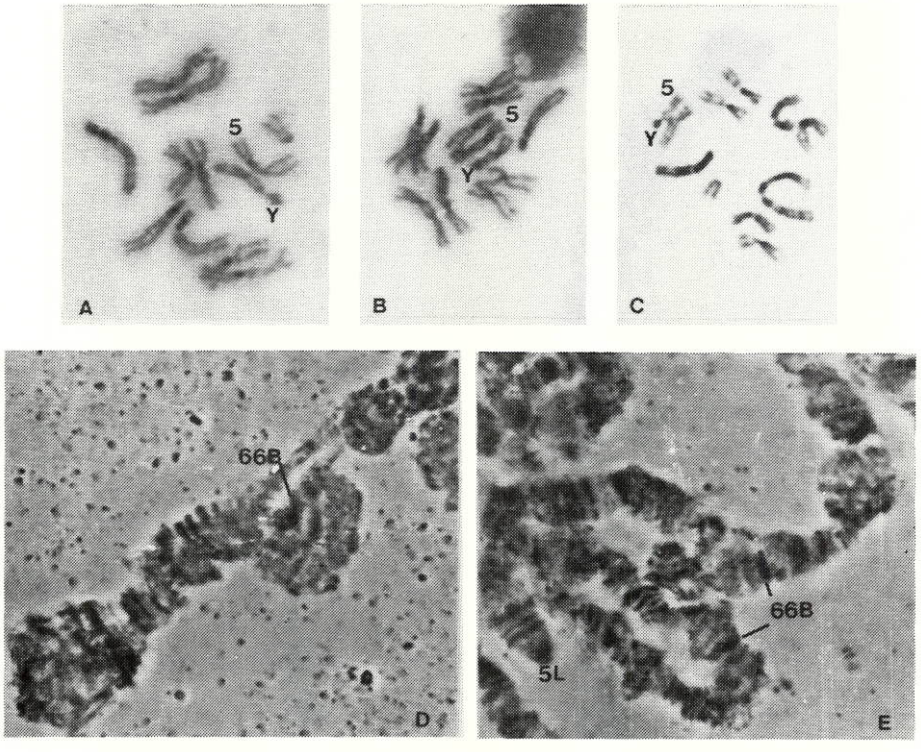


FIG. 11. (A) Air dried mitotic metaphase in a male translocation strain wp23. *(B) A mitotic metaphase in a male showing a partially trisomic karyotype. (C) C banded mitotic metaphase from the same line. (D) A region of the polytene element 61–80 showing an abnormal synapsis. (E) A duplication for a region in the same polytene element.*

the autosome breakpoint is on the long arm and at a site near the middle of the arm. Some of the dissected larvae showed a partially trisomic karyotype (Fig. 11). The abnormal synapsis of the polytene chromosomes (Fig. 11D) revealed that the element 61–80 is involved in the translocation. A duplication for a part of the long arm was also observed (Fig. 11E). On the basis of these findings, this polytene element was correlated to the first pair of the two smallest autosomes, the fifth.

Finally, by using the Y–5 translocation in the strain *wp23*, we could map the *wp* locus on the fifth chromosome. Thus, linkage group B [11] correlates to chromosome 5 and to the polytene element 61–80.

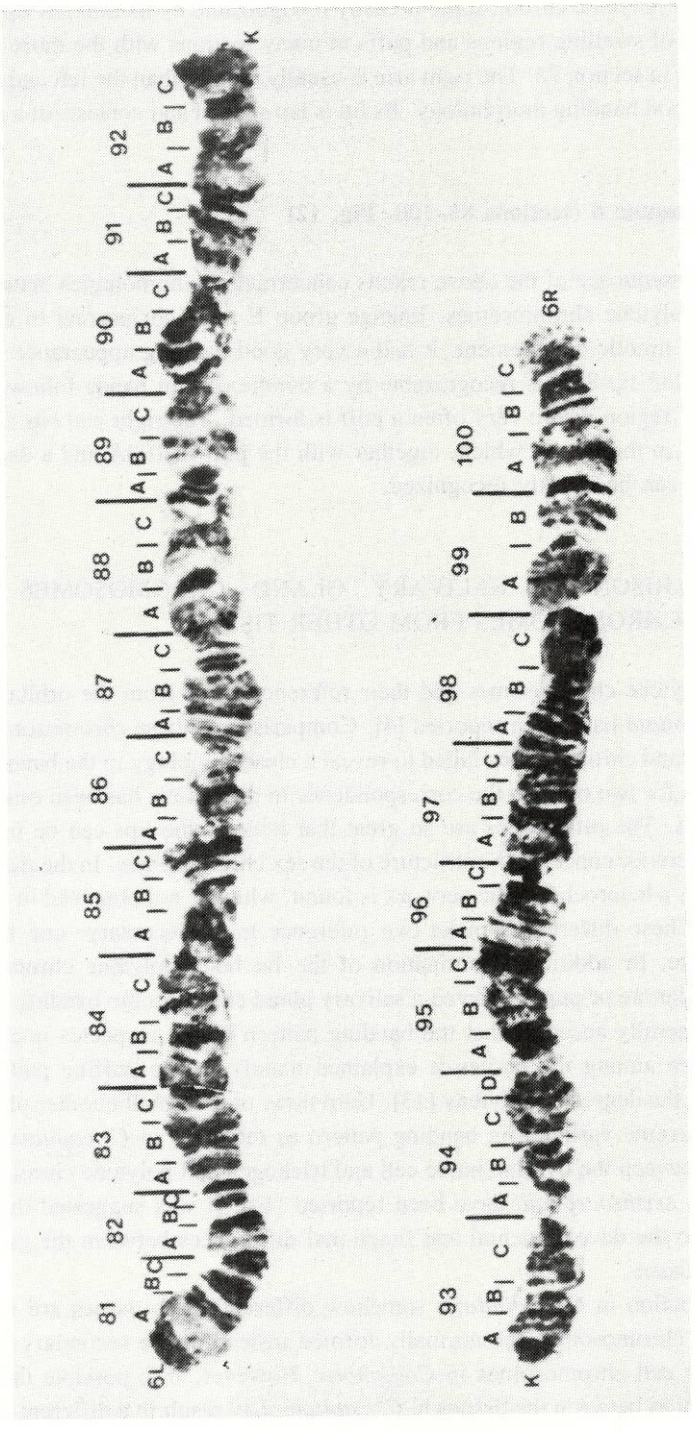


FIG. 12. Polytene chromosome 6, sections 81–100.

The fifth polytene chromosome is easily recognizable by its thin left tip in 61A and a number of swelling regions and puffs in many sections with the more characteristic feature in section 72. The right arm is usually thinner than the left and characterized by a good banding morphology. Its tip is tap shaped and consists of a number of thin bands.

3.5. Chromosome 6 (sections 81–100, Fig. 12)

As a consequence of the above results concerning the homologies between the mitotic and polytene chromosomes, linkage group E must correspond to chromosome 6 of the mitotic complement. It had a very good banding appearance in most regions. The left tip 81A is recognizable by a bundle of thin bands followed by a lightly stained region where very often a puff is formed. The right end has a unique fan like shape in the tip, by which, together with the puff in 100A and a dark band close to it, it can be quickly recognized.

4. COMPARISON OF SALIVARY GLAND CHROMOSOMES WITH POLYTENE CHROMOSOMES FROM OTHER TISSUES

The polytene chromosomes and their reference maps from the orbital bristle cells in *C. capitata* have been reported [4]. Comparison of these chromosomes with the salivary gland chromosomes failed to reveal a clear homology in the banding pattern, although for two of them the correspondence to the mitotic has been established in both tissues. The differences are so great that none of the tips can be matched. A second difference concerns the structure of the sex chromosomes. In the male orbital bristle cells a heterochromatic network is found, which is not observed in salivary gland cells. These differences make two reference maps necessary: one for each polytene tissue. In addition, examination of the fat body polytene chromosomes derived from larvae or pupae showed a salivary gland chromosome banding pattern.

It is generally accepted that the banding pattern within a species is constant. Some variation among the tissues is explained mainly by the puffing pattern and differences in the degree of polyteny [13]. Until now, only a small number of reports show such extreme variation in banding pattern as there are in *C. capitata.* Large differences between the ovarian nurse cell and trichogen cell polytene chromosomes in *Calliphora erythrocephala* have been reported [14]. It was suggested that these may be due to the developmental and functional differences between the germ line and somatic tissue.

The situation in *C. capitata* is somehow different. Both tissues are somatic, and polytene chromosomes are normally formed instead of the secondary induced ovarian nurse cell chromosomes in *Calliphora.* However, it is possible that functional differences between the tissues in *C. capitata* may result in a different banding

pattern. This suggestion is in agreement with the model proposed by Zhimulev and Belyaeva [15].

5. CONCLUSIONS

Salivary gland chromosomes of *C. capitata* provide good material for cytogenetic and genetic studies in this very important species. Although their quality is not as good as in orbital bristle cells, the study of these chromosomes is important, especially in the light of results presented here. Combination of the two polytene systems provides a wide ranging polytene chromosome analysis. These studies may prove very useful in the genetic analysis of this species and may also lead to better understanding of the underlying mechanisms of the organization and development of polytene chromosomes.

ACKNOWLEDGEMENTS

I should like to thank Drs. Y. Rössler, A. Robinson, M. Riva and E. Busch-Petersen for valuable help in supplying *Ceratitis* strains. Thanks are also due to A. Papatheou for technical assistance and to Drs. M. Ashburner and C. Christodoulou for useful discussions. The FAO/IAEA Research Contract is gratefully acknowledged.

REFERENCES

[1] ZAPATER, M., ROBINSON, A.S., Sex chromosome aneuploidy in a male linked translocation in *Ceratitis capitata*, Can. J. Genet. Cytol. **281** (1986) 161–167.

[2] ZACHAROPOULOU, A., Cytogenetic analysis of mitotic and salivary gland chromosomes in the medfly, *Ceratitis capitata*, Genome **29** (2987) 67–71.

[3] BEDO, D.G., Polytene and mitotic chromosome analysis in *Ceratitis capitata* (Diptera: Tephritidae), Can. J. Genet. Cytol. **28** (1986) 180–188.

[4] BEDO, C.G., Polytene chromosome mapping in *Ceratitis capitata* (Diptera: Tephritidae), Genome **29** (1987) 598–611.

[5] RÖSSLER, Y., Automated sexing of *Ceratitis capitata:* the development of strains with inherited sex limited pupal colour dimorphism, Entomophaga **24** (1979) 11–416.

[6] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*, a suitable case for genetic sexing, Genetica **58** (1982) 224–237.

[7] ROBINSON, A.S., RIVA, M.E., ZAPATER, M., Genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata*, using the alcohol dehydrogenase locus, Theor. Appl. Genet. **72** (1986) 455–457.

[8] BUSCH-PETERSEN, E., SOUTHERN, D.I., Induced suppression of genetic recombination in females of the Mediterranean fruit fly, *Ceratitis capitata*, by translocation heterozygosity, Genetica **72** (1987) 161–169.

[9] BEDO, D.G., C, Q, and H-banding in the analysis of Y chromosome rearrangements in *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae), Chromosoma **77** (1980) 299–308.

[10] BEDO, D.G., ZACHAROPOULOU, A., Inter-tissue variability of the polytene chromosome banding pattern, Trends Genet. **4** (1988) 90–91.

[11] MALACRIDA, A.R., GASPERI, G., MILANI, R., "Genome organization of *Ceratitis capitata:* linkage groups and evidence for sex ratio distorters", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 169–176.

[12] SAUL, S.H., RÖSSLER, Y., Genetic markers of the autosomal linkage groups of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Ann. Entom. Soc. Am. **77** (1984) 323–327.

[13] BEERMAN, W., " Chromosomes and genes", Results and Problems in Cell Differentiation, Vol. 4, Springer Verlag, Berlin (1972) 1–33.

[14] RIBBERT, D., Chromomeres and puffing in experimentally induced polytene chromosome in *Calliphora erythrocephala*, Chromosoma **74** (1979) 269–289.

[15] ZHIMULEV, I.F., BELYAEVA, E.S., Proposals to the problem of structural and functional organization of polytene chromosome, Theor. Appl. Genet. **45** (1975) 335–340.

POLYTENE CHROMOSOME ANALYSIS IN RELATION TO GENETIC SEX SEPARATION IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)*

P. KERREMANS, E. BUSCH-PETERSEN
FAO/IAEA Entomology Unit,
IAEA Seibersdorf Laboratory,
Vienna

Abstract

POLYTENE CHROMOSOME ANALYSIS IN RELATION TO GENETIC SEX SEPARATION IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

The development of stable genetic sexing strains in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is hampered by the presence of low levels of male recombination. Such recombination may be reduced by minimizing the distance between the translocation breakpoint and the translocated 'sexing' allele. Cytogenetic analysis of mitotic/meiotic and polytene chromosomes could provide information on the selection of such potentially stable genetic sexing strains. Translocation breakpoints in two genetic sexing strains in the medfly, based on a white female/brown male pupal colour dimorphism, have been determined. Preliminary results are described and the advantages and limitations of polytene chromosome analysis for the isolation of stable genetic sexing strains of the medfly are discussed.

1. INTRODUCTION

The efficiency of the sterile insect technique (SIT) may be improved by eliminating the female insects prior to the release of the sterile males [1–3]. Such sexual separation has important advantages in SIT programmes against the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) [4]. First, by eliminating female neonate larvae only half the amount of larval diet would be required, leading to a large reduction in the cost of larval rearing. Secondly, the number of matings between released males and wild females has been found to increase by 50–100% in field cages [5], thus making the released males more effective. Thirdly, the oviposition punctures normally caused by sterile females are eliminated. Owing to bacterial and fungal infection of such punctures, this problem is commonly so severe that the SIT approach to medfly control will not be considered for use in some countries unless females can be eliminated prior to the release of the sterile males [2].

---

* This work forms part of a joint FAO/IAEA research programme on the development of genetic sexing mechanisms in *C. capitata*.

Three types of workable genetic sexing mechanisms are at present available for the medfly. The first such mechanism, developed by Rössler [6], is based on a 'dark pupae' mutation. This strain produces brown (wild type) male pupae and black female pupae. The second mechanism, also based on a pupal colour dimorphism, was developed by Robinson and van Heemert [7] and by Busch-Petersen et al. [8]. These strains both utilize the pupal colour mutation 'white pupae' *(wp)*, and result in the production of white female and brown male pupae. The last mechanism is based on purine sensitivity [9] and, while the pupal colour strains allow only separation in the pupal stage, this mechanism enables females to be eliminated in the earlier larval stage.

The most suitable type of genetic sexing mechanism, from both the economic and the mass rearing points of view, would be one that enables females to be separated early in development through exposure to environmental stress, such as, for example, temperature or alcohol. The development of a temperature system is being attempted now [10]. Induction of recessive temperature sensitive lethal *(tsl)* mutations is carried out with ethyl methanesulphonate (EMS) [11]. Subsequently, the effectively dominant allele of this gene will be translocated onto the male-determining Y chromosome [4]. These two steps are being performed in reverse order by Manso et al. [12], who proposed a screening method involving first the isolation of a translocation and then the induction of a *tsl* mutation.

2. STABILITY OF Y-AUTOSOME TRANSLOCATIONS

Genetic sexing strains are constructed by means of translocating the effectively dominant genetic sexing allele onto the Y chromosome. However, subsequent male recombination in the chromosomal region between the translocation breakpoint and the genetic sexing allele will return this allele to the autosome, thus inevitably leading to the breakdown of the genetic sexing strain. Male recombination exists at very low levels in higher Diptera, including the medfly [13,14]. Rössler [14] found that, while the two linked mutations, 'white pupae' and 'orange-red', segregated almost independently in females, crossing over occurred at a level of up to 1.05% in males with a normal chromosome complement. As male recombination frequency in Y translocated strains is generally related to the chromosomal distance between the breakpoint and the translocated allele, the occurrence of such recombination may be minimized by keeping this distance as small as possible.

Recombination is commonly reduced around translocation breakpoints owing to disturbed pairing between homologous chromosomes. However, increases in male recombination frequency have also been observed in medfly strains containing Y-autosome translocations [13]. The recombination frequency between the mutations 'apricot eye' and 'double chaetae' is 18.25% in females [15]. However, whereas only 0.14% recombination could be detected between these genes in medfly

males carrying a normal chromosome complement, a recombination frequency of 1.98% to 4.0% was observed in males which contained a Y translocation not involving the *ap-dc* chromosome [13]. The presence of a Y–autosome translocation thus appears to induce crossing over in males, at least in these particular strains, even if the translocation involves another member of the chromosome complement. Such increase in male recombination has also been found in the housefly, *Musca domestica* [16], and in the sheep blowfly, *Lucilia cuprina* [17].

Not only translocations may influence male recombination frequency. Such recombination is also found in wild type strains of *Drosophila* [18]. Most observations in *D. melanogaster* are related to the phenomenon of hybrid dysgenesis, which involves the action of mobile genetic elements [19,20]. In the sheep blowfly, flies were found which had a structurally normal configuration, thus leading to the conclusion that translocation reversal had played a role [17].

Theoretically, a low level of male recombination is not in itself a major problem in maintaining the stability of genetic sexing strains. Simulation studies by Hooper et al. [21] and by Rössler [22] showed that a recombination value of 0.05% would result in about 2% aberrant females after 50 generations. The problem is, however, aggravated by the differential viability of recombinant and non-recombinant flies, as the carrier of the effectively dominant allele may commonly be expected to have a selective advantage over the recessive mutant allele. Once the former allele is transferred by the recombinant event to the autosome, the mutant allele will be eliminated from the population at a rate depending upon the differential genetic load of the two alleles. Unless male recombination can be fully eliminated, mutant genes which show high viability values should therefore be chosen.

Male recombination may be further reduced or eliminated by the use of chromosomal inversions. In *Anopheles albimanus* a single inversion eliminated recombination in a genetic sexing strain based on propoxur resistance [23,24], whereas in *An. quadrimaculatus* recombination was suppressed by three small paracentric inversions [25].

3. SELECTION OF POTENTIALLY STABLE GENETIC SEXING STRAINS

To assess the stability of genetic sexing strains, it is often necessary to place them under mass rearing conditions. Recombination in such strains is generally very low and commonly undetectable in laboratory populations of less than 1000 flies. The medfly genetic sexing strain, T:Y(wp^+)101 [26], showed no sign of instability after approximately 36 generations in the laboratory [21]. However, after only two generations of mass rearing, more than 5% aberrant females were found [21,27]. Similar observations were made with a genetic sexing strain of the sheep blowfly [17].

Screening, by means of mass rearing each potential genetic sexing strain, is a cumbersome and lengthy procedure, commonly taking several months and requiring a substantial investment in money and manpower. Moreover, the appearance of aberrant females in the colony may not always provide conclusive data on the mechanism of breakdown [21,27]. A potentially more rapid way to assess the probable stability of genetic sexing strains is to determine directly the distance between the translocation breakpoint and the genetic sexing gene through cytogenetic analysis of the polytene chromosome complement. The polytene chromosomes of the medfly have recently been described by Bedo [28], who analysed the orbital bristle chromosomes, and by Zacharopoulou [29], who analysed the salivary gland chromosomes. The salivary gland chromosomes show high levels of ectopic pairing with many constrictions and weak points in the chromosomes [29]. Attempts to separate and spread these chromosomes is therefore rather difficult. In addition, the sex chromosomes appear to be totally absent [29]. The polytene chromosomes of the trichogen cells spread more easily, although these chromosomes still show some tendency to fragment at certain weak points. The main advantage is, however, that the sex chromosomes are present in these cells, albeit as heterochromatic bodies with a nucleolus, thus facilitating the analysis of Y linked translocations. The major disadvantage of using trichogen cell chromosomes is that only two trichogen cells are present in each male, while females contain none. Several preparations are therefore necessary before complete, well spread chromosomes are found.

4. ANALYSIS OF TWO GENETIC SEXING STRAINS IN THE MEDFLY

The stability of two genetic sexing strains, each based on a white/brown pupal colour dimorphism, had previously been analysed under mass rearing conditions [21,27]. In each strain the dominant brown allele of the *wp* gene had been translocated onto the Y chromosome to produce white female and brown male puparia. Strain T:Y(wp^+)101 was isolated from a backcross of irradiated wild type males with homozygous *wp* females [26], while T:Y(wp^+)30C was derived from a line in which female recombination was suppressed [8]. T:Y(wp^+)101 had twice proved to be unstable under mass rearing conditions and contained after five generations between 28% [21] and 41% [27] females among brown pupae. The second strain, T:Y(wp^+)30C, was more stable and contained 0.6% and 6.9% brown female puparia after five and ten generations, respectively. The breakdown of T:Y(wp^+)101 is probably the consequence of recombination [21,27], whereas the pattern of breakdown of T:Y(wp^+)30C suggested contamination with wild type flies [27]. T:Y(wp^+)30C is now being used in field experiments to determine its suitability for release in medfly SIT programmes.

We analysed the polytene chromosomes of the above two genetic sexing strains. As previously mentioned, the analysis of Y–autosome translocations is

particularly difficult because the X and Y chromosomes do not polytenize. The Y chromosome in these preparations consists of a large heterochromatic body together with the nucleolus [30]. In Y–autosome translocations this nucleolus is often closely associated with the translocated autosome, thus indicating the position of the breakpoint. The linkage between the Y chromosome and the autosome, however, is very loose and often breaks during manipulation of the cells. Thus, in many preparations the position of the Y chromosome may not be determined at all. Despite these difficulties it was possible to preliminarily assign the chromosomal breakpoints in T:Y(wp^+)101 and T:Y(wp^+)30C to sections 52a-b and 52b, respectively, on chromosome 5, through the association between the nucleolus of the Y chromosome and the relevant polytene chromosome bands. However, this still requires confirmation.

Bedo [30] first described the polytene chromosomes in the medfly and numbered them according to their length. According to this numbering sequence the autosome involved in the Y linked translocations analysed here spans the bands 44a to 62e. It has subsequently become known that this chromosome contains the *wp* gene and that, using the nomenclature of the medfly linkage group and the meiotic, mitotic and salivary gland polytene chromosomes, it should therefore be labelled as chromosome 5 [31]. Bedo [30] allocated the centromere of this chromosome to sections 55c/d. If this position is indeed correct, it would place the translocation breakpoint in T:Y(wp^+)30C about one third of the length of this chromosome arm from the centromere. However, it is not known whether *wp* is physically linked to the Y chromosome in either of the two strains.

A correlation between the stability of genetic sexing strains and the distance between the translocation breakpoint and genetic sexing locus may be made only if the cytological position of both factors is known. Thus, the next step will be the localization of the 'white pupae' locus. This information will be obtained by the study of chromosomal deletions, which may be detected in polytene chromosomes where one or more bands are missing by the formation of loops in the chromosomes of the heterozygous karyotype. In addition, the varying methods of numbering the trichogen cell chromosomes indicate an urgent need to correlate the trichogen cell with the salivary gland chromosomes. This is now being attempted through hybridization studies of existing medfly clones to both types of polytene chromosomes.

ACKNOWLEDGEMENTS

We wish to thank Dr. D.I. Southern of the Department of Cell and Structural Biology, School of Biological Sciences, University of Manchester, United Kingdom, for training P. Kerremans. We thank Dr. A. Zacharopoulou, Department of Biology, University of Patras, Greece, for her assistance in determining the autosomal breakpoint of T:Y(wp^+)30C. The technical assistance of E. Schorn, A. Hafner and I. Szabo is gratefully acknowledged.

REFERENCES

[1] Report on Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Vienna, Mar. 1983, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[2] Report on Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Vienna, July 1985, obtainable from the Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[3] Report on Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Colymbari, Crete, Sep. 1986, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[4] BUSCH-PETERSEN, E., "Genetic sex separation in sterile insect technique pest management programmes, with special reference to the medfly, *Ceratitis capitata* (Wied.)", Fruit Flies of Economic Importance (CEC/IOBC Int. Symp. Rome, 1987), (CAVALLORO, R., Ed.), Balkema, Rotterdam (in press).

[5] ROBINSON, A.S., CIRIO, U., HOOPER, G.H.S., CAPPARELLA, M., Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, *Ceratitis capitata*, Entomol. Exp. Appl. **41** (1986) 231–235.

[6] RÖSSLER, Y., Automated sexing of *Ceratitis capitata* (Diptera: Tephritidae): the development of strains with inherited, sex-linked pupal color-dimorphism, Entomophaga **24** (1979) 411–416.

[7] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*: a suitable case for genetic sexing, Genetica **58** (1982) 23–24.

[8] BUSCH-PETERSEN, E., RIPFEL, J., PYREK, A., KAFU, A., "Isolation and mass rearing of a pupal genetic sexing strain of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)", Modern Methods of Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 211-219.

[9] SAUL, S.H., Genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae): Conditional lethal translocations that preferentially eliminate females, Ann. Entomol. Soc. Am. **77** (1984) 280–283.

[10] BUSCH-PETERSEN, E., "Temperature sensitive lethal factors and puparial colour sex separation mechanisms in the Mediterranean fruit fly *Ceratitis capitata* (Wied.)", these Proceedings.

[11] BUSCH-PETERSEN, E., PYREK, A., RIPFEL, J., RUHM, E., Efficiency of different methods of EMS application for the induction of dominant lethals in germ cells of medfly, *Ceratitis capitata* (Wied.), males, Mutat. Res. **163** (1986) 247–254.

[12] MANSO, F.C., CLADERA, J.L., LIFSCHITZ, E., "Screening for a female-limited temperature-sensitive lethal mutation induced on a Y-autosome translocated strain in *Ceratitis capitata*", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 219–225.

[13] RÖSSLER, Y., Genetic recombination in males of the Mediterranean fruit fly, and its relation to automated sexing methods, Ann. Entomol. Soc. Am. **75** (1982) 28–34.

[14] RÖSSLER, Y., Recombination in males and females of the Mediterranean fruit fly (Diptera: Tephritidae) with and without chromosomal aberrations, Ann. Entomol. Soc. Am. **75** (1982) 619–622.

[15] BUSCH-PETERSEN, E., SOUTHERN, D.I., Induced suppression of genetic recombination in females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), by translocation heterozygosity, Genetica **72** (1987) 161–169.

[16] LESTER, D.S., CROZIER, R.H., SHIPP, E., Recombination in the male housefly, *Musca domestica*, Experientia **35** (1978) 175–176.

[17] FOSTER, G.G., MADDERN, R.H., MILLS, A.T., Genetic instability in mass-rearing colonies of a sex-linked translocation strain of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) during a field trial of genetical control, Theor. Appl. Genet. **58** (1980) 169–175.

[18] HIRAIZUMI, Y., Spontaneous recombination in *Drosophila melanogaster* males, Proc. Natl. Acad. Sci. U.S.A. **68** (1971) 268–270.

[19] BREGLIANO, J.-C., KIDWELL, M.G., "Hybrid dysgenesis determinants", Mobile Genetic Elements (SHAPIRO, J.C., Ed.), Academic Press, New York (1983) 363–404.

[20] KIDWELL, M.G., KIDWELL, J.F., SVED, J.A., Hybrid dysgenesis in *Drosophila melanogaster*: A syndrome of aberrant traits including mutation, sterility and male recombination, Genetics **86** (1971) 813–833.

[21] HOOPER, G.H.S., ROBINSON, A.S., MARCHAND, R.P., "Behaviour of a genetic sexing strain of Mediterranean fruit fly, *Ceratitis capitata*, during large scale rearing", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 349–362.

[22] RÖSSLER, Y., 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, Ann. Entomol. Soc. Am. **78** (1985) 265–270.

[23] KAISER, P.E., SEAWRIGHT, J.A., DAME, D.A., JOSLYN, D.J., Cytology of a genetic sexing system for *Anopheles albimanus*, Can. J. Genet. Cytol. **21** (1979) 201–211.

[24] KAISER, P.E., SEAWRIGHT, J.A., JOSLYN, D.J., Development of a genetic sexing system for *Anopheles albimanus*, J. Econ. Entomol. **71** (1978) 766–771.

[25] SEAWRIGHT, J.A., KIM, S.S., NARANG, S.K., "Current research relevant to genetic sexing at the Insects Affecting Man and Animals Laboratory", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 203–208.

[26] ROBINSON, A.S., Unexpected segregation ratios from male-linked translocations in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Genetica **62** (1984) 209–215.

[27] BUSCH-PETERSEN, E., KAFU, A., Stability of two mass reared genetic sexing strains of *Ceratitis capitata* (Diptera: Tephritidae) based on pupal color dimorphisms, Environ. Entomol. **18** (1989) 315–322.

[28] BEDO, D.G., Polytene and mitotic chromosome analysis in *Ceratitis capitata* (Diptera: Tephritidae), Can. J. Genet. Cytol. **28** (1986) 180–188.

[29] ZACHAROPOULOU, A., Cytogenetic analysis of mitotic and salivary gland chromosomes in the Medfly, *Ceratitis capitata*, Genome **28** (1987) 67–71.

[30] BEDO, D., The orbital bristle polytene chromosomes of *Ceratitis capitata* (Diptera: Tephritidae), Genome **29** (1987) 598–611.

[31] ZACHAROPOULOU, A., "Salivary gland polytene chromosome maps in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann): Correlation between linkage group and chromosome", these Proceedings.

GENETICS OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), AS A TOOL IN THE STERILE INSECT TECHNIQUE*

Y. RÖSSLER, H. ROSENTHAL
Citrus Agrotechnical Service,
"Israel Cohen" Institute for Biological Control,
Rehovot, Israel

Abstract

GENETICS OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), AS A TOOL IN THE STERILE INSECT TECHNIQUE.

The report covers a period of five years of studies on the genetics of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.), and genetic sexing. Fourteen morphological mutants were isolated during that period, including *gr*, *ru*, *ro*, *Sr*, *Sp*, *ew*, *br*, *sb* and six yet unstudied mutants. Additional data were accumulated on genetic recombination between the various marked loci in males and females, and genetic maps were constructed. Recombination in males was found to be rather common in the medfly and not associated with the presence of chromosomal aberrations or with a particular chromosome. It seemed, however, that the dominant mutants that have been studied had a higher frequency of recombination in males, which almost matched the recombination levels encountered in the females. Initial steps towards the construction of genetic sexing strains were conducted. Selection for resistance to certain chemicals (potassium sorbate, Avermectin and Cyromazine) was carried out with limited success. Lines with high immunity to the three chemicals were established, and the mode of inheritance to Cyromazine and potassium sorbate was studied. Indications were that Cyromazine resistance was recessive and governed by a single gene whereas potassium sorbate resistance seemed to be a quantitative trait.

1. INTRODUCTION

During the years 1984–1988 we isolated and studied a number of morphological mutants and established multiple morphological markers for almost all the autosomes of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). We also continued and concluded our studies on genetic recombination in males and found it to be common and not related to the presence of chromosomal aberrations. We attempted to develop genetic sexing strains through selection for resistance to certain chemicals, and aimed to incorporate the resistant traits through translocations

* This research was supported by IAEA Research Grant No. 2797/RB and US-Israel BARD Research Grant No. US-816-84.

onto the male's Y chromosome. These attempts were not concluded successfully, although we were able to develop strains with resistance to potassium sorbate, Avermectin^{R} and Cyromazine^{R}.[1]

2. MORPHOLOGICAL MUTANTS

By 1984 we had 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 [1]. The mutants were later assigned to chromosomes in co-operation with laboratories in Patras (Greece) and Pavia (Italy). Chromosomes follow the numbering by Radu et al. [2].

During the period 1984–1988 we isolated 14 more morphological mutants. Eight were studied and some were reported on in publications. The others are still under initial investigation (Table I). The established mutant strains (*gr*, *Sr*, *ro*, *Sp*, *ru*, *br*, *sb*, *ew*) are described in this paper; the others are only named.

2.1. Eye shape and colour mutants

(a) Garnet eye colour (*gr*)

Adults with garnet eye colour were found in Jan. 1984 in a laboratory strain of the medfly. The colour matches Plate 11, E8, in the Methuen Handbook of Colour [3]. The trait is autosomal, linked to *bo* (linkage group E) and recessive. The trait was described by Rössler and Rosenthal in 1988 [5].

(b) Ruby eye colour (*ru*)

A single female with ruby coloured eyes was found in Mar. 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 colour mutants. The name 'ruby' is therefore temporary since 'reflectionless', which seems more appropriate, is already allocated to a mutant found by Carante in France [8]. It is possible that these two mutants are identical. Ruby is autosomal, recessive and in linkage group B (chromosome 5).

[1] Avermectin is a streptomycete derived macrocyclic lactone produced by Merck, Sharp & Dome Ltd. Cyromazine is an s-triazine compound, 2-cyclopropylamino-4,6-diamino-5-triazine, produced by CIBA-GEIGY.

TABLE I. MORPHOLOGICAL MUTANTS OF THE MEDFLY IN THE BIOLOGICAL CONTROL LABORATORY, REHOVOT, ISRAEL

Symbol	Name	Mode	Linkage group	Chromosome	Ref.
	Group A. Studied and published				
ap	Apricot eye	r	A	4	[4]
B	Bar eye	D	A	4	[5]
dc	Double SFO[a] bristle	r	A	4	[4]
or	Orange eye	r	B	5	[6]
wp	White pupa	r	B	5	[7]
Cy	Curly wings	D	B	5	[5]
dp	Dark pupa	r	C	3	[4]
bo	Brown orange eye	r	E		[5]
gr	Garnet eye	r	E		[5]
	Group B. Studied and not yet published				
Sp	Spotty abdomen	D	A	4	
Sr	Sergeant	D	A	4	
h	Harpoon SFO[a] bristle	r	B	5	
ro	Rough eye	r	B	5	
ru	Ruby eye	r	B	5	
ew	Eroded wings	r	C	3	
	Group C. Isolated — still under study				
br	Branched SFO[a] bristle	r			
sb	Stout bristles on thorax and pleura	r			
	Bulgy eyes				

[a] SFO: superior fronto-orbital bristle (males' 'cera').

(c) Rough eye (*ro*)

Three females with rough eyes were found in Nov. 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 owing to very low egg hatch. Egg hatch of sib-mating 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 wild type females, resulting in 87.4% egg hatch. This problem disappeared eventually and the line is maintained in our laboratory with satisfactory egg hatch and reproduction.

2.2. Abdominal mutants

(a) Sergeant (*Sr*)

Two females, with three intersegmental dorso-lateral grey lines on the abdomen instead of the normal two, were found in Nov. 1986 in the laboratory strain. The trait is autosomal, dominant and in linkage group A (chromosome 4). Wild type flies are still being produced by 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 carried out in June 1987 which produced 26 *Sr* and one wild type progeny. The F_2 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.

(b) 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 with 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.

2.3. Various mutants

(a) Eroded wings (*ew*)

A single male with eroded wings was found in Sep. 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. The trait is autosomal, recessive and in linkage group C (chromosome 3).

(b) Branched (*br*)

A single male with a spear like appendage on the stalk of the male's superior fronto-orbital (SFO) bristle was found in Dec. 1987 in the laboratory strain. The trait seems to be autosomal and recessive. The line is, however, not pure yet, and yielded 50% wild type flies in Mar. 1988.

(c) Stout bristles (*sb*)

Medfly adults with short, 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 females' ovaries and the males had viable and motile sperm. Reproduction became normal eventually, and a true breeding culture was obtained in Apr. 1988. The trait seems to be autosomal and recessive and is still being studied.

Additional 'variants' were found during the project period (and are still at a very early stage of rearing and studies). These were: male *dc* with normal (non-*sk*) eyes; bulgy eyes; 'wide-bar' eyes and an additional 'sergeant' mutant.

3. RECOMBINATION IN MALES AND FEMALES

The existence of genetic recombination (crossing over) between loci in males of the medfly was first suspected when genetic sexing lines involving a translocation between pupal colour loci and the male's Y chromosome 'deteriorated', producing both wild type and mutant males and females [9]. The occurrence of genetic recombination in males of that species was subsequently studied and substantiated (Table II) [5, 6, 10–12]. Four questions are addressed here:

(1) Is there any relation between recombination levels in males and females?
(2) Are high recombination levels in males related to certain chromosomes or to particular loci?
(3) Does the heterozygous configuration (repulsion or coupling) affect the level of genetic recombination (in males and females)?
(4) Do chromosomal aberrations enhance genetic recombination in males [13]?

During the study period we accumulated data on the genetic recombination in males and females in 23 pairs of loci. A linear regression analysis showed the lack of dependence between the males' and females' recombination levels ($R^2 = 0.03$,

TABLE II. RECOMBINATION IN MEDFLY MALES AND FEMALES

Loci pairs	Recombination %		
	Males	Females	Ratio females:males
Chromosome 3			
ew–dp	0.14	21.57	155
Chromosome 4			
ap–B	0	0.53	
ap–sk/dc	0.96	18.80	19.85
ap–Sr	0	45.10	
ap–Sp	0	40.16	
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
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
Chromosome 5			
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
Chromosome 6			
bo–gr	3.80	11.10	2.92

X coefficient (slope) = 0.117, SE (standard error) = 0.141, Y intercept = 0.696). We also noted that most of the loci in our study had rather high recombination levels in females. In the 23 pairs studied the average recombination levels in females was 32.51% ± 15.97.

Only two chromosomes with multiple marker (chromosomes 4 and 5) were available for comparison studies. Chromosome 5 seemed to possess relatively more loci with recombination in the male gender (in 5 out of 7 loci as compared to 7 out of 13 loci in chromosome 4). A regression analysis (between male and female recombination levels) on each of the two chromosomes resulted in the following data:

- Chromosome 4: R^2 = 0.062; X coefficient = 0.184 and Y intercept = –0.606.
- Chromosome 5: R^2 = 0.211; X coefficient = –0.242 and Y intercept = 13.727.
- The slope (X coefficient) in chromosome 5 is negative, suggesting a negative relation between the female and male recombination levels.

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 three out of four dominant loci (with *Sp, lt, B*), recombination in males almost matched the females.

TABLE III. EFFECT OF HETEROZYGOTE CONFIGURATION (COUPLING/REPULSION) AND PRESENCE OF CHROMOSOMAL TRANSLOCATION ON THE RECOMBINATION LEVEL IN MEDFLY MALES AND FEMALES

Genotype	Loci pair	Recombination %	
		Males	Females
ap dc$^+$*/ap*$^+$ *dc*	*ap dc*	0.96	21.60
ap$^+$ *dc*$^+$*/ap dc*	*ap dc*	0.14	18.80
wp$^+$ *or/wp or*$^+$	*wp or*	1.05	51.22
wp$^+$ *or*$^+$*/wp or*	*wp or*	0.40	45.98
T(Y:dp$^+$*); ap*$^+$ *dc*$^+$*/ap*$^+$*dc*	*ap dc*	4.00	—
T(Y:dp$^+$*); ap*$^+$ *dc*$^+$*/ap dc*	*ap dc*	1.98	—
T(Y:ap$^+$ *dc*$^+$*)/ap dc*	*ap dc*	0.30	—

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

In conclusion, recombination in medfly males is common; it 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 the sterile insect technique (SIT) has already been extensively discussed [6, 10–12].

4. SELECTION FOR RESISTANCE AND GENETIC SEXING

The ultimate goal of the co-ordinated research project was to develop a genetic sexing strain as part of future medfly SIT projects. Such a strain should possess a sex linked conditional lethal trait, so that invoking the lethal factor will produce an all male brood for release purposes. A search for resistance to chemicals (including insecticides) was initiated, and the modes of inheritance of established resistance traits were studied. We aimed to find a dominant single gene for resistance, translocate it to the male's Y chromosome, and produce a strain with resistant males and susceptible females to the given chemical.

4.1. Materials and methods

A series of chemicals, including insecticides, was utilized in our studies. We discuss only three chemicals, which showed some promise in our studies:

(a) Potassium sorbate, a food preservative, which was incorporated in the larval diet;
(b) Avermectin^R, a streptomycete derived macrocyclic lactone discovered by Merck, Sharp & Dome (1.8% AI);
(c) Cyromazine^R, a CIBA-GEIGY s-triazine compound, 2-cyclopropyl-amino-4,6-diamino-5-triazine, which shows insect growth regulation (IGR) as well as some chemosterilant effect on insects.

A dose–mortality curve was established, exposing eggs or larvae. We used the highest dose possible (LC_{90} or more) to mass expose eggs or larvae of our laboratory reared flies, which we replenished periodically with field material. Survivors were continuously reared on the selective agent, and doses were increased gradually

in the selection process. When some immunity of flies to the chemical became evident, family lines were established by repeated single pair crosses and were continuously exposed to the selective agent. When a certain plateau had been reached, we initiated studies on the mode of inheritance to the selective agent. These studies involved crosses and backcrosses with the wild type (non-selected) strain and subsequent exposure of the progeny to discriminating doses of the selective agent. We also initiated crosses with the available morphological mutants to attempt linking the suspected resistant trait to a known and marked chromosome.

4.2. Results and discussion

4.2.1. Potassium sorbate

Eggs and larvae were exposed to concentrations of potassium sorbate (PS) ranging from 0.1% to 0.4%; the LC_{50} for eggs was found to be between 0.4% and 0.45% and for larvae 0.25%. Only 3% of the larvae survived in the diet with 0.4% PS and that dose was consequently used for mass exposure of eggs. Larvae became increasingly immune to PS, but egg hatch remained very low and erratic. The effect of PS on egg hatch increased with the decrease of the pH of the diet, being more effective at pH of 3 and almost non-effective at pH = 6. Medfly diets require a pH of 4 to prevent bacterial contamination. We therefore changed the exposure method from the 18th generation and exposed only larvae to PS. We raised the concentration of PS to 0.7% following 21 generations of exposure and it has remained so until now (following 62 generations). Single family lines were produced in the first generation after the initial mass exposure and also after 30 generations of selection. The line which is maintained now originated from a single family (line 16). Survival of larvae (Abbott's corrected formula from hatched eggs to pupae) reached a plateau of above 75% from the 37th generation on (16th exposure to 0.7%) and remained so until the present time (Table IV).

The mode of inheritance of the trait was studied three times during the selection process [14] and revealed similar patterns of resistance of larvae to PS, the highest being of the PS × PS crosses, whereas the lowest was of the wild type × wild type crosses. Crosses between wild type and PS flies showed intermediate resistance. Sib-crosses between the hybrids yielded progeny with intermediate resistance, similar to or somewhat less than that of the hybrids themselves. Backcrosses of the hybrids to the wild type flies yielded progeny with reduced resistance, only a little higher than that of the wild type parents. Progeny of backcrosses of the hybrids and the PS flies had a level of resistance almost similar to the PS flies themselves.

Crosses of the PS flies with the *ap*, *dp*, *wp* and *bo* mutants (representing all the available marked chromosomes), subsequent backcrosses to these mutants and exposure to 0.7% PS did not yield any conclusive results.

TABLE IV. SURVIVAL OF LARVAE[a] OF POTASSIUM SORBATE (PS) SELECTED AND WILD TYPE MEDFLIES ON PS-CONTAINING DIET

Conc. PS (%)	PS line		Wild type line survival (%)
	Generation	Survival (%)	
0.4	7	20.6	3.5
0.7	23 (2)[b]	37.8	0
0.7	37 (16)	81.6	1.5
0.7	39 (18)	79.5	4.9
0.7	42 (21)	76.1	—
0.7	43 (22)	76.0	—
0.7	48 (27)	115.3	11.2
0.7	83 (62)	78.9	17.1

[a] Abbott's corrected.
[b] In parentheses: generations exposed to 0.7% PS.

TABLE V. DOSE-MORTALITY RESPONSE OF MEDFLY PRE-PUPAL STAGES TO IMMERSION OF EGGS IN AVERMECTIN SOLUTIONS

Avermectin (ppm a.i.)	Survival (%)[a]			
	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

[a] Abbott's corrected.

It seems that the PS resistance is semi-dominant and involves more than one locus. However, the fact that resistance to PS could be developed and maintained warrants further studies of that phenomenon.

4.2.2. Avermectin^R

Avermectin^R in the larval diet was totally ineffective. Hence, immersion of eggs or third instar larvae in Avermectin solution was used as a possible method for exposure and selection. We found that freshly oviposited eggs responded best to the treatment. Older eggs responded much less and third instar larvae did not respond at all. Two tests were carried out with freshly laid eggs (Table V) and we placed the LC_{50} around 5 ppm active ingredient (a.i.) Avermectin and the LC_{90} close to 50 ppm a.i. Avermectin reduced egg hatch and also caused delayed mortality of larvae. Selection was therefore initiated by immersing eggs for two hours in 50 ppm a.i. Avermectin solution. Egg hatch was reduced to 36.1% in the first exposure and increased to 90% and above in subsequent exposures. Further studies showed that it was not a response of the medfly line to selection, and that Avermectin had no effect on egg hatch, but rather a delayed effect during the larval stages. Survival of the pre-pupal stages reached or exceeded 90% after 14 generations of exposure, and the selective dose was increased to 100 ppm a.i., where it remains until today.

The results, which seemed promising, became rather puzzling during the selection period. A positive response of the selected line to Avermectin was evident, but the wild type line, used as controls in our studies, also showed substantial immunity to the chemical later during the project. Thus, exposure of the Avermectine selected line to 100 ppm and 200 ppm a.i. Avermectin yielded survival levels of 64.3% and 80.6% respectively, but at the same time the survival levels of the wild type line were 22.4% and 45.3% respectively. The immunity of the Avermectin selected line was higher than that of the wild type line, but the latter far exceeded the values obtained in the initial stages of the study. We therefore continued to maintain the Avermectin selected line on 100 ppm a.i. Avermectin, but discontinued our studies or efforts to investigate the line further.

4.2.3. Cyromazine

Cyromazine (CYR) was incorporated into the medfly larval diet, and medfly eggs were placed on top of the diet on black blotting paper. Eggs and first instar larvae were not affected by the chemical. The lowest dose causing mortality of larvae and pupae was 2 ppm a.i. (Table VI); at 4 ppm a.i. mortality was complete and no larvae pupated. Larval diets containing 3 ppm CYR were used initially for selection. The medfly responded quite quickly to the selection pressure, and survival (Abbott's corrected) reached 57.6% after six generations of selection (Table VII). The dose

TABLE VI. DOSE–MORTALITY RESPONSE OF THE MEDFLY TO CYROMAZINE IN THE LARVAL DIET (% SURVIVAL OF STAGE)

Conc. (ppm a.i.)	Hatch (%)	Pupation (%)	Adults (%)	Total (%)
0	95.7	86.8	93.5	77.6
1	95.9	95.6	95.8	87.9
2	95.2	62.5	84.0	50.1
3	95.0	5.4	61.9	3.2
4	95.4	0.0	0.0	0.0

TABLE VII. SELECTION OF MEDFLY TO CYROMAZINE IN THE LARVAL DIET: % SURVIVAL OBTAINED IN THE PROCESS

Selective dose (ppm a.i.)	Generation	Survival (%)[a]		
3	6	55.0	92.8	57.6
5	4 (10)	94.1	107.7	104.8
10	4 (14)	91.6	91.7	82.8
20	9[b] (23)	75.5	89.0	68.8

[a] Abbott's corrected.

[b] In Aug. 1988 we completed 39 generations of selection on 20 ppm a.i. CYR.

was increased to 5 ppm a.i., and then to 10 ppm a.i. with equally good response (Table VII). After 14 generations the dose was raised to 20 ppm a.i., at which it now remains. It seemed that the survival of the flies at 20 ppm a.i. reached a plateau of about 70% and remained stable there. We have maintained the line on 20 ppm a.i. CYR for 34 generations. Throughout the selection process we also compared the duration of the development period from egg to pupa (at 24°C) and found no significant change.

TABLE VIII. MODE OF INHERITANCE OF CYROMAZINE RESISTANCE IN THE MEDFLY: SURVIVAL IN CROSSES AND BACKCROSSES OF THE CYROMAZINE SELECTED LINE AND WILD TYPE FLIES

Cross type	Survival from egg to pupa (%)	
	Test I 5 ppm, F_{10-11}	Test II 20 ppm, F_{19-20}
CYR × CYR	86.9–106.4	62.7–63.3
CYR × F_1	19.8–34.9	17.4–24.1
CYR × wild type	16.8–18.9	0
F_1 × F_1	11.6–16.0	—
F_1 × wild type	0–1.1	0
Wild type × wild type	0	0

The mode of inheritance was studied twice during the selection process, in the F_{10-11} of the selected line on 5 ppm a.i. CYR, and in the F_{19-20} on 20 ppm a.i. CYR. The results of these crosses between the selected line and wild type (non-selected) flies, as well as backcrosses of the hybrids to the respective parental lines, are presented in Table VIII. We pooled the reciprocal crosses and the replicates of each of the two tests to a single figure and added the range of survival rates. Abbott's correction formula was used to present the survival in Table VIII.

The results for the 5 ppm a.i. diet indicated the recessiveness of the CYR resistance trait. However, crosses between CYR selected and wild type flies did leave a few survivors on the CYR-containing diet. Backcrosses between hybrids and wild type flies also left very few survivors. The data were more conclusive with the 20 ppm a.i. diet, where the only survivors were found in the cross between the CYR selected flies or between hybrids and CYR selected flies. No survivors were obtained in any of the crosses involving wild type flies. This was a strong indication of the recessiveness of the trait.

An attempt was made to locate the chromosome carrying the CYR resistant trait. CYR flies were crossed to each of the following mutant flies:

ap *dc*
or *w*
dp
bo

representing four marked chromosomes. The F_1 females were then backcrossed to the respective mutant males and the F_2 mutant flies were sib-mated and their progeny reared on CYR-containing diet. It was expected that among the mutants flies those 'homozygous' to the CYR trait will survive the CYR treatment. These would later be crossed to heterozygous 'CYR mutant' males and their progeny reared on CYR diet. Linkage between the CYR trait and the given mutation would result in predominantly wild type progeny. Non-linkage would result in a ratio of almost 1:1 wild type and mutant flies. When this series of crosses was conducted, no progeny of the F_2 sib-mating survived the CYR treatment, and the test was, of course, discontinued.

5. CONCLUSIONS

The attempt to develop a medfly strain with dominant resistance to a given chemical did not succeed. However, the flies responded to the selection pressure. The only promising trait, resistance to Cyromazine, is probably recessive and could not be used in the construction of a genetic sexing strain of the medfly.

ACKNOWLEDGEMENT

The authors wish to thank R. Akiva for technical help throughout the project.

REFERENCES

[1] SAUL, S.H., RÖSSLER, Y., Genetic markers of the autosomal linkage groups of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Ann. Entomol. Soc. Am. **77** (1984) 323.

[2] RADU, M., RÖSSLER, Y., KOLTIN, Y., The chromosomes of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.): karyotype and chromosomal organization, Cytologia **40** (1975) 823.

[3] KORNERUP, A., WANCHER, J.H., Methuen Handbook of Colour (2nd edn), Methuen, New York (1967).

[4] RÖSSLER, Y., KOLTIN, Y., The genetics of the Mediterranean fruit fly, *Ceratitis capitata:* three morphological mutations, Ann. Entomol. Soc. Am. **69** (1976) 604.

[5] RÖSSLER, Y., ROSENTHAL, H., Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): eye color, eye shape and wing mutations, Ann. Entomol. Soc. Am. **81** (1988) 350.

[6] RÖSSLER, Y., Recombination in males and females of the Mediterranean fruit fly (Diptera: Tephritidae) with and without chromosomal aberrations, Ann. Entomol. Soc. Am. **75** (1982) 619.

[7] RÖSSLER, Y., The genetics of the Mediterranean fruit fly: a "white pupa" mutant, Ann. Entomol. Soc. Am. **72** (1979) 583.

[8] CARANTE, J.P., Genetics of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), a 'reflectionless' eye mutant, Ann. Entomol. Soc. Am. **75** (1982) 613.

[9] RÖSSLER, Y., "69-apricot", a synthetic strain of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera:Tephritidae) with sex-limited pupal color and eye color marker, Entomophaga **25** (1980) 275.

[10] RÖSSLER, Y., "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 (1982) 291.

[11] RÖSSLER, Y., Genetic recombination in males of the Mediterranean fruit fly, and its relation to automated sexing methods, Ann. Entomol. Soc. Am. **75** (1982) 28.

[12] RÖSSLER, Y., 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, Ann. Entomol. Soc. Am. **78** (1985) 265.

[13] THOMPSON, J.N., Jr., WOODRUFF, R.C., Chromosome breakage: a possible mechanism for diverse genetic events in outbred populations, Heredity **40** (1978) 153.

[14] RÖSSLER, Y., "Selection for resistance in the Mediterranean fruit fly for genetic sexing in sterile insect technique programmes", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 229.

THE CONTRIBUTION OF FORMAL GENETIC STUDIES TO THE CHARACTERIZATION OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

A.R. MALACRIDA, G. GASPERI, L. BARUFFI, R. MILANI
Dipartimento di Biologia Animale,
Università di Pavia,
Pavia, Italy

Abstract

THE CONTRIBUTION OF FORMAL GENETIC STUDIES TO THE CHARACTERIZATION OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

Twenty-eight functional loci and four morphological gene markers have been assigned to five of the six linkage groups of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). The linkage group labelling system, proposed by Saul and Rössler, has been adopted. Map distances, obtained for twenty markers, showed the marked loci to be distributed over wide map intervals in all five autosomal linkage groups. The available information appears adequate for determining the position of chromosomal characteristics peculiar to each chromosome.

1. INTRODUCTION

The use of biochemical variants as tools for genotype analysis has greatly improved our knowledge of the inheritance [1], population structure [2, 3], comparative genetics, taxonomy [4, 5] and applied genetics [6] of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). This paper provides information on the linkage groups and presents data on the mapping of 28 biochemical and functional markers.

2. RESULTS

At present, 28 functional gene markers are known in *C. capitata*; they have been assigned to five of the six linkage groups. These groups include structural genes coding for enzyme functions, sex ratio distortion and developmental proteins. Four of the groups also carry one or more morphological markers. The relationship between linkage groups (A–E) and the biochemical and functional markers are shown in Table I. The linkage groups are labelled according to the system proposed

TABLE I. SUMMARIZED DATA SHOWING CORRELATION BETWEEN MORPHOLOGICAL LINKAGE GROUPS AND BIOCHEMICAL AND FUNCTIONAL LOCI IN *C. capitata*

	A (*ap*)	B (*wp*)	C (*dp*)	D (*ry*)	E (*bo*)
Hk-2, Est-1, Sd-1, Est-2, Pgi, β-Est	+	—	—	—	—
Hk-1, Zw, Pgd, Fh, Had, LspIII	—	+	—	—	—
Got-2	—	—	+	—	—
Acon-1, Aox, Xdh, Mdh-2, LspI Mpi, Est-6, Adh-1, Adh-2	—	—	—	?	—
Idh, Pgm, Sd-2, Got-1, Gox, LspII	—	—	—	—	+

Note: + means linkage; — means free assortment; ? means not tested.

by Saul and Rössler [7]. A direct test with the rosy eye (*ry*) marker has yet to be performed, but on the evidence of free assortment of *Xdh* with all other visible markers, *Xdh* appears to belong to the same group as *ry*. An allelism test for *ry* and *Xdh* would be particularly informative, because in *Drosophila melanogaster* the rosy eye colour has been interpreted as being the result of a pleiotropic effect of the *Xdh* gene [8].

Four of the linkage groups have already been assigned to the corresponding chromosomes. Linkage group A (*ap*) corresponds to chromosome 4 [9], B (*wp*) to chromosome 5 [10], C (*dp*) to chromosome 3 [10] and *Adh-1* to chromosome 2 [11].

Each linkage group carries some structural genes coding for enzyme functions constituting important steps in the energetics metabolism and in detoxification processes. These groups of genes are also conserved in other Diptera [1].

Map distances, determined for 20 markers on five linkage groups, showed that the marked loci are distributed over wide map intervals. Therefore, sufficient loci are now available for extending the physical chromosome map to include chromosomal characters also, such as centromere position, cross-over distorters, etc.

2.1. Linkage group A: *ap* ... *β-Est* (chromosome 4)

The map of this linkage group has already been described [12]. It includes a lethal factor (*l*) and the sex ratio distorter gene *Sd-1*. This latter gene occurs widely both in laboratory and field populations [13].

The linkage interval between the two most widely spaced loci (*ap* and *Pgi*) is 56.1 recombination units (Mu); within this interval, the coincidence between expected and observed double cross-overs approaches a value of 1, indicative of very low interference or none. A single instance of male recombination has been observed in the interval *Hk-2–Est-1*, which cover 26.69 ± 0.02 Mu [12]. No evidence of factors blocking recombination in this linkage group has been encountered. Early evidence of genetic disturbance in the *ap* chromosomal region has been confirmed and seems to reveal a point of special interest for genetic manipulation of this chromosome in relation to improving knowledge of its structure.

2.2. Linkage group B: *wp ... LspIII* (chromosome 5)

The six loci mapped on chromosome 5 are distributed over a map interval of 112.3 Mu. The occurrence of double cross-overs is affected only slightly by interference. The loci *Hk-1*, *Zw* and *Pgd* code for sequentially related metabolic functions in the energetic metabolism. The white pupae gene (*wp*) is used for automated genetic sexing. Chromosome 5 (*wp ... Hk-1 ... Zw ... Pgd*) might possibly be used as a tool in building conditional lethal systems, exploiting the very low activity levels of some *Pgd* alleles.

2.3. Linkage group C: *dp ... Got-2* (chromosome 3)

This linkage group comprises the two very loosely linked loci *dp–Got-2*. Cross-over rates reach the level of free assortment.

2.4. Linkage group D(?): *Acon-1 ... Lspl* (chromosome 2)

The nine loci of this group reassort freely with the morphological markers of the A, B, C and E linkage groups and they therefore probably belong to linkage group D. However, direct evidence, as provided by direct tests with the *ry* reference marker, has yet to be obtained. The map distances of five of these markers are given in Table II.

This linkage group contains the two loci *Adh-1* and *Adh-2*, which, in *C. capitata*, control alcohol degradation. It also includes the locus *Mpi*, which controls the mannose phosphorylation and shows seasonal frequency fluctuations in a Mediterranean population on Procida Island [2]. The *Mpi* locus is highly differentiated in terms of its fixation index (F_{ST} = 0.509) among samples of the Procida population collected at different seasons of the year [3, 14].

TABLE II. RECOMBINATION DISTANCES IN LINKAGE GROUP D(?) OF *C. capitata*

Map interval	Recombination (%)
Est-6–Mdh-2	45.2 ± 0.031
Xdh–Mpi	28.4 ± 0.028
Adh-1–Mpi	40.0 ± 0.031
Est-6–Mpi	30.0 ± 0.029

2.5. Linkage group E: *bo ... LspII*

This group comprises the sex ratio distorter gene *Sd-2* [1]. By three-point test crosses it has been shown that the *Idh*, *Pgm* and *Got-1* loci are located in that order on the chromosome, that recombination frequencies in the two intervals are 15.29 ± 0.22 and 35.68 ± 0.029, respectively, and that the occurrence of double cross-overs in the interval *Idh–Got-1* is affected only slightly by interference. Cross-over rates of 0.67 and 38.5 have been found in the intervals *Sd-2–Got-1* and *Sd-2–Idh*, respectively [14]. The close linkage between a sex ratio distorter gene and an enzymatic marker might be suitable for exploitation for genetic sexing purposes.

3. CONCLUSIONS

The allocation of the described mutants and variants to their respective map positions provides a picture of the genetic structure of *C. capitata*, which is sufficiently detailed to allow genetic manipulation for applied purposes and to obtain reliable data both on intraspecific variability and on conservative aspects characterizing the genotypes of various related taxa.

Detailed genetic maps will facilitate the recognition of synonyms which may currently inflate the list of known morphological loci.

REFERENCES

[1] MALACRIDA, A.R., GASPERI, G., MILANI, R., "Genome organization of *Ceratitis capitata:* Linkage groups and evidence for sex ratio distorters", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 169.

[2] GASPERI, G., MALACRIDA, A.R., MILANI, R., "Protein variability and population genetics of *Ceratitis capitata*", ibid., 149.

[3] GASPERI, G., MALACRIDA, A.R., GUGLIELMINO, C.R., CANGEMI, M.G., MILANI, R., A populational study on the Medfly, *Ceratitis capitata*, Atti Assoc. Genet. Ital. **34** (1988).

[4] MALACRIDA, A.R., GASPERI, G., BISCALDI, G.F., MILANI, R., Persistence of linkage relationships among enzyme loci in some Dipteran species, Atti Assoc. Genet. Ital. **32** (1986) 121.

[5] MALACRIDA, A.R., GASPERI, G., GUGLIELMINO, C.R., BARUFFI, L., MILANI, R., "Genetical approach to systematics and phylogenesis of Trypetinae (Diptera: Tephritidae)", in Proc. 2nd Natl Congr. Ist. Zoologica, Univ. Pavia (1988).

[6] ROBINSON, A.S., Sex ratio manipulation in relation to insect control. Annu. Rev. Genet. **17** (1983) 191.

[7] SAUL, S.H., RÖSSLER, Y., Genetic markers for the autosomal linkage groups in the Mediterranean fruit fly, *Ceratitis capitata*, Ann. Entomol. Soc. Am. **77** (1984) 323.

[8] O'BRIEN, S.J., "Genetics and biochemistry of enzymes and specific proteins of *Drosophila*", Genetics and Biology of *Drosophila* (ASHBURNER, M., WRIGHT, I.R.F., Eds), Academic Press, New York (1987) 490.

[9] BUSCH-PETERSEN, E., SOUTHERN, D.I., "Induced suppression of genetic recombination of females of the Mediterranean fruit fly, *Ceratitis capitata*, by translocation heterozygosity", Genetica **72** (1987) 161.

[10] ZACHAROPOULOU, A., Univ. of Patras, Greece, personal communication.

[11] ZAPATER, M., ROBINSON, A.S., Sex chromosome aneuploidy in a male linked translocation in *Ceratitis capitata*, Can. J. Genet. Cytol. **28** (1986) 161.

[12] GASPERI, G., MALACRIDA, A.R., TOSETTI, M., MILANI, R., "Enzyme variability: A tool for investigating the genome organization and the population structure of *Ceratitis capitata*", Fruit Flies of Economic Importance 84 (Proc. Working Group Mtg Hamburg, 1984), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1986) 153.

[13] MILANI, R., GASPERI, G., MALACRIDA, A.R., "Biochemical genetics", Fruit Flies, Their Biology, Natural Enemies and Control (ROBINSON, A.S., HOOPER, G.H.S., Eds), in press.

[14] MALACRIDA, A.R., GASPERI, G., BARUFFI, L., BISCALDI, G.F., MILANI, R., "Updating of the genetics of *Ceratitis capitata* (Wied.)", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 221.

ELECTROPHORETIC MULTILOCUS ANALYSIS FOR THE STUDY OF NATURAL POPULATIONS OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

G. GASPERI*, A.R. MALACRIDA*, C.R. GUGLIELMINO**, R. MILANI*

* Dipartimento di Biologia Animale, Università di Pavia, Pavia, Italy

** Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy

Abstract

ELECTROPHORETIC MULTILOCUS ANALYSIS FOR THE STUDY OF NATURAL POPULATIONS OF THE MEDITERRANEAN FUIT FLY, *Ceratitis capitata* (Wied.).

Data concerning spatial and/or temporal variation among 29 samples of four populations of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) were obtained by computation of gene frequency values at 25 biochemical loci. The four populations came from Africa (Kenya and Réunion) and from the Mediterranean basin (Sardinia and Procida Island). Statistical parameters of genetic variation included average heterozygosity per locus, proportion of polymorphic loci and average number of alleles per locus. The data were analysed using Principal Component Analysis and Wright's fixation index. Significant differences in genetic heterogeneity were observed on a regional scale in relation to the dispersion of the fly from its supposed area of origin (East Africa) towards the periphery (Mediterranean region). The samples from Procida, collected at different seasons for four consecutive years (1983–1986), provided consistent indications of temporal changes in the genetic structure of this population, and permitted evaluation of the efficiency of a sterilized male strain (T-101) released during a sterile insect technique programme on Procida in 1986.

1. INTRODUCTION

Knowledge of the genetic structure and dynamics of natural populations can provide information of essential importance for population control strategies [1]. In recent decades, the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), has extended its range to a variety of ecologically different zones in tropical and subtropical regions of the world [2]. It is therefore important to evaluate

geographical divergence among local populations and to acquire information concerning host specificity and other eco-ethological features. Such information is vital for the success of biological control programmes.

Here we present data concerning spatial variation among four populations originating from geographically and ecologically distinct areas. Temporal variation has been registered in one of these populations.

2. MATERIAL AND METHODS

The four populations examined were: Kenya (two samples), Réunion (two samples) and, in the Mediterranean basin, Sardinia (three samples) and Procida Island, near Naples, Italy (twenty-two samples).

In the Procida population, temporal variation was analysed over four successive years (1983–1986). Each year, samples were taken at different seasons. The impact on the population of the release of sterilized males of the genetic sexing strain, T-101, was considered.

The genetic variations present in samples from natural populations were calculated, using the allele frequency information provided by electrophoresis, of the following 25 enzyme loci: *Hk-1, Hk-2, Pgm, Pgi, Pgk, Zw, Pgd, α-Gpdh, Idh, Fh, Mdh-1, Mdh-2, Ak-1, Ak-2, Adh-2, Aox, Est-1, Est-2, Est-6, Got-1, Got-2, Gpt, Had, Me, Mpi.* Of these, *Ak-1, Me* and *α-Gpdh,* were found to be uniformly monomorphic.

The parameters used to estimate the genetic variation were: H (mean heterozygosity per locus), P (proportion of polymorphic loci), A (average number of alleles per locus), and F_{ST} (Wright's fixation index) [3]. A comprehensive method of multivariate statistical analysis (Principal Component Analysis) was used to synthesize the information in the entire set of data composed of a total of 29 samples.

3. RESULTS AND DISCUSSION: GEOGRAPHICAL DIFFERENTIATION AMONG POPULATIONS

A general trend characterized the species distribution of *C. capitata*: Mediterranean populations were less polymorphic than subtropical and tropical populations. This was confirmed by all three types of parameters calculated. In particular, the variability expressed by mean heterozygosity ranged from 2.2% to 9.2% on Procida Island, from 6.6% to 11.4% in Sardinia, from 6.9% to 16.3% in Réunion and from 16.6% to 18% in Kenya. Similarly, the proportion of polymorphic loci was never larger than 36% on Procida Island, while it reached 64% in Kenya. This latter population also had the highest average number of alleles per locus

(A = 1.88). These findings are in agreement with the theory of decreasing genetic variability as the populations move from the area of origin towards the periphery of their geographical range [4–6].

The proportion of heterozygosity attributable to differentiation among populations is given by Wright's fixation index. This index is a measure of the standardized genetic differentiation between populations. Among the loci with the highest values of F_{ST} we found in decreasing order: *Est-2* (0.685), *Est-1* (0.613), *Hk-2* (0.219), and *Got-2* (0.183). Loci, such as *Est-6* and *Mpi*, which were very polymorphic, showed an intermediate value of F_{ST} (0.126 and 0.108, respectively). Conversely, *Est-1* and *Est-2*, which displayed the highest level of differentiation between populations, showed rather low levels of heterozygosity.

A large proportion of the interpopulation differentiation was contributed by the Réunion population. If this population is left out of the calculation, the high level of F_{ST} for *Est-2* and *Est-1* disappears. Réunion showed significantly more differentiation at the two *Est* loci than all the other populations, owing to the presence of alternative fixed alleles.

The level of heterozygosity and the degree of polymorphism can provide a good estimate of differentiation if the fixation index is known. The fixation of alternative alleles enables the relative loss of heterozygosity, and thus the real degree of polymorphism, to be assessed.

The information obtained on all 29 population samples was synthesized by using Principal Component Analysis (PCA) of allele frequencies. The relative position of the four populations with respect to the two first principal axes, which account globally for almost 50% of total variation, is shown in Fig. 1. The first principal component (I PC), the abscissa axis, which accounts for 28.5% of the total variation, separates samples largely on a geographical basis. Réunion, Kenya and Sardinia appear on the right side of the plot, while Procida samples occupy the left half. The two Procida samples in the right half of the plot were those collected in December and February (i.e. the overwintering and the early season generations).

In Subsections A and B below we discuss the meaning of the synthetic variables represented by the two principal axes, with respect to the possible source of variability among the Procida samples.

A. Procida: annual variation and bottleneck effect

It is interesting to note the significant correlation between the first principal component and a parameter of variability, such as the average number of heterozygous individuals detected on Procida ($r = 0.76$, $p < 0.01$). This correlation may be connected with the annual increase in variability. This is confirmed by the increase in annual average heterozygosity, as observed in Procida samples collected from 1983 to 1985 (Table I). The genetic sexing strain, T-101, was released on Procida in 1986 [7]. The mean heterozygosity of the samples collected

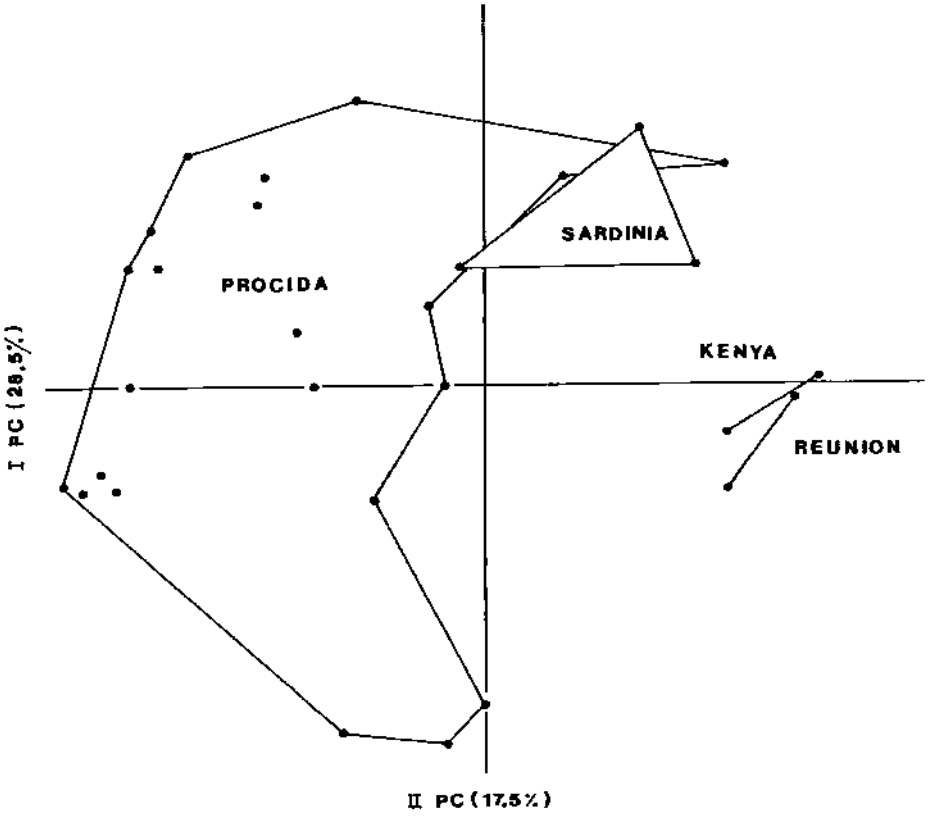


FIG. 1. Plot of the 29 population samples of C. capitata *with respect to the plane of the two first principal components (PCs).*

TABLE I. ANNUAL MEAN VALUES OF HETEROZYGOSITY (H) RECORDED IN THE PROCIDA POPULATION

Year	No. of samples examined	H	(± s.d.)
1983	4	0.031	(0.010)
1984	4	0.042	(0.016)
1985	8	0.066	(0.018)
1986	5	0.045	(0.018)

during that year and after the release of the sexing strain was similar to that of 1984. It therefore seems reasonable to assume that the observed reduction in genetic variability was related to the impressive reduction of the Procida population during 1986. The efficiency of the genetic sexing strain used on Procida could thus be determined both by its direct effect on the population size and, indirectly, by the reduction of genetic variability in the post-treatment population.

B. Procida: seasonal variation

The second principal component, represented by the ordinate axis in Fig. 1 (II PC), which accounts for 17.5% of the total variation, appears to be correlated with a seasonal trend among the Procida samples. Overwintering and early season populations are located in the top half, July and August in the lower half, and September around the middle. The correlation coefficient ($r = 0.62$, $p < 0.01$) of the II PC with the changing seasons supports this hypothesis.

The seasonal pattern of variation in Procida populations is highlighted by an analysis of F_{ST} values which summarizes the variability on a per locus basis. When we compare the Procida samples between years, there is no differentiation in terms of the fixation index. However, when samples are compared between seasons (i.e. samples collected from December to April are compared with samples collected from June to August and with September samples), a very high degree of differentiation appears for the *Mpi* locus. In fact, the F_{ST} value, between seasons, for this locus was 0.509, while the corresponding value between years was 0.017. Seasonal variation at this locus was also expressed by changes in allele frequencies [6].

4. CONCLUSIONS

The present data consistently suggest that, within the species range, populations of *C. capitata* are genetically different. The Afrotropical origin of *C. capitata* is supported by the decreasing variability away from the tropical and subtropical populations towards the Mediterranean region. Genetic bottlenecks are likely to have been associated with the geographical colonization [8], and founder effects are probably responsible for the particular processes of geographical differentiation shown in the Réunion population.

The temporal variation observed on Procida is related both to annual and seasonal events. The population size, which in a temperate climate undergoes dramatic fluctuations, may very likely account for the observed changes in the genetic structure. Immigration of flies from the nearby mainland [9] could explain the annual genetic fluctuations of the Procida population. Some particular loci, such as *Mpi*, appear to be heavily influenced by seasonal changes.

REFERENCES

[1] BUSH, G.L., HOY, M.A., "Evolutionary processes in insects", Ecological Entomology (HUFFAKER, C.B., RABB, R.L., Eds), Wiley, New York (1984) 247.

[2] SAUL, S.H., "Genetics of the Mediterranean fruit fly (*Ceratitis capitata*) (Wiedemann)", Agricultural Zoology Reviews (RUSSEL, G.E., Ed.), Intercept-Ponteland, Newcastle–upon–Tyne, UK (1986) 73.

[3] NEI, M., CHESSER, R.K., Estimation of fixation indices and gene diversities, Ann. Hum. Genet. **47** (1983) 253.

[4] HUETTEL, M.D., FUERST, P.A., MARUYAMA, T., CHAKRABORTY, R., Genetic effects of multiple population bottlenecks in the Mediterranean fruit fly (*Ceratitis capitata*), Genetics **945** (1980) 47.

[5] LOUKAS, M., "Population genetic studies of fruit flies of economic importance, especially medfly and olive fruit fly, using electrophoretic methods", Proc. Int. Symp. Electrophoresis Studies on Agricultural Pests, Rothamsted, UK, in press.

[6] GASPERI, G., MALACRIDA, A.R., MILANI, R., "Protein variability and population genetics of *Ceratitis capitata*", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 149.

[7] CIRIO, U., CAPPARELLA, M., ECONOMOPOULOS, A.P., "Control of medfly (*Ceratitis capitata* Wied.) by releasing a mass-reared genetic sexing strain", ibid., p. 515.

[8] MAYR, E., in Evolution as a Process (HUXLEY, J., HARDY, A.C., FORD, E.B., Eds), George, Allen and Unwin, London (1954) 167.

[9] CIRIO, U., DE MURTAS, I.D., "Status of Mediterranean fruit fly control by the sterile-male technique on the Island of Procida", The Sterile-Insect Technique and its Field Applications (Proc. Panel Vienna, 1972), IAEA, Vienna (1974) 5.

Part II

GENETIC SEXING OF *Ceratitis capitata* BY MORPHOLOGICAL, BIOCHEMICAL AND OTHER MEANS

GENETIC OR MECHANICAL SEXING SYSTEM FOR THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)*

J.M.M. WALDER
Centro de Energia Nuclear na Agricultura,
Universidade de São Paulo,
Piracicaba,
São Paulo, Brazil

Abstract

GENETIC OR MECHANICAL SEXING SYSTEM FOR THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

A black puparium, monofactorial mutant was isolated in 1983 from a laboratory colony of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). The mutant was used to construct a genetic sexing strain based on pupal sorting. Translocations were induced in wild male adults, 48 hours old, by gamma radiation (55 Gy; ^{60}Co). These males were crossed to black pupae females and produced two pupal sorting strains (T-44 and T-213) in 1987. These strains were lost after six generations. In another series of translocation inductions the strain T-87B was screened. Rearing the strain for eight generations in the laboratory provided no indication of instability in the strain. T-87B is now being mass reared.

1. INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) is a major pest with a tremendous destructive potential. The larvae attack about 200 varieties of fruit and vegetables [1].

The medfly in Brazil occurs more frequently in the south and southeast regions, where the climate is cooler and where there are larger plantations of introduced fruit species such as citrus, peach, apple, pear and coffee [2].

The Entomology Section at the Centro de Energia Nuclear na Agricultura (CENA) started research in 1973 on the application of the sterile insect technique (SIT) for eradication or control of the medfly. The objective of these studies was to develop the necessary methodology to improve systems of mass rearing and sterilization tests and to develop new diets [3–5]. Field studies were also conducted to observe the population density and biotic potential [4, 6].

* The research was supported partly by IAEA Research Contract No. 4132/RB, partly by Financiadora de Estudos e Projetos (FINEP) 42.86.0735.0, and partly by Comissão Nacional de Energia Nuclear (CNEN).

In a suppression programme by means of SIT, the removal of females prior to release is very advantageous [7, 8]. Several prototype genetic sexing systems were developed [9–11] using the principle of male linked translocations.

2. MATERIAL AND METHODS

2.1. Strains

Two medfly strains were used in this study:

— **CENA strain:** a laboratory colony maintained on a large scale since 1981;
— **Black pupae:** spontaneous mutant isolated from the CENA strain in 1983 and maintained as a homozygous stock.

2.2. Rearing conditions

All experiments were carried out at 26°C ± 1 and relative humidity 70–80%. The larvae were reared on the following diet: brewer's yeast 3.69%; wheat flour 11.06%; sugar 6.45%; ascorbic acid 0.18%; Nipagin 0.22%; antibiotic 0.03%; water 17.52%; acid solution (hydrochloric acid + benzoic acid, pH4.7) 51.63%; and dry sugar cane bagasse 9.22%.

Adults were fed a mixture of sugar and yeast hydrolysate (3:1). Water was provided by a moisture cotton and offered to the flies from the outside top of the cages. Light (14L:10D) was provided by 20 W or 40 W fluorescent lights fixed close to the cages.

Mass rearing was carried out in trapeziform cages (0.04 m^3) with oviposition net at two sides. Family rearing units followed the units described by Rössler [12], but Nadel traps were used.

2.3. Irradiation and mating schemes

All irradiations were carried out on two day old adult males from CENA stock, using a ^{60}Co irradiator. Doses of 50 Gy and 55 Gy were used. The irradiated males were crossed to virgin females from the mutant black pupa stock, and the F_1 males were individually backcrossed (1 male:3 females) to mutant females. The F_2 offspring were reared in family groups and scored for the presence of translocations as indicated by pseudolinkage between pupal colour and sex.

3. RESULTS AND DISCUSSION

Conventional genetic studies showed the black puparium gene to be autosomal and recessive. This mutant could be identical to dark pupae (*dp*) [13] or to black pupae (*nig*) [14].

Some irradiation tests were made using 50 Gy of gamma radiation, but none of the resultant F_2 families showed any pseudolinkage between *bp* and sex. The radiation dose was increased to 55 Gy, and two lines with wild type males and black pupae females were isolated [11]. These lines remained true breeding up to six generations, but yielded no adult offspring in the seventh generation.

A new translocation test was conducted. One hundred and ninety adult males (48 hours old) from CENA strain were irradiated (55 Gy) and crossed to 224 virgin black pupae females. The radiation resulted in egg sterility and distorted sex ratio in the F_1 generation and had no effect on pupal survival (Table I). Similar results were obtained in previous studies [10].

From a series of 155 families set up, three lines (1.93%) produced wild type males and black pupae females. The rate was similar to the values of 2.3% obtained by Rössler [9] and 2–3% by Robinson and Van Heemert [10].

The history of the translocated lines in each generation is presented in Tables II–IV. Wild type males were outcrossed to black pupae females in every generation. The T-12B line yielded no adults by the fifth generation and was lost (Table II). The T-37B line was lost when the F_5 adults laid sterile eggs (Table III).

The remaining line (T-87B) was checked for eight generations for undesirable recombinants, with negative results. There was, however, an excess of males in three out of eight generations (Table IV).

When this translocated line is well established in the laboratory, we shall increase the fly production for biological studies (fecundity, fertility, pupal yield,

TABLE I. EFFECT OF 55 Gy ON EGG AND PUPAL SURVIVAL AND ADULT SEX RATIO AFTER IRRADIATION OF 48 HOUR OLD MALE *C. capitata*

Treatment	Survival (%)		Sex ratio
	Egg	Pupae	Males : Females
55 Gy	7.3	93.3	193 : 127
Control	87.4	97.0	170 : 171

TABLE II. HISTORY OF LINE T-12B UP TO FIFTH GENERATION

Generation	No. of eggs	Hatch (%)	Pupae harvested			Adults from wild type pupae			Adults from black pupae		
			Total	Wild	Black	%	Males	Females	%	Males	Females
F_1	n.a.[a]	n.a.	26	11	15	72.7	8	0	46.7	0	7
F_2	n.a.	n.a.	60	19	41	47.4	9	0	31.7	0	13
F_3	n.a.	n.a.	43	11	13	81.8	9	0	23.1	0	3
F_4	567	15.9	24	14	10	64.3	9	0	57.1	0	8
F_5	211	25.6	14	2	12	0.0	0	0	0.0	0	0

[a] n.a. — not available; eggs were oviposited directly onto the larval diet and were not counted.

TABLE III. HISTORY OF LINE T-37B UP TO SIXTH GENERATION

Generation	No. of eggs	Hatch (%)	Pupae harvested			Adults from wild type pupae			Adults from black pupae		
			Total	Wild	Black	%	Males	Females	%	Males	Females
F_1	n.a.[a]	n.a.	10	4	6	100	4	0	66.7	0	4
F_2	n.a.	n.a.	186	94	92	86.2	81	0	77.2	0	71
F_3	n.a.	n.a.	91	47	44	82.9	39	0	63.1	0	28
F_4	1162	12.8	18	9	9	66.7	6	0	66.7	0	6
F_5	243	70.0	110	57	63	80.7	46	0	85.7	0	54
F_6	35	0.0	—	—	—	—	—	—	—	—	—

[a] n.a. — not available; eggs were oviposited directly onto the larval diet and were not counted.

TABLE IV. HISTORY OF LINE T-87B UP TO EIGHTH GENERATION

Generation	No. of eggs	Hatch (%)	Pupae harvested			Adults from wild type pupae			Adults from black pupae			Adult sex ratio	χ^2
			Total	Wild	Black	%	Males	Females	%	Males	Females		
F_1	n.a.[a]	n.a.	51	30	21	73.3	22	0	71.4	0	15	1.47	1.32
F_2	n.a.	n.a.	110	56	54	62.5	35	0	42.6	0	23	1.52	2.48
F_3	n.a.	n.a.	33	18	15	38.9	7	0	46.7	0	7	1.00	0.00
F_4	264	3.4	7	3	4	66.7	2	0	75.0	0	3	0.67	0.20
F_5	37	27.0	4	3	1	100	3	0	100	0	1	3.00	1.00
F_6	913	29.0	66	42	24	71.4	30	0	45.8	0	11	2.73	8.80**
F_7	5371	38.0	482	311	171	46.9	226	0	60.8	0	104	2.17	45.00***
F_8	7267	39.9	875	598	277	72.4	433	0	81.4	0	225	1.92	65.75***

[a] n.a. — not available; eggs were oviposited directly onto the larval diet and were not counted.

adult emergence, total yield, development time, longevity and gamma radiation effects). After these studies, a seed sorting machine will be tested for sexing [7, 15], and sterile males could be tested for medfly control or eradication [16].

4. CONCLUSIONS

A genetic sexing strain of Mediterranean fruit fly was obtained, with females of black pupal colour, and males (wild type) of brown pupal colour. If this technique should prove workable it could be used in a SIT field test as part of an integrated control programme in Brazil.

This classical method of using male linked translocation to obtain a sex sorting strain was very valuable since basic information was obtained from it. This first positive result encouraged us to continue the search for new techniques of genetic sexing systems as a means of genetic control.

ACKNOWLEDGEMENT

Special thanks are due to Mr. Luis A. Lopes for technical assistance throughout the study.

REFERENCES

[1] DIRECCION GENERAL DE SANIDAD VEGETAL, Programa mosca del Mediterráneo — Manual de operación, Talleres Gráficos de la Nación, Mexico (1981)

[2] WALDER, J.M.M., "Prospects for genetic control of fruit flies in Brazil", Report of Advisory Group Meeting on Genetic Methods of Insect Control, Vienna, 1987, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[3] TORNISIELO, V.L., SGRILLO, R.B., WALDER, J.M.M., WIENDL, F.M., ZOCA, W., Nova dieta para criação da mosca-do-mediterrâneo, *Ceratitis capitata* (Wied.), Ciênc. Cult. **27** (1977) 760.

[4] WALDER, J.M.M., Uso de Técnicas Nucleares no Estudo e Controle de mosca-do-mediterrâneo, *Ceratitis capitata* (Wied.), Rep. CNPq/301008-80, Piracicaba, São Paulo (1983).

[5] ALMEIDA, M.S.P., Uso de radiação gama e gases inertes na esterilização de *Ceratitis capitata* (Wied.) com vistas a aplicação da TME, Thesis, ESALQ, Piracicaba, São Paulo.

[6] WIENDL, F.M., WALDER, J.M.M., SGRILLO, R.B., PACHECO, J.M., EPIPHANIO, J.C.N., Estudo de soltura e recaptura de mosca do mediterrâneo, *C. capitata*, marcada com fósforo radioativo em café e citrus, Ciênc. Cult. **29** (1977) 475.

[7] WHITTEN, M.J., Automated pupal sexing and its usefulness in control by sterile insects, J. Econ. Entomol. **62** (1969) 272.

[8] LACHANCE, L.E., "Genetic strategies affecting the success and economy of the sterile insect release method", Genetics in Relation to Insect Management (Proc. Conf. Bellagio, 1978), Rockefeller Foundation, New York (1979) 8.

[9] RÖSSLER, Y., Automated sexing of *Ceratitis capitata* (Dip.: Tephritidae): the development of strains with inherited, sex-limited pupal color dimorphism, Entomophaga **24** (1979) 411.

[10] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*, a suitable case for genetic sexing, Genetica **58** (1982) 229.

[11] WALDER, J.M.M., "Genetic method for separation of males and females of the medfly on the basis of pupal colour", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 205.

[12] RÖSSLER, Y., Rearing unit for single-pair or small cultures of the Mediterranean fruit fly (Diptera: Tephritidae), J. Econ. Entomol. **77** (1984) 556.

[13] RÖSSLER, Y., KOLTIN, Y., The genetics of the Mediterranean fruit fly, *Ceratitis capitata*: Three morphological mutations, Ann. Entomol. Soc. Am. **69** (1976) 604.

[14] LIFSCHITZ, E., "New mutations in the medfly (*Ceratitis capitata*)", Report of Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and Other Genetic Measures, Vienna, 1985, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[15] SMITTLE, B.J., SEAWRIGHT, J.A., BIRKY, B.K., Use of a commercial color sorter to separate sexes of stable fly (Dip.: Muscidae) pupal from a genetic sexing strain, J. Econ. Entomol. **70** (1986) 877.

[16] ROBINSON, A.S., CIRIO, U., HOOPER, G.H.S., CAPPARELLA, M., Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, *Ceratitis capitata*, Entomol. Exp. Appl. **41** (1986) 231.

TWO NEW PUPAL SEXING STRAINS IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)*

M. ZAPATER
Facultad de Agronomía,
Universidad de Buenos Aires,
Buenos Aires, Argentina

Abstract

TWO NEW PUPAL SEXING STRAINS IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

A genetic sexing system in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is urgently required in order to reduce the costs of mass rearing and to prevent punctures in fruit made by the ovipositors of sterilized females. Two genetic sexing strains, T(Y,5)122; T:Y(*wp*), white pupae, and T(Y,3,5)11; T:Y(dp^+ wp^+), were isolated and studied in connection with their possible use in a mass rearing programme. Both strains have males emerging from wild type brown pupae and females emerging from mutant pupae; they are stable up to generation 22. The strain T(Y,5)122 has a translocation linking the Y chromosome with the autosome carrying the *wp* locus. The strain T(Y,3,5)11 has a translocation linking the Y chromosome and the two chromosomes carrying the *wp* and the *dp* loci. The egg fertility of the strain T(Y,5)122 was 52% and that of T(Y,3,5)11 was 48%. Larval survival of the latter line was 79%. The technical advantages of these strains are discussed in this paper. The strain T(Y,4)116; T:Y(ap^+), apricot eye, is characterized by wild type males and apricot eye females, as well as apricot eye sterile males. A model explaining the appearance of these *ap* males is proposed. Isolation and preliminary fertility studies of six sex linked multiple translocations are presented. Each of these strains has three translocations involving the chromosomes Y, 3, 4 and 5.

1. INTRODUCTION

The sterile insect technique (SIT) has been demonstrated to be an effective control measure against the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) [1]. A genetic sexing system based on the early elimination of females from mass rearing would also enable SIT to be used in areas where it cannot be used at present because sterilized females can damage fruit by puncturing it with their ovipositor. Some further advantages of genetic sexing are:

(a) A change in the mating pattern from intrastrain to interstrain mating following release [2];

* This work was partially supported by the IAEA under Contract No. 4816/RB and by the University of Buenos Aires under Contract No. UBACyT: AG 009.

(b) Reduction of the sterilizing dose, resulting in more competitive males [3];
(c) No accidental releases of non-sterilized females.

The first attempts to produce a pupal colour genetic sexing strain were developed in *Lucilia cuprina* [4]. Rössler [5] and Robinson and Riva [6] continued this approach in *C. capitata*. Further promising work on genetic sexing based on ethanol tolerance of males is also being pursued. This method has an advantage over the pupal colour system in that young female larvae can be eliminated.

Data on two new genetic sexing strains and their stability are presented here.

2. MATERIALS AND METHODS

2.1. Strains

Wild type provided by the Departamento de Patología Vegetal, Instituto Nacional de Tecnología Agropecuaria, Castelar, Argentina.

wp ap dp: homozygous multiple marker stock; the markers are on chromosomes 5, 4 and 3 respectively. The strain has grey-bronze pupae.

2.2. Rearing

All stages were reared at 25°C ± 1, 65% ± 10 relative humidity, and 146:100 photophase.

2.3. Isolation of translocations

Sixty wild type males, two days old, were irradiated with 3 krad of X rays and mass mated to 150 virgin *wp ap dp*. A total of 115 F_2 families were obtained by backcrossing one F_1 male with three *wp ap dp* females. The pupae were separated by colour and the association of eye colour, pupal colour and sex was recorded.

2.4. Fertility, survival and sex ratio

Single pair crosses were set up between translocation males and *wp* females. Eggs were collected on black filter paper over a two day period and transferred to larval medium. The hatched eggs were counted 72 hours later. The pupae were counted 14 days later, and pupal emergence was subsequently measured. Survival is defined as the percentage of eggs that become adults.

2.5. Stability of the translocation strains

At generations 1, 4, 8, 11, 17 and 22, samples of pupae were taken from the translocation strains, separated according to pupal colour, and the sex of the emerging adults checked.

TABLE I. TRANSLOCATIONS INDUCED IN *C. capitata* IRRADIATED WITH 3 krad OF X RAYS

Chromosomes	No. of translocations
Y,3,5	1
5,4,3	1
Y,5	2
Y,3	4
Y,4	3
5,3	7
3,4	7
5,4	2
	27

3. RESULTS

3.1. Isolation of translocations

A total of 27 translocations were isolated, comprising 10 Y linked, 17 autosomal and 2 double translocations (Table I); 9 male linked and 10 autosomal translocations are being maintained. Two strains were chosen for further study: T(Y,5)122 and T(Y,3,5)11. The latter strain was also outcrossed to *wp* females so that it produced white pupae females (11a) whereas the original strain produced grey-bronze females (11b).

In total, 23% of F_1 males carried a translocation between the marked chromosomes; this is similar to figures found in other studies [7].

TABLE II. EGG, LARVAL AND PUPAL SURVIVAL FOR TWO TRANSLOCATION STRAINS AND ONE WILD TYPE IN *C. capitata*

Strain	No. of families	Egg hatch	Larval survival	Pupal survival		Overall survival	Sex ratio	
							Male	Female
Wild type	7	90	86.2	97.8		76.6	—	—
T(Y,5)122	20	47.2	88.6	80.9	98.3	37.0	191	174
T(Y,5,3)11	8	44.3	68.2	93.8	92.4	28.2	61	49

TABLE III. STABILITY OF TWO Y LINKED TRANSLOCATIONS IN *C. capitata*

Strain	Generation	No. of wild type pupae sampled	No. of mutant pupae sampled	No. of recombinants
T(Y,5)122	1	40	40	0
	4	100	100	0
	8	100	100	0
	11	236	177	0
	17	150	150	0
	22	150	130	0
T(Y,3,5)11	1	40	40	0
	4	100	100	0
	8	100	100	0
	11	65	53	0
	17	150	120	0
	22	135	141	0

3.2. Fertility, survival and sex ratio

Egg, larval and pupal survival for the three strains, T(Y,5)122, T(Y,3,5)11 and wild type, are shown in Table II. Egg hatch in the two translocation strains is reduced by about half; in the multiple translocation strain, larval survival was clearly lower than in the wild type or the single translocation strain. This perhaps indicates that some duplication/deficiency zygotes are surviving to the larval stage.

In strain T(Y,5)122 the pupal phenotypic ratio was significantly distorted ($\chi^2 = 5.32$) in favour of wild type, but the low emergence of these pupae led to a final adult sex ratio not significantly different from 1:1.

3.3. Stability of the translocation strains

Recombination can rapidly destabilize a genetic sexing line and is an important factor in the suitability of a strain for mass rearing. Table III gives some data on the stability of these strains. In the small samples taken, no recombinant individuals were found. This is quite encouraging as the populations were propagated with about 400 individuals per generation.

3.4. Genetic studies of the strain T(Y,4)116

This strain is characterized by having *ap* (apricot eye) females, wild type males, and *ap* males. The latter express high mortality in the pupal stage (Table IV) or survive as deformed and sterile adults. Owing to this unusual segregation, this strain was further studied. The origin of the *ap* males can be explained as follows. These males represent surviving aneuploid zygotes containing the non-translocated chromosome 4 and a portion of the Y chromosome. It can be inferred that the break

TABLE IV. PUPAL SURVIVAL PERCENTAGE OF THREE GENOTYPES FROM STRAIN T(Y,4)116

	Wild type	*Ap* males	*Ap* females
Pupae not emerged	8.0	76.0	16.0
Pupae half emerged	2.5	97.5	0.0
Pupae emerged	39.1	23.3	37.6
Distribution	34.1	32.6	33.3

in chromosome 4 must be in the proximal part of the chromosome. Small duplications and deficiencies can easily survive as viable zygotes in *C. capitata* [8] and *Lucilia cuprina* [9].

3.5. Reirradiation of translocations

Males of the strain T(Y, 3, 5)11 were irradiated with 2 krad of X rays. Six translocations involving four chromosomes (Y, 3, 4 and 5) were isolated. All strains, as expected, show a reduced fertility of about 25% (this work was done in collaboration with J. Mazzoli).

4. DISCUSSION

Pupal sexing promises to become the system used for mass rearing programmes in the coming years. This system should be used until the moment that a discriminating system operating at early developmental stages is available. The results of the present work indicate that both induced translocations show potential as genetic sexing strains. The fertility of a genetic sexing strain at a given irradiation dose is the product of its fertility vis à vis the wild type strain and the fertility of the latter line at that given dose [3]. Thus, a lower dose is needed to achieve a comparable sterility in the genetic sexing line and, as a result, the competitiveness of the released males will increase.

The quadruple translocation induced by reirradiating established translocation strains offers an alternative approach to control. If the strains could be mass reared economically they could be released following substerilizing doses of radiation or perhaps without being irradiated at all. In this scenario, suppression of the pest population below the economic threshold, rather then eradication, is the objective.

Pupal sexing, and other systems such as *tsl* lethals, ADH activity or resistance to insecticide, all use a translocation linking the Y chromosome with the autosome carrying the discriminating factor. Cytological studies of translocations [10] and, more recently, analysis of polytene chromosomes [11, 12], have made it possible for the rearrangement of the translocations to be studied and their breakpoint to be determined.

Recombination could be avoided or reduced when the locus of the discriminating factor is close to the breakpoint. Recombination has been observed in stable laboratory strains when mass reared [13], with consequent breakdown of the strain. All studies related to recombination should be encouraged.

In conclusion, the work presented here has demonstrated once again that genetic sexing based on pupal colour is a workable system in *C. capitata*. It is now essential that one of these systems should undergo extensive field testing.

ACKNOWLEDGEMENTS

I should like to express my gratitude to Professor Alberto Prina for advice and encouragement, and to Dr. Alan Robinson for constructive criticism of the manuscript.

REFERENCES

[1] ORTIZ, G.P., et al, "Mediterranean fruit fly, *Ceratitis capitata*: present status of the eradication program in southern Mexico," Fruit flies of Economic Importance 84 (Proc. CEC/IOBC Working Group Hamburg, 1984), (CAVALLORO, R. Ed.), Balkema, Rotterdam (1986) 101–110.

[2] ROBINSON, A.S., CIRIO, V., HOOPER, G.H.S., CAPPARELLA, M., Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, *Ceratitis capitata*, Entomol. Exp. Appl. **11** (1986) 231–235.

[3] STEFFENS, R., Combination of radiation and translocation induced sterility for genetic control of fruit flies, Entomol. Exp. Appl. **33** (1983) 253–258.

[4] WHITTEN, M., Automated sexing of pupae and its usefulness in control by sterile insects, J. Econ. Entomol. **62** (1969) 272–273.

[5] RÖSSLER, Y, Automatic sexing of the Mediterranean fruit fly: the development of strains with inherited, sex-limited pupal colour dimorphism, Entomophaga **24** (1979) 411–416.

[6] ROBINSON, A.S., RIVA, M.E., "Male-linked translocations in *Ceratitis capitata* for genetic sexing and irradiation", Fruit Flies of Economic Importance (Proc. 1st Int. Symp. Athens, 1982), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1983) 190–197.

[7] ROBINSON, A.S., ZAPATER, M., "Genetic and cytological studies of translocations in *Ceratitis capitata*", Fruit Flies of Economic Importance 84 (Proc. CEC/IOBC Working Group, Hamburg, 1984), (CAVALLORO, R., Ed.), (1986) 149–153.

[8] ROBINSON, A.S., Unexpected segregation ratios from a male-linked translocation in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Genetica **62** (1984) 209–215.

[9] KONOVALOV, C.A., FOSTER, G.G., WHITTEN, M.J., Viability and fertility of sex-linked autosomal duplications in *Lucilia cuprina* (Wied.), Theor. Appl. Genet. **65** (1983) 9–16.

[10] ZAPATER, M., ROBINSON, A.S., Sex chromosome aneuploidy in male-linked translocations in *Ceratitis capitata*, Can. J. Genet. Cytol. **28** (1986) 161–167.

[11] BEDO, D., Polytene chromosome mapping in *Ceratitis capitata* (Diptera: Tephritidae), Genome **29** (1987 598–611.

[12] ZACHAROPOULOU, A., Cytogenetic analysis of mitotic and salivary gland chromosomes in the Medfly, *Ceratitis capitata*, Genome **29** (1987) 67–71.

[13] STEFFENS, R., Methodology of translocations production and stability of translocations in the Mediterranean fruit fly, *Ceratitis capitata*, Z. Angew. Entomol. **95** (1983) 181–188.

TEMPERATURE SENSITIVE LETHAL FACTORS AND PUPARIAL COLOUR SEX SEPARATION MECHANISMS IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

E. BUSCH-PETERSEN
FAO/IAEA Entomology Unit,
IAEA Seibersdorf Laboratory,
Vienna

Abstract

TEMPERATURE SENSITIVE LETHAL FACTORS AND PUPARIAL COLOUR SEX SEPARATION MECHANISMS IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

A programme to develop genetic sexing mechanisms in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), was initiated at the IAEA Laboratories, Seibersdorf, in 1983. Because of the potential benefits arising from the elimination of females early in the developmental cycle, combined with the anticipated relative ease of inducing temperature sensitive lethal (*tsl*) factors, it was decided to attempt to induce and isolate *tsl* factors active in the egg or early larval stages. Initially, five recombination suppressor (RS) strains were isolated. The degree of recombination suppression ranged from 77.6% to 99.1%. The viability of each of the five RS strains was assessed and RS 30/55 was selected as the most suitable strain. Ethyl methanesulphonate (EMS) was used to induce the *tsl* factors, by feeding two-day old adult males with a suspension of EMS in a 10% solution of sugar in the drinking water supply. Temperature tolerance tests indicated a discriminating temperature of 32°C when isolating *tsl* factors active in the egg stage and 35°C when isolating such factors in the early larval stage. A total of 39 and 22 *tsl* factors have been isolated in the two stages, respectively. However, none has yet proved stable. Induction of *tsl* factors with a reduced dose of EMS is now being attempted. An alternative genetic sexing programme was initiated in 1985, based on the use of pupal colour dimorphisms. Previously, a genetic sexing strain, T:Y(wp^+)101, based on a white female/brown male puparial colour dimorphism, had twice been assessed for stability under mass rearing conditions. In both cases the sexual colour dimorphism disintegrated immediately. Another similarly dimorphic strain, T:Y(wp^+)30C, was developed. This strain remained stable for seven generations of mass rearing, after which it started to disintegrate. Disintegration of this strain was probably caused by accidental contamination by wild type medflies. The overall quality of T:Y(wp^+)30C was not significantly different from that of the newly colonized, wild type 'Sohag' strain, except that egg production was reduced to 56% when compared to the currently well adapted Sohag strain.

---

* This work forms part of a Joint FAO/IAEA Research Programme on the development of genetic sexing mechanisms in *C. capitata*.

1. INTRODUCTION

The release of irradiation sterilized insects has proved a very powerful tool in the control or eradication of a range of insect species around the world [1, 2]. This technology involves the colonization, mass rearing, sterilization and release of the target species into the target area. When the released sterile insects mate with the native ones a reduction in the reproductive potential of the native population occurs. Continuous releases of sufficient numbers of sterile insects will eventually lead to eradication of the target population [3]. The sterile insect technique (SIT) was effectively employed to eradicate, among others, the screw-worm fly, *Cochliomyia hominivorax* (Coq.), from southern USA and Mexico, and the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.), from southern Mexico and parts of Guatemala. Plans are now under way to eradicate the medfly from the whole of Central America and Panama.

Generally, only the released sterile males contribute effectively towards the induction of sterility into the natural target population, whereas the effects of the sterile females, when both sexes are released together, may range from slightly positive to strongly detrimental. Invariably, however, the sterile females will attempt to oviposit in host fruit, thus making the fruit prone to bacterial and fungal infections. Other potential advantages of rearing and releasing only males include financial implications, increased effectiveness of the SIT, and increased potential for SIT pest management [4]. The advantages of separating males and females prior to release are therefore vast. However, because of the inability to perform such separation automatically and reliably in most pest species, males and females are commonly released together.

Genetic sexing mechanisms have been developed in 15 insect species through the translocation of the dominant allele of a chosen 'sexing' gene onto the male determining chromosome [4]. When the programme reported here was initiated, such a system existed also in the medfly and was based on a black female/brown male puparial colour dimorphism [5]. However, because of the potential financial savings arising from the ability to eliminate the female sex prior to larval development, it was decided to attempt to induce and isolate temperature sensitive lethal factors in the egg or very early larval stages of the medfly. Subsequently, when Robinson et al. [6] showed that the absence of sterile females increased the mating efficiency of the sterile males with the wild females in field cages by 50–100%, this programme was extended to include research on pupal colour genetic sexing mechanisms.

2. INDUCTION OF TEMPERATURE SENSITIVE LETHAL FACTORS

Temperature sensitive lethal (*tsl*) mutations are very common in microorganisms [7], insects [8, 9] and mammals, and have commonly been shown to be

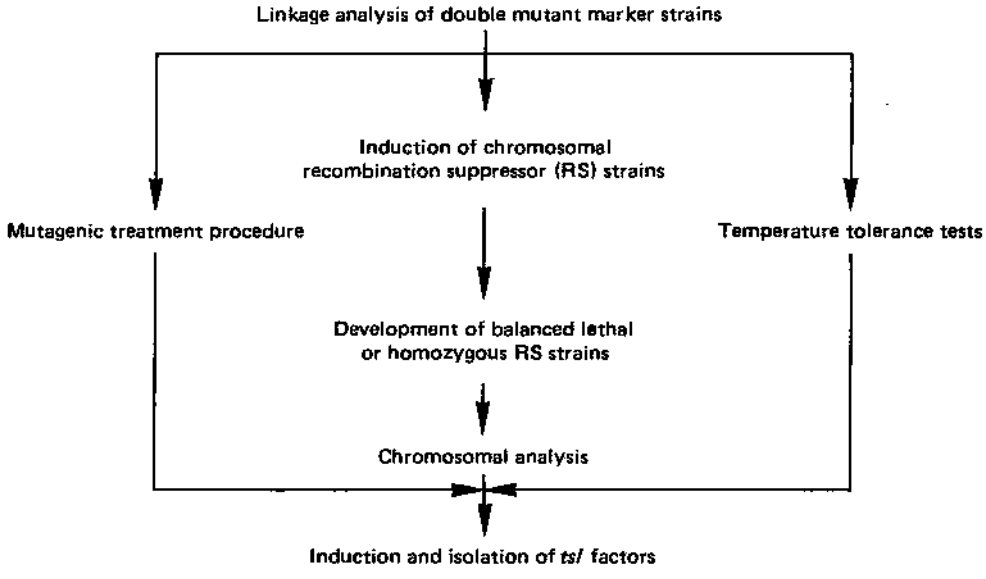


FIG. 1. Flow chart for the development of a genetic sexing mechanism for the medfly based on temperature sensitive lethal (tsl) *factors.*

the consequence of a single amino acid substitution in a polypeptide [10] which alters the biological activity of proteins at different temperatures [11]. Theoretically, it should therefore be possible to induce a *tsl* mutation at any locus involved in the production of a protein, thus rendering such loci potentially very common throughout the length of any one chromosome. Indeed, Suzuki [12] recorded 150 *tsl* factors on the *Drosophila melanogaster* X chromosome. Temperature sensitive lethal mutations are equally common on the autosomes and have been found to account for 10–12% of all ethyl methanesulphonate (EMS) induced lethal mutations [12]. In a study on the characterization of *tsl* mutations in *Musca domestica*, McDonald and Overland [13] recovered 10 *tsl* mutations among 900 EMS treated chromosomes. It is thus obvious that *tsl* factors are both quite common and rather easily induced with EMS. For these reasons, it was decided to pursue a programme on *tsl* induction and isolation, for subsequent employment as a genetic sexing mechanism for medflies mass reared for release in SIT programmes. The *tsl* induction and isolation programme was pursued as outlined in Fig. 1.

2.1. Linkage analysis of double mutant marker strains

The breeding scheme (Fig. 1) [4] required the availability of two linked mutant markers to enable the mutagenized chromosome to be tracked. Because of the low

number of morphological mutants available in the medfly, the choice was limited to either the apricot eye (*ap*)–double chaetae (*dc*) linkage group or to the orange eye (*or*)–white pupae (*wp*) linkage group. The distance between *ap* and *dc* is 18.25 recombination units and between *or* and *wp* 41.3 units. The *or–wp* linkage group appeared the more suitable as it would allow tracking of *tsl* mutations induced on a larger piece of the chromosome. However, the colour of the *or* phenotype was highly variable (probably affected by the rearing conditions) and was often difficult to distinguish from the wild type eye colour. The *ap–dc* linkage group was therefore selected.

2.2. Temperature tolerance tests

In attempting to isolate *tsl* factors it is obvious that the longer the duration of exposure to the discriminating temperature during the *tsl* isolation phase, the higher are the chances of isolating an appropriate allele during that particular phase. The limit to the duration of exposure is therefore determined mainly by the constraints imposed by the use of the prospective allele as a genetic sexing mechanism in a mass rearing operation. For this reason it was decided to attempt the induction only of *tsl* alleles which would be active during the egg and/or very early larval stages.

After a suitable linkage group had been selected, the temperature tolerances of the double mutant strain, the two single mutant strains, and the wild type EgII strain were determined. Eggs were collected over a period of 16–18 hours, placed on filter paper in 9 cm diameter Petri dishes with larval diet, and counted. The Petri dishes were then placed in temperature controlled cabinets for two, three or

TABLE I. TOLERANCE OF EGGS/EARLY INSTAR LARVAE TO TEMPERATURE WHEN EXPOSED FOR 2 TO 4 DAYS IN LARVAL REARING CONTAINERS

Strain[a]	LT_{50}[b]	SE	LT_{10}[b]	SE	b	r
EgII	35.3 b	0.07	33.5 a	0.09	56.2	0.96
ap	34.3 a	0.03	33.0 a	0.05	73.3	0.99
dc	34.7 ab	0.05	32.7 a	0.08	50.7	0.94
ap–dc	34.3 a	0.03	33.0 a	0.08	74.0	1.00

[a] See text for detail.

[b] Same letter after LT values indicates no significant difference (t test) between respective values.

four days at a range of temperatures (±0.1°C). After exposure the dishes were returned to normal rearing conditions (25°C ± 1). At this time egg hatch was scored and all unhatched eggs were removed. Mortality was assessed in both the pupal and adult stages. No difference in mortality was observed between exposures of two, three and four days duration, suggesting that the more sensitive stage is within the first two days of egg collection. The data for all three durations are therefore combined in Table I. Mortality, when scored at the pupal stage, was not significantly different from that recorded in the adult stage.

The mutant strains were all found to exhibit very similar tolerance levels to temperature treatment, whereas the LT_{50} of the wild type EgII strain was about 0.6–1.0°C higher than the LT_{50} of the three mutant strains. All strains produced full mortality at 36°C but none at 32°C. The LT_2 of *ap–dc*, the most sensitive strain, was located slightly above 32°C. This temperature was used as the discriminating temperature for the isolation of *tsl* factors in the egg stage.

Later in the *tsl* programme it was decided to transfer selection from the egg stage to the first and second instar larvae. Preliminary experiments (unpublished) indicated that the highest temperature tolerated by newly hatched larvae was 35°C, when exposed over a period of three days, whereas 37.5°C would cause over 80% mortality in all strains. A discriminating temperature of 35°C was therefore used during the latter part of the programme.

2.3. Mutagenic treatment procedures

EMS has been used extensively to induce mutations in *Drosophila*, either by injection of EMS into male adults [14] or by feeding of adult flies [15, 16]. Larval feeding or dipping have been shown to be rather inefficient in inducing sex linked recessive lethals [17]. EMS has been widely used in attempts to induce mutations in the medfly, but only Lifschitz [18] has reported any success. We therefore investigated a series of EMS application procedures in order to assess their efficiency in inducing dominant lethal mutations in medfly males.

A total of ten treatment procedures was assessed that involved 'egg/larval feeding', feeding of third instar larvae, dipping of pre-pupae, dipping of pupae (8 days old) with and without 1% demethyl sulphoxide (DMSO), exposure of pupae (8 days old) to vapour, exposure of adults (2 days old) to vapour, feeding of adults (2 days old) with 1% and 10% sugar water and injection of adults [19].

The uniformity with which the EMS was taken up by the treated males was measured by mating the treated males individually to single females. On the basis of the arbitrary criterion that at least 80% of these females should show some degree of fertility when mated to males treated with the concentration of EMS causing 80% dominant lethality, only the procedures involving feeding of third instar larvae, dipping of pre-pupae and feeding of adults using both 1% and 10% sugar proved effective. However, of these four procedures, the EMS caused an unacceptably high

level of mortality in the treated parental males when administered to third instar larvae and to pre-pupae, leaving only the two adult feeding procedures as potential candidates. A direct correlation between the concentration of EMS administered and the subsequent F_1 dominant lethality was observed when the adult males were fed with EMS containing 10% sugar ($\chi^2 = 2.29$; $P > 0.1$) [20], but not when they were fed EMS containing 1% sugar. Feeding of adults with EMS containing 10% sugar in the drinking water supply was therefore utilized for the subsequent induction of *tsl* factors.

2.4. Induction and isolation of recombination suppressors

Genetic recombination in the medfly occurs in the female, whereas recombination in the male was, until recently [21, 22], thought to be totally absent. Such recombination has the effect of severing the linkage between the EMS treated chromosome segment and the marker genes used for tracking that segment through the appropriate breeding schemes. It was therefore necessary to prevent or reduce the appearance of recombinant types in the medfly.

The breeding scheme used for the isolation of induced recombination suppressors (RS) [23] involved crossing gamma irradiated males of the *dc/dc* strain to *ap/ap* females. The resultant F_1 females were then crossed in single pairs to *ap–dc/ap–dc* males and the F_2 offspring were screened for families showing absence of recombinant phenotypes. When such families were observed, the *dc* phenotypes were further inbred in single pairs in order to produce a true breeding strain homozygous for the RS factor.

Five RS strains were isolated from a total of 570 screened chromosomes. The recombination frequency between the *ap* and *dc* genes in the five strains ranged from 0.17% to 4.09% in the RS heterozygotes, as compared to a normal frequency of 18.25%. Thus, the degree of recombination suppression ranged from 77.6% to 99.1%.

The viability of the five RS strains, measured as percentage egg hatch and as adult emergence, was assessed both in the homozygous and the heterozygous conditions, and compared with that of the *dc/dc* strain. Egg hatch in the five homozygous RS strains ranged from 55.0% to 80.7% as compared to 77.2% in *dc/dc*, while adult emergence was not significantly reduced in any of the strains. In the heterozygous condition egg hatch was significantly reduced in all RS strains while adult emergence was significantly reduced only in RS 30B, RS 109 and RS 30/55 [24].

The most efficient reduction of heterozygous recombination suppression was observed in strains RS 30/55 and RS 30B, which suppressed recombination between *ap* and *dc* by 98.1% and 99.1%, respectively. However, whereas RS 30B showed an overall corrected egg–adult viability in the heterozygous condition of 53.3%, the corresponding viability in RS 30/55 was 61.1%. RS 30/55 was therefore subsequently employed in the *tsl* induction programme.

2.5. Chromosomal analysis of RS strains

Meiotic and mitotic analysis of the RS strains showed that all strains contained a reciprocal autosomal translocation [23]. All translocations involved the long arm of chromosome 4, thus indicating that the *dc* gene is located in this region. Reciprocal chromosome arrangements also involved chromosomes 2, 5 and 6.

Reciprocal autosomal translocations may suppress recombination either through the creation of unbalanced gametes when recombination takes place in the interstitial segment between the breakpoint and the centromere in the translocation heterozygote and is followed by alternate segregation [25], or through interference in the translocation heterozygote with the initiation or maintenance of cross-over synapsis [26]. In fact, no consistent correlation was seen between the location of the breakpoint on chromosome 4 and the degree of recombination suppression, thus suggesting that at least some of the translocations interfered with the initiation or maintenance of cross-over synapsis [23].

2.6. Induction and isolation of *tsl* factors

A total of 39 promising F_3 families was obtained from 3416 screened chromosomes, all families showing clear evidence of temperature sensitivity. However, although single pair sib-selection was performed in these families for up to a further seven generations, this sensitivity was invariably lost and it proved impossible to isolate a pure breeding line. It was therefore decided to place the selection pressure on early instar larvae rather than, as previously, in the egg stage, in the hope that induced *tsl* factors would be more stable in this stage.

Until July 1988, 22 promising *tsl* families were isolated from 1955 screened F_3 chromosomes. Five of these families are still under investigation, whereas those remaining again proved unstable. There appears to be no fundamental difference in the behaviour of *tsl* alleles induced in the egg stage and in the early larval stage; the percentage of promising families (1.14% and 1.13%, respectively) was also similar.

The reason why all the isolated *tsl* families have so far proved unstable is unknown. However, it appears that we may be inducing a very high proportion of non-*ts* lethals which accumulate upon inbreeding. We are now attempting induction of *tsl* factors with reduced dosage of EMS.

3. DEVELOPMENT, STABILITY AND QUALITY CONTROL OF TWO PUPAL GENETIC SEXING STRAINS

A major reason for our interest in and efforts to develop a genetic sexing mechanism that will allow the separation of male and female medflies early in development arises from the cost, time and space involved in larval rearing. If the

female sex could be eliminated at an early stage, nearly 50% of the normally consumed larval diet could be saved. This again would lead to a corresponding saving in rearing space, time and manpower. Although this justification is still valid, the finding by Robinson et al. [6], that the release in field cages of only the male sex increased the mating competitiveness of these males by 50–100%, suggests that a similar cost reduction may also be obtained by releasing sterile flies separated sexually at later stages in development. For this reason we also started investigating the possibility of creating sexual dimorphisms in the pupal stage.

Genetic sexing in the medfly was pioneered by Rössler [5], who translocated the wild type brown allele of a black pupae gene onto the Y chromosome. This was later followed by the development of a mechanism based on a brown/white pupal colour dimorphism [27]. Our involvement has been restricted to work on two such strains, namely T:Y(wp^+)101 and T:Y(wp^+)30C [28].

3.1. Stability of T:Y(wp^+)101 under mass rearing conditions

The white female/brown male puparia strain [29] was brought up from a single true breeding pair of flies and mass reared for five generation in 1985. Mass rearing was performed as described by Hooper [30] with some minor modifications [31]. To assess the frequency of aberrant phenotypes, two samples of 100 brown and 100 white pupae were taken in each of generations F_1 to F_5. Aberrant phenotypes, i.e. females from brown puparia and males from white puparia, were isolated as virgin flies within 24 hours of emergence and outcrossed individually to a standard white pupae (*wp/wp*) strain [32] in order to confirm their respective genotypes [31]. Generations were kept separate and aberrant types were not removed when adult mass rearing cages were set up.

Aberrant female phenotypes were observed from generation 1 onwards and increased steadily to reach 32.0% of all females by generation 5. Of 117 aberrant females observed, 65% reproduced and were all confirmed as carrying the wp^+ allele. Only heterozygous wp^+/wp were observed during the first three generations of mass rearing but, in generations 4 and 5, 11.1% and 19.0%, respectively, of female aberrant types were confirmed as being wp^+/wp^+ homozygotes.

Aberrant male (white pupae) phenotypes were first confirmed in generation 1 but an increase in frequency was not seen until generation 3. By generation 5, the level of observed aberrant male phenotypes had reached 16.5% of all males screened. Of the 75 aberrant males observed throughout the experiment, 69.3% reproduced and all were confirmed as being homozygous *wp/wp*.

When releasing genetically sexed flies in an SIT programme, the factor of overriding importance is that of females emerging from brown puparia. In T:Y(wp^+)101, this level reached 40.7% in generation 5, while the corresponding figure for males emerging from white puparia was 19.9%.

The rapid breakdown of this genetic sexing strain led to the conclusion that the instability was most likely caused by a relatively low frequency of male recombination in the region between the translocation breakpoint and the *wp* allele, probably below 0.05%, combined with a rather high genetic load on the *wp* allele. The acceleration of the rate of breakdown by a low level of wild type contamination could not be excluded [31]. Hooper et al. [33] reached a similar conclusion from an earlier attempt in 1983 to mass rear this strain (for a more detailed analysis and discussion of the results see Ref. [31]).

3.2. Stability of T:Y(wp^+)30C under mass rearing conditions

The T 30C strain [23] was isolated as part of the programme to induce recombination suppressors in medfly females and subsequently outcrossed to *wp/wp* females in order to develop the T:Y(wp^+)30C strain, in which females emerge from white puparia and males from brown (for a complete history of this strain see Ref. [28]). Mass rearing was performed over ten generations in 1987, as described above, and stability was assessed in the same manner as for T:Y(wp^+)101, except that more samples were analysed.

The first aberrant female phenotypes were observed in generation 3, and from generation 4 onwards their numbers increased steadily to reach 6.8% in generation 10. Of the 195 aberrant females observed throughout the experiment, 44.1% reproduced. All were confirmed as being heterozygous wp^+/wp, except for one wp^+/wp^+ which was observed in each of the last three generations.

Aberrant male phenotypes were found in each generation except the fourth; the highest level was observed in generation 7 at 0.89% of the total males screened. Although 27 of the total 37 aberrant males were found in the last five generations, their numbers fluctuated erratically and no correlation could be established with generation progression. A total of 35.1% of the aberrant males reproduced; all were confirmed as being wp^+/wp.

The level of females emerging from brown puparia reached 6.9% in generation 10, while that of males emerging from white puparia reached 0.5%.

The pattern of instability in T:Y(wp^+)30C differed substantially from that of T:Y(wp^+)101. Firstly, T:Y(wp^+)30C remained stable over a longer period of time. Secondly, although the levels of female aberrant types were similar in the two strains in generation 7 of the former strain and generation 1 of the latter, it had reached 6.9% in T:Y(wp^+)30C, and 19.0% in T:Y(wp^+)101 three generations later. Thus, the breakdown rate was more pronounced in T:Y(wp^+)101. Thirdly, no increase in the level of aberrant male types was observed in T:Y(wp^+)30C, whereas these types increased at about half the rate of the female types in T:Y(wp^+)101.

The last feature in particular argues against male recombination as the cause of the instability in T:Y(wp^+)30C. If recombination had indeed occurred then the resultant T:Y(*wp*) males would initially produce all white puparia males, thus

making this type common in the population. This was not observed in T:Y(wp^+)30C. A more likely explanation for the observed instability is accidental contamination by a wild type medfly strain. Experimental laboratory scale contamination of this strain provided comparable data when the strain was contaminated either with females alone or with a low level of mixed sexes (unpublished data). In addition, computer simulations by Hooper et al. [33] on the effects of contamination by females alone confirms this suggestion. Busch-Petersen and Kafu [31] thus concluded that the instability of T:Y(wp^+)30C was most likely caused by contamination of the genetic sexing strain (for a more detailed analysis and discussion of the results see Ref. [31]).

3.3. Quality control of T:Y(wp^+)30C under mass rearing conditions

During the ten generations of mass rearing of T:Y(wp^+)30C, several quality control parameters were assessed. These were compared with corresponding values of the newly colonized, wild type Sohag strain [30] or with the currently reared Sohag strain. The parameters included egg production, egg hatch, pupal recovery, pupal size, pupal colour ratio, adult emergence and flight ability. All parameters were assessed as described by Busch-Petersen and Kafu [34].

Egg production in T:Y(wp^+)30C was significantly reduced as compared to the currently reared Sohag strain, but increased from 34% during the initial generation of mass rearing to a mean of 56% during the last three generations. It thus appears that the mass rearing procedure selects heavily for increased egg production. However, as comparable data are not available for the newly colonized Sohag strain, a valid comparison could not be made.

The mean egg hatch of 68.4% was below the 77.6% of Sohag, but well within the range expected of a strain carrying a male linked translocation. The same applied to the mean pupal recovery, although the mean recovery in Sohag was still within the tolerance limit of T:Y(wp^+)30C. In addition, pupal recovery also increased substantially with selection throughout the period of mass rearing.

Pupal size, adult emergence and flight ability in T:Y(wp^+)30C were all higher than the corresponding values for Sohag. Again, flight ability increased with selection. The total mean recovery of flying adults from hatched eggs was about 31.1% in Sohag [30] and 29.7% in T:Y(wp^+)30C [34]. Thus, although there were variations within each quality control parameter, the overall quality was not significantly different in the two strains. It was therefore concluded that the T:Y(wp^+)30C strain shows good potential for utilization in SIT programmes depending upon the elimination of females prior to release of the sterile males [34] (for a more detailed analysis and discussion of the results see Ref. [34]). Field cage studies and experimental field releases with this strain are now being planned.

ACKNOWLEDGEMENTS

I wish to thank Drs. L.E. LaChance, D.A. Lindquist, R.E. Gingrich and A.P. Economopoulos of the Joint FAO/IAEA Division for their support and encouragement in this work, and the participants of the FAO/IAEA Co-ordinated Research Programme for their valuable suggestions and advice. I also wish to thank Dr. D.I. Southern of the Department of Cell and Structural Biology, School of Biological Sciences, University of Manchester, United Kingdom, for his chromosomal analysis of the recombination suppressor strains. The technical assistance of A. Kafu, J. Ripfel[1], A. Pyrek[1], K. Kada, E. Schorn, A. Hafner, I. Szabo[1], S. Zorbic[1] and H. Baumgartner is gratefully acknowledged.

REFERENCES

[1] LINDQUIST, D.A., BUSCH-PETERSEN, E., "Applied insect genetics and IPM", Integrated Pest Management: Quo Vadis? (Proc. Parasitis 86 Mtg Geneva, 1986), (DELUCCHI, V., Ed.), (1987) 237–256.

[2] CURTIS, C.F., Genetic control of insect pests: growth industry or lead balloon?, Biol. J. Linn. Soc. **26** (1985) 359–374.

[3] KNIPLING, E.F., Possibilities of insect control or eradication through the use of sexually sterile males, Science **130** (1955) 902–904.

[4] BUSCH-PETERSEN, E., "Genetic sex separation in sterile insect technique pest management programmes, with special reference to the medfly, *Ceratitis capitata* (Wied.)", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Rome, 1987), (CAVALLORO, R., Ed.), Balkema, Rotterdam (in press).

[5] RÖSSLER, Y., Automated sexing of *Ceratitis capitata* (Dip.: Tephritidae): the development of strains with inherited, sex-limited pupal color dimorphism, Entomophaga **24** (1979) 411–416.

[6] ROBINSON, A.S., CIRIO, U., HOOPER, G.H.S., CAPPARELLA, M., Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, *Ceratitis capitata*, Entomol. Exp. Appl. **41** (1986) 231–235.

[7] EDGAR, R.S., LIELAUSIS, I., Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization, Genetics **49** (1964) 649–662.

[8] SUZUKI, D.T., et al., Temperature-sensitive mutations in *Drosophila melanogaster* — I. Relative frequencies among gamma-ray and chemically induced sex-linked recessive lethals and semilethals, Proc. Natl. Acad. Sci. U.S.A. **57** (1967) 907–912.

[9] SMITH, R.H., Dominant and recessive lethal mutations induced by mitomycin C in *Habrobracon* oocytes and sperm, Genetics **56** (1967) 591 (abstract only).

[10] WITTMANN, H.G., WITTMANN-LIEBOLD, B., JAUREGUI-ADELL, J., Die primäre Proteinstruktur temperatur-sensitiver Mutanten des Tabakmosaikvirus, Z. Naturforschg. B **20** (1965) 1224–1234.

[1] Former staff members of the Joint FAO/IAEA Division.

[11] JOCKUSCH, H., Relations between temperature sensitivity, amino acid replacements, and quaternary structure of mutant proteins, Biochem. Biophys. Res. Commun. **24** (1966) 577–583.

[12] SUZUKI, D.T., Temperature-sensitive mutations in *Drosophila melanogaster*, Science **170** (1970) 695–706.

[13] McDONALD, I.C., OVERLAND, D.E., Temperature-sensitive mutations in the house fly: the characterization of heat-sensitive recessive lethal factors on autosome III, J. Econ. Entomol. **65** (1972) 1364–1368.

[14] IKEBUCHI, M., NAKAO, Y., Storage effects on translocations and dominant lethals induced by ethyl methanesulfonate (EMS) in *Drosophila melanogaster*, Jap. J. Genet. **54** (1979) 133–137.

[15] BARNETT, B.M., MUÑOZ, E.R., Recessive lethals induced by ethyl methanesulfonate and diethyl sulfate in *Drosophila melanogaster* post-meiotic male cells pretreated with butylated hydroxytoluene, Mutat. Res. **110** (1983) 49–57.

[16] MITCHELL, J.A., Fitness effects of EMS-induced mutations on the X chromosome of *Drosophila melanogaster* — I. Viability effects and heterozygous fitness effects, Genetics **87** (1977) 763–774.

[17] NASRAT, G.E., NAFET, H., AHMED, K.A., ABDEL-RAHMAN, A.H., Efficiency of different methods of application of EMS in inducing mutations in *Drosophila melanogaster*, Alex. J. Agric. Res. **26** (1978) 181–190.

[18] LIFSCHITZ, E., "New mutants in *Ceratitis capitata*", Report on Research Coordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Vienna, July 1985, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[19] BUSCH-PETERSEN, E., PYREK, A., RIPFEL, J., RUHM, E., Efficiency of different methods of EMS application for the induction of dominant lethals in germ cells of medfly, *Ceratitis capitata* (Wied.), males, Mutat. Res. **163** (1986) 247–254.

[20] BUSCH-PETERSEN, E., "Development of induced sex-separation mechanisms in *Ceratitis capitata* (Wied.): EMS tolerance and suppression of female recombination", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 209–216.

[21] RÖSSLER, Y., Genetic recombination in males of the Mediterranean fruit fly, and its relation to automated sexing methods, Ann. Entomol. Soc. Am. **75** (1982) 28–31.

[22] RÖSSLER, Y., Recombination in males and females of the Mediterranean fruit fly (Diptera: Tephritidae) with and without chromosomal aberrations, Ann. Entomol. Soc. Am. **75** (1982) 619–622.

[23] BUSCH-PETERSEN, E., SOUTHERN, D.I., Induced suppression of genetic recombination in females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), by translocation heterozygosity, Genetica **72** (1987) 161–169.

[24] BUSCH-PETERSEN, E., PYREK, A., RIPFEL, J., Viability and behaviour of translocations that suppress female recombination in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), Theor. Appl. Genet. 74 (1987) 38–41.

[25] STRICKBERGER, M.W., Genetics (2nd edn), MacMillan, New York (1976) 914 pp.

[26] ROBERTS, P.A., Screening for X-ray-induced cross-over suppressors in *Drosophila melanogaster*: prevalence and effectiveness of translocations, Genetics **65** (1970) 429–448.

[27] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata* — a suitable case for genetic sexing, Genetica **58** (1982) 229–237.

[28] BUSCH-PETERSEN, E., RIPFEL, J., PYREK, A., KAFU, A., "Isolation and mass rearing of a pupal genetic sexing strain of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)", Modern Methods of Insect Control: Nuclear Techniques and Biotechnology (Proc. Int. Symp. Vienna, 1987), IAEA, Vienna (1988) 211–219.

[29] ROBINSON, A.S., Unexpected segregation ratios from male-linked translocations in the Mediterranean fruit fly, *Ceratitis capitata*, Genetica **62** (1984) 209–216.

[30] HOOPER, G.H.S., Application of quality control procedures to large scale rearing of the Mediterranean fruit fly, Entomol. Exp. Appl. **44** (1987) 161–167.

[31] BUSCH-PETERSEN, E., KAFU, A., Stability of two mass reared genetic sexing strains in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), based on pupal colour dimorphisms, Environ. Entomol. **18** (1989).

[32] RÖSSLER, Y., The genetics of the Mediterranean fruit fly: a "white pupae" mutant, Ann. Entomol. Soc. Am. **72** (1979) 583–585.

[33] HOOPER, G.H.S., ROBINSON, A.S., MARCHAND, R.P., "Behaviour of a genetic sexing strain of Mediterranean fruit fly, *Ceratitis capitata*, during large scale rearing", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P. Ed.), Elsevier, Amsterdam and New York (1987) 349-362.

[34] BUSCH-PETERSEN, E., KAFU, A., Assessment of quality control parameters during mass rearing of a genetic sexing strain of the Mediterranean fruit fly, based on a pupal colour dimorphism, Entomol. Exp. Appl., in press.

EVALUATION OF A WHITE FEMALE PUPA GENETIC SEXING STRAIN OF MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), UNDER SELECTION PROCEDURES

A.P. ECONOMOPOULOS
FAO/IAEA Entomology Unit,
IAEA Seibersdorf Laboratory,
Vienna

Abstract

EVALUATION OF A WHITE FEMALE PUPA GENETIC SEXING STRAIN OF MEDITERRANEAN FRUIT FLY, *Ceratatis capita* (Wied.), UNDER SELECTION PROCEDURES.

During seven consecutive generations of mass rearing of a white female, brown male, genetic sexing strain of the Mediterranean fruit fly, *Ceratatis capitata* (Wiedemann), isolated at Seibersdorf (T:Y*(wp*$^{+}$*)*30C, hereafter referred to as *SWFP*), only eggs produced during oviposition days 7–9 (adult age of 9–11 days, respectively) were seeded into the larval medium. Data on the quality of the various life stages were compared with similar data from the 'Sohag' normal colour strain collected throughout 1987. The Sohag strain was mass reared at the FAO/IAEA Seibersdorf Laboratory from October 1983 until December 1987 when it was replaced by the *SWFP* strain. The mean amount of eggs collected daily per large colony cage of *SWFP* ranged between 40 mL and 62 mL in the successive generations, as compared to about 105 mL for the Sohag strain. Egg hatch in the *SWFP* successive generations varied between 71% and 76% as compared to about 85% for Sohag. Successful larval development in the artificial medium (percentage pupae from hatched eggs) fluctuated between 30% and 50%, while the average recovery in the Sohag strain was about 50%. Pupal size in the *SWFP* successive generations was always higher than in Sohag, i.e. 5.6 to 5.9 versus approx. 5.3 (size 5 on pupal day 4 corresponds to about 8.2 mg). Breakdown of the strain, as measured by the percentage of females emerging from brown pupae of the two highest larval collection days, was very low; increasing to only 1.2% by generation 7. Adult emergence and flight ability improved with the successive generations of *SWFP* from 64% to about 90% and from 50% to about 75%, respectively, while adult emergence and flight ability in the Sohag strain were around 93% and 90%, respectively.

1. INTRODUCTION

The development of genetic sexing strains in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), has received much attention in the last decade. The effort was motivated by the assumption that separation of the sexes, if possible at an early immature stage, and release of only sterilized

males would reduce the overall cost of the control operation, avoid puncturing of fruit by the released sterile females and, probably most important, would improve the effectiveness of the method. The latter because all the mating activity of the sterile males would concentrate on the wild females. At present, the only practical separation method available for the medfly involves strains with pupal colour sexual dimorphism. Rössler [1] first isolated a strain which had brown male and black female pupae. He also isolated a white pupa strain [2]. Robinson and Van Heemert [3], using this white pupa strain, induced and isolated several strains having brown male, white female pupae, which could be separated by an electronic colour separator (seed sorter) [4]. One of the white pupae strains [T:Y(wp^+)101] was subsequently mass produced and used for field studies [5, 6 and Economopoulos, unpublished]. Although the T:Y(wp^+)101 strain was stable under small scale laboratory rearing [7], it broke down rapidly under mass rearing [5]. Recently, a new white female strain of high stability (T:Y(wp^+)30C, hereafter referred to as *SWFP)* was induced and isolated at the FAO/IAEA Seibersdorf Laboratory [8] from Rössler's white pupa strain. When the *SWFP* strain was first compared with a wild type strain ('Sohag'), which had been mass reared at Seibersdorf for almost three and a half years, it was inferior in fecundity, fertility, larval growth, adult emergence and flight ability (first *SWFP* generations of this study). Preliminary results are presented here from the first seven consecutive generations of mass rearing the *SWFP* strain under selection procedure in which eggs collected only several days after females started ovipositing were used. The present mass rearing and selection study of *SWFP* will continue for some further generations. Field studies on the dispersal, survival and mating of *SWFP* males are also under way.

2. MATERIALS AND METHODS

Full scale mass rearing of the *SWFP* strain was initiated in Feb. 1988 when it was in the third generation. It started from a single pair and increased to relatively large numbers during the first generation (Busch-Petersen, personal communication). Then it was transferred to the medfly mass rearing facility of the Seibersdorf Entomology Unit, where it was further increased during the second generation. By the third generation the first full scale mass rearing generation was possible. The mass rearing generations 1–7 of Table I thus correspond to strain generations 4–10. From Oct. 1983 to Dec. 1987, the Sohag normal strain had been mass reared in the facility. This started from pupae collected from guava in the Sohag Governate, Egypt. After the Sohag strain was discontinued in Dec. 1987, to allow mass rearing of *SWFP*, no simultaneous comparison between the two strains was possible. Instead, data from the successive mass rearing generations of *SWFP* were compared with means of control treatments from 16 different experiments or routine productions of Sohag throughout 1987. In this way, the successive mass rearing generations

of a newly established genetic sexing strain were compared with a normal strain that had been mass reared for more than three consecutive years.

In each successive mass rearing generation of *SWFP*, four large cages (2.0 m × 1.8 m × 0.2 m) were each loaded with 4.5 L (about 260 000) pupae. This resulted in about 170 000 – 230 000 adults per cage. The number increased with the generations because of an increase in adult emergence (Table I). The cages were maintained for two weeks (12 egging days) at 26°C ± 2, 60% ± 5 RH and 14 h:10 h light:dark photoperiod. For selection purposes, only eggs collected during oviposition days 7–9 (i.e. adult days 9–11) were seeded into the larval diet [9]. In each successive generation the following quality control parameters were studied: number of eggs laid and their hatch, larval survival and pupation, pupal size, strain breakdown (percentage females from brown pupae), and adult emergence and flight ability (for more information on the mass rearing and laboratory quality control procedures followed see Refs [10] and [11]). Data are presented as means ± their standard deviations (SD).

3. RESULTS AND DISCUSSION

The summarized results are presented in Table I.

3.1. Egg collection

During the first three generations less than 50 mL of eggs were collected daily per mass rearing cage of *SWFP* (eggs were collected for 12 days; 1 mL is equivalent to about 25 000 eggs). By generation 6, egg production increased to about 60 mL. The drop seen in generation 7 was probably caused by an increase in temperature which occurred in the egging room during days 3–6 of that generation. The increased volume of eggs collected in generations 4–6 could be partly related to the increase in adults, because of increased adult emergence in those generations. The mean daily egg collection observed in the Sohag strain during 1987 was about double that observed in *SWFP* generations 4–6.

3.2. Egg hatch

Two 100-egg samples were examined daily from each mass rearing cage. The mean percentage egg hatch (mean of 12 day means of four cages) was between 71% and 76% throughout the first six generations. In the seventh generation it dropped to 64%, probably again owing to the increased temperature. In the Sohag strain the mean percentage egg hatch was remarkably stable at about 85% throughout 1987. Data in Table I show that there was no improvement in the egg hatch of the *SWFP* strain during the first seven generations of mass rearing.

TABLE I. LABORATORY PERFORMANCE OF A WHITE FEMALE PUPA MEDFLY STRAIN UNDER A MASS REARING SELECTION SYSTEM (MEAN ± SD)

(to produce the successive generations, only eggs collected during oviposition days 7–9 were seeded onto the starter larval medium)

Generation of mass rearing[a]	Eggs collected per large cage per day (12 collection days) (mL)	Egg hatch (4 replications) (%)	Recovery of hatched eggs to pupae (6–9 replications) (%)	Pupal size, only males (6–9 replications)	Females emerged from male pupae of the two highest larval collection days (6 replications)		Emergence from male pupae of 2nd larval collection day (6 replications) (%)	Flight ability of males of 2nd larval collection day (6 replications) (%)
					Day 1 (%)	Day 2 (%)		
1	47.6 ± 4.8	72.3 ± 1.6	40.9 ± 1.8	5.9 ± 0.01	0.0	0.4 ± 0.7	64.3 ± 4.5	50.5 ± 13.1
2	41.3 ± 2.7	75.7 ± 1.9	34.5 ± 3.0	5.6 ± 0.02	0.4 ± 0.9	0.4 ± 0.6	74.3 ± 6.5	50.3 ± 7.9
3	43.5 ± 2.9	71.8 ± 2.1	44.9 ± 6.5	5.8 ± 0.02	0.7 ± 0.9	0.0	87.7 ± 2.2	52.9 ± 6.5
4	61.3 ± 3.2	72.9 ± 2.4	30.0 ± 8.5	5.7 ± 0.04	0.2 ± 0.4	0.7 ± 0.6	89.5 ± 0.8	68.8 ± 9.9
5	50.5 ± 4.1	72.9 ± 1.3	43.6 ± 1.5	5.7 ± 0.01	0.9 ± 1.4	0.4 ± 0.6	91.7 ± 2.4	64.7 ± 11.3
6	62.5 ± 1.5	71.4 ± 1.2	42.7 ± 1.7	5.6 ± 0.00	0.7 ± 1.3	0.6 ± 0.6	92.8 ± 3.3	81.1 ± 7.4
7[b]	40.1 ± 1.7	63.7 ± 8.0	51.3 ± 2.2	5.5 ± 0.01	1.2 ± 1.2	1.1 ± 1.6	87.0 ± 1.4	72.2 ± 4.1
Control[c]	106.9 ± 15.8	85.2 ± 3.4	51.4 ± 10.9	5.3 ± 0.21	—	—	93.5 ± 2.3	89.9 ± 7.4

[a] Each generation: 4 large cages.

[b] High temperature in adult colony room during egg collection days 3–6.

[c] Means of 16 experimental control treatments or routine production logs of the Sohag normal mass rearing colony throughout 1987. The Sohag strain was replaced by the white female pupa strain in Jan. 1988.

3.3. Larval growth and pupation

The successful growth of the *SWFP* larvae in the standard larval medium (with starter) used at Seibersdorf was determined from the percentage of newly hatched larvae that completed their growth and pupated successfully. This was determined from 6–9 units, each consisting of 10 larval medium trays prepared in every generation. Each tray contained 5 kg of larval medium. Five mL of 48 hour old eggs (about 125 000 eggs) were seeded in each tray. During the first four generations the pupal recovery fluctuated between 30% and 45%. In the subsequent generations it was constantly about 40%, and reached 51% in generation 7. The latter was similar to the mean recovery of Sohag during 1987.

3.4. Pupal size

For each of the four successive larval collection days (grown larvae emigrating to pupation medium), 20 mL samples of pupae from every 10 tray unit were pooled together. A 20 mL sample of pupae was then separated from this pooled sample and was used to measure mean pupal size and number of pupae per mL for each of the four larval collection days. Subsequently, the above measurements were used to estimate the weighed mean pupal size and total number of pupae produced from each 10 tray rearing unit. The pupal size so estimated was between 5.5 and 5.9 in all generations (size 5 measured on 4 day old pupae is equivalent to about 8.2 mg). Size fluctuated from 5.9 in the first generation to 5.5 in the seventh. The mean size of Sohag strain pupae was smaller than that recorded in any generation of *SWFP*.

3.5. Strain breakdown

Breakdown was measured as percentage of females that emerged from brown pupae. Six samples of 100 brown pupae were taken from pooled samples of the two highest larval collection days (usually first and second collection days). Initially, no females, or very few, emerged from the above samples. However, by generation 7 the number of females that emerged from brown pupae increased to 1.2%. It was noted that the percentage of females that emerged from brown pupae was usually considerably higher in the third and fourth larval collection days than in the first and second presented in Table I. If we compare the above breakdown with that observed in the T:Y(wp^+)101 strain mass reared in Seibersdorf during 1983, i.e. 28% by generation 5 and 44% by generation 9 [5], we conclude that the *SWFP* strain is remarkably stable.

3.6. Adult emergence

The percentage of adult emergence from brown pupae was measured in six samples of 100 brown pupae taken from the pooled sample of the second larval collection day (this was usually the day of highest larval collection). It increased progressively from 64% to around 90%, a level similar to the Sohag strain.

3.7. Flight ability

Six 100-pupae samples were taken from the pooled sample of pupae of the second collection day. During the first three generations the percentage flight ability was about 50%, but increased thereafter to levels between 60% and 80% as compared to about 90% of the Sohag strain. The improvement observed was probably related to the selection procedure followed, by which only the flies that survived long enough and were able to reach water and food in the large crowded cages produced the eggs seeded into the larval medium. A supplementary, small cage experiment, conducted during the third mass rearing generation of *SWFP*, clearly indicated reduced mobility and reactivity of the fly. In the first generations it had also been observed that three days after adult emergence the flies concentrated in the lower part of the large cages. The crowding changed with the progress of generations so that by the sixth generation it occurred only after adult days 4–5. The concentration of flies in the lower part of cages was never reported with the Sohag strain.

4. CONCLUSIONS

Following seven generations of mass rearing under selection procedures, the volume of eggs collected per cage remained lower in the *SWFP* strain than in the Sohag strain. Also, there was no improvement in the percentage egg hatch of the *SWFP* strain, which was also substantially lower than in the Sohag strain. The percentage of pupal recovery appeared to have improved only in the seventh generation and should be verified in subsequent generations. The male pupal size in the *SWFP* strain was always larger than in the Sohag strain. This was possibly related to the reduced crowding of *SWFP* larvae in the medium because of reduced egg hatch. The breakdown of the *SWFP* strain was low until the seventh generation. Finally, after selection a clear improvement was observed in the adult emergence and flight ability, the former reaching normal levels while the latter was still substantially lower than in the Sohag strain. The percentage of flight ability of the *SWFP* males observed up to generation 7 of this study would be unacceptable by the required quality specifications for the medfly (for details of test at the fly production facility see Ref. [12]).

It is understood that, for a thorough evaluation of the suitability of the *SWFP* strain for application to the sterile insect technique (SIT), studies on dispersal, sur-

vival and mating in the field must also be undertaken, in addition to the laboratory studies, using *SWFP* males subject to the usual SIT procedures, i.e. sorting, storage, irradiation, marking, packaging, transport and release.

ACKNOWLEDGEMENTS

I wish to thank R.E. Gingrich and D.A. Lindquist for support and for reading this manuscript. I should also like to thank L. Lang, V. Wornoayporn, H. Görz and H. Gensthaler for technical help.

REFERENCES

[1] RÖSSLER, Y., Automated sexing of *Ceratitis capitata* (Diptera: Tephritidae): the development of strains with inherited, sex-limited pupal color dimorphism, Entomophaga **24** (1979) 411–416.

[2] RÖSSLER, Y., The genetics of the Mediterranean fruit fly, *Ceratitis capitata:* a "white pupae" mutant, Ann. Entomol. Soc. Am. **72** (1979) 583–585.

[3] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*, a suitable case for genetic sexing, Genetica **58** (1982) 229–237.

[4] ROBINSON, A.S., RIVA, M.E., "Male-linked translocations in *Ceratitis capitata* for genetic sexing and irradiation", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Athens, 1982), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1983) 190–196.

[5] HOOPER, G.H.S., ROBINSON, A.S., MARCHAND, R.P., "Behaviour of a genetic sexing strain of Mediterranean fruit fly, *Ceratitis capitata*, during large scale rearing", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 349–362.

[6] CIRIO, U., CAPPARELLA, M., ECONOMOPOULOS, A.P., "Control of medfly *Ceratitis capitata* (Wied.) by releasing a mass-reared genetic sexing strain", ibid., pp. 515–522.

[7] ROBINSON, A.S., Unexpected segregation ratios from male-linked translocations in the Mediterranean fruit fly, *Ceratitis capitata*, Genetica **62** (1984) 209–216.

[8] BUSCH-PETERSEN, E., RIPFEL, J., PYREK, A., KAFU, A., "Isolation and mass rearing of a pupal genetic sexing strain of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 211–219.

[9] FAY, H.A.C., A starter diet for mass-rearing larvae of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), J. Appl. Entomol. **105** (1988) 496–501.

[10] HOOPER, G.H.S., Application of quality control procedures to large scale rearing of the Mediterranean fruit fly, Entomol. Exp. Appl. **44** (1987) 161–167.

[11] ECONOMOPOULOS, A.P., et al., "Recent developments in medfly mass rearing", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 285–292.

[12] BRAZZEL, J.R., CALKINS, C., CHAMBERS, D.L., GATES, D.B., Required Quality Control Tests, Quality Specifications, and Shipping Procedures for Laboratory Produced Mediterranean Fruit Flies for Sterile Insect Control Programs, Rep. USDA/APHIS/PPQ, APHIS 81-51, US Dept of Agriculture, Washington, DC (1986).

GENETIC SEXING OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), IN HAWAII: PROBLEMS AND PROSPECTS

D.O. McINNIS*, D. HAYMER**, S.Y.T. TAM*, S. THANAPHUM**, C. GRACE*

*Tropical Fruit and Vegetable Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Honolulu, Hawaii

**Department of Genetics, University of Hawaii (Manoa), Honolulu, Hawaii, United States of America

Abstract

GENETIC SEXING OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), IN HAWAII: PROBLEMS AND PROSPECTS.

Research is continuing towards the ultimate goal of developing an efficient system of separating the sexes of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). The authors are evaluating existing pupal colour sexing strains, as well as the potential of genetic engineering in creating a strain with useful genetic sexing properties. Collaborative research is under way between the United States Department of Agriculture/Agricultural Research Service (Honolulu) and the University of Hawaii (D. Haymer) regarding molecular approaches to the problem. Two pupal colour sexing strains are being compared: one of pure European stock and one backcross Hawaiian strain derived from the former. Results are presented for laboratory viability and quality parameters between the two strains, and further comparisons are made for behaviour in the field, including mating cage and free release assays. To date, the results indicate that the Hawaiianized strain is very competitive with normal (non-translocated) strains, while the pure foreign strain performs at a substandard level in Hawaii. After three years and over 25 000 embryos injected, there is still no evidence for genomic transformation of the medfly using *Drosophila* p elements. On the basis of positive evidence from a recently developed assay with the oriental fruit fly, *Dacus dorsalis*, micro-injections with this species have been initiated. In the medfly, however, there is evidence for both apparent cytoplasmic inheritance of the neomycin resistance gene and bona fide transient expression of this gene. Currently being investigated are an alternative potential gene transfer system, concatemerized linear DNA of the neomycin structural gene, and metallothionein gene resistance as an alternative to neomycin resistance. Long range research has also been initiated to search for potential transposable vectors present in tephritids themselves.

1. INTRODUCTION

During the period of the principal investigator's research contract with the IAEA (1985–1988) we have focused research on two areas of study of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann): genetic sexing and genetic improvement by artificial selection. Because the former area is of primary importance to our work and is the basis for the current research agreement with the IAEA, the discussion below will be limited to matters relating to genetic sexing alone. Work on genetic sexing has progressed in three phases: (1) an evaluation of Dr. Alan Robinson's imported pupal sexing strain and other pure or hybrid strains under quarantine conditions; (2) laboratory and field tests involving normal and genetic sexing strains outside quarantine; and (3) an attempt to create a genetic sexing strain by p element mediated gene transformation in collaborative efforts with Dr. Krishna Kumaran and colleagues of Marquette University (Wisconsin, USA) and with Dr. David Haymer of the University of Hawaii.

The discussion begins with the pupal colour sexing strains [1] and leads into a consideration of our efforts at genetic engineering using *Drosophila* p element based genome transformation [2, 3].

Almost five years ago we imported a medfly pupal colour sexing strain, *wp-23*, from Dr. Alan Robinson (Research Institute ITAL, Netherlands). In addition, a second pupal colour sexing strain has been created with a Hawaiian medfly genetic background by repeated backcrossing of *wp-23* (or hybrid) males to Hawaii Laboratory females. The two sexing strains, *wp-23* and *wp-23* × Hawaii, hereafter called pure and Hawaiianized Robinson strains, respectively, have been compared with each other and with the standard laboratory strain regarding the following parameters: rearing (laboratory), mating competitiveness (field cage), dispersal (field) and longevity (field).

Rearing data were presented in detail at an IAEA Research Co-ordination Meeting in Vienna in 1985 [4]. The pure Robinson strain, under our laboratory conditions, has had significantly lower rates of fecundity and pupal yield than either the Hawaii Laboratory or the backcross hybrid strain, Hawaiianized Robinson. As expected, the egg fertility rates were significantly lower (60–80%) for both translocation based sexing strains than the normal Hawaii Laboratory strain (90+%). With regard to the stability of the sexing strains — males and females emerging from normal brown and mutant white pupae, respectively — both the Robinson and backcross hybrid lines have consistently shown excellent purity, i.e. about 1 per 400 of undesired brown pupae females or white pupae males. However, the inherent partial sterilities of the two strains make them susceptible to contamination by fertile flies originating either from the outside or from inherent rare genetic recombination which may or may not eliminate the translocation. Such contamination could pose a serious problem for a mass rearing operation, especially when the production facility is surrounded by a resident medfly population as in Hawaii.

TABLE I. MASS REARING OF PURE AND HAWAIIANIZED PUPAL COLOUR SEXING STRAINS OF *Ceratitis capitata* (Honolulu, HI 1988)

Generation	Pure Robinson			Hawaiianized Robinson		
	Yield (L)	Contaminants		Yield (L)	Contaminants	
		White	Brown		White	Brown
P	0.05	0.000	0.000	0.10	0.000	0.003
G_1	0.20	0.000	0.000	0.60	0.005	0.003
G_2	0.70	0.002	0.005	2.80	0.002	0.005
G_3	3.30	0.000	0.050	5.20	0.000	0.007
G_4	5.40	*0.470*	*0.530*	3.90	*0.003*	*0.003*

In the last six months we have begun mass rearing both pupal colour strains (Table I). As production approached 5 L per generation (about 300 000 pupae), the frequency of contaminants (white male, brown female) rose dramatically for the pure Robinson strain in the last (G4) generation. Coincidentally, the ratio of brown: white pupae increased steadily for the pure Robinson strain throughout the test, reaching about 3:1 in the G_4 generation. Outside contamination can be virtually excluded because of a tightly controlled quarantine situation and enclosed oviposition of lines rather than external (screen) collection of eggs.

The experiment with the pure Robinson strain was terminated after the G_4 generation because the contamination level reached about 50% for both pupal colours. The reason(s) for the dramatic breakdown is (are) not known, though the data suggest that a combination of high genetic recombination coupled to high fitness of contaminant recombinants (including a high relative viability of brown versus white) led to the breakdown. On the other hand, the Hawaiianized Robinson strain has not broken down through the G_4 generation; indeed, it shows no sign of breakdown — the levels of contaminants are low, characteristic of small scale populations (about 5000 pupae/generation). If this strain continues to show good mass rearing purity we shall examine it by means of a photoelectric mass sorter, and then potentially in a mass release sterile insect technique (SIT) programme in Hawaii. If this line should also eventually break down, we shall try to correct the problem, if possible.

We have been field testing the pure and Hawaiianized pupal colour strains in outdoor cages as well as under free release conditions. The outdoor cages (3 m × 3 m × 2.5 m high) are each placed over a single guava tree in an orchard at a University of Hawaii field experiment station. Observations on fly behaviour

TABLE II. CAGE MATING, DISPERSAL AND LONGEVITY IN THE FIELD OF PUPAL COLOUR SEXING STRAINS COMPARED WITH NORMAL HAWAII STRAINS (Honolulu, HI 1985–1988)

Strain	Cage mating		Dispersal, males (m)	Longevity, males (d)
	Males	Females		
1. Robinson	0.30±0.07	0.32±0.09	54.1±8.8	4.2±0.4
2. Hawaii Robinson	1.53±0.3	1.23±0.2	82.7±4.5	5.0±0.4
3. Hawaii wild	2.1±0.6	1.80±0.3	92.2±4.7	5.2±0.6
4. Hawaii Lab.	(1.0)	(1.0)	62.2±8.1	4.2±0.2

inside these cages have been made in a continuing series of studies on oviposition rates [5] and on mating and survival rates of normal and sterile flies under various sex ratios [6]. In the present experiments, gravid virgin flies of both sexes (100–200/sex) for the two sexing strains, Hawaii wild flies (G_1 to G_3 generations), and the 30 year old Hawaii laboratory strain, were released inside cages, and fly couples were collected during the normal morning mating hours (approx. 7–11 a.m.). Mating partners were identified by the presence of different fluorescent dyes, one for each strain. Relative mating frequency for each strain and sex were expressed as a proportion of the level for the Hawaii Laboratory strain (index = 1.0) (see Table II). The pure Robinson strain has mated about one third to one quarter as well as any of the other three strains, while the Hawaiianized Robinson has in general mated better than the Hawaii Laboratory strain, but less than the wild strain. This pattern held for both sexes. We have run six releases of dye marked adults in a University of Hawaii experimental arboretum containing many host trees. Approximately 5000 pupae per strain were released from a single location and emerging flies (males) were trapped (Steiner traps) over a two week period. Forty traps were set out in a circular pattern as far as about 250 m from the release point every 3–4 days for a 24 hour period. Trapped flies were identified according to strain by examination of head capsule dye under UV light. The pure Robinson strain has dispersed significantly less than either the Hawaiianized Robinson or wild strains (Table II). Survivals of all four strains were not significantly different from each other, but more test replicates might show a difference. Future plans call for: (1) releasing and comparing males only, females only, or bisexual populations; (2) releasing and comparing radiation sterilized or normal populations. If the Hawaiianized Robinson strain continues to look good it will be used in larger scale SIT pilot tests in Hawaii and perhaps elsewhere.

Worldwide research on medfly pupal colour sexing strains is important because such strains remain to date the only viable means to achieve males-only release populations under mass rearing conditions. Quantitative assessments of the role of the sterile female are needed under free release conditions, especially in the light of field cage results, which demonstrated a significant positive sterile female role [6]. Continued efforts to improve the quality of pupal colour sexing strains should continue because of the uncertain outcome of currently existing genetic engineering approaches to sexing tephritids. On the basis of a growing body of data, it appears that the backcross hybrid sexing strain is superior to the original, purely European strain (*wp-23*) for both mass rearing and field viability parameters, at least in Hawaii. Obviously, much more research is needed to discuss whether or not our experience in this matter can be generalized.

2. GENETIC ENGINEERING

For three years now, our laboratory has been working on a genetic engineering approach to genetic sexing under a co-operative agreement which began with Dr. Krishna Kumaran and colleagues at Marquette University. A *Drosophila* p element, flanking a bacterial gene which codes for resistance to the antibiotic neomycin, is being microinjected into medfly embryos in the hope of genetically transforming the medfly genome. Specifically, it is hoped that an active neomycin gene will insert itself into the male Y chromosome directly, in order that only males survive in larval diet containing neomycin. However, since the Y chromosome represents only about 5% of the total medfly genome, and because the Y chromosome is largely heterochromatic [7], p element insertion, if any, would be likely to occur on another chromosome. In such an event, the resistance gene could then be translocated to the Y chromosome following routine irradiation induction.

The sequence of steps in the entire process is as follows:

(1) p element construction and availability,
(2) Development of microinjection procedures for tephritids,
(3) Development of neomycin (geneticin) bioassay,
(4) Assessment of degree of p element expression, if any,
(5) Assessment of degree of genetic sexing breakdown.

The p element we are using was developed by Steller and Pirrotta [3] and was provided by courtesy of Dr. Krishna Kumaran. The p element is carried by a plasmid vector (pUChsneo) and contains a bacterial structural gene which codes for the detoxification of the antibiotic geneticin (closely related to neomycin). Activity of this gene has been greatly stimulated by a *Drosophila* heat shock promoter in successful transformation experiments with *Drosophila* [3]. Several laboratories continue to utilize p elements worldwide in attempts to transform various tephritid

TABLE III. FIRST GENERATION (G_0) MICROINJECTION OF *Ceratitis capitata* AND *Dacus dorsalis* EMBRYOS WITH *Drosophila* p ELEMENTS (Honolulu, HI)

Time period	No. of eggs injected	No. of eggs hatched	No. of pupae	No. of adults
1. 22 Nov. 85 to 20 Mar. 87	12 218[a]	648 (0.053)	283	223
2. 20 Mar. 87 to 21 Aug. 87	3 446[a]	674 (0.196)	231	179
3. 21 Aug. 87 to 21 Aug. 88	*9 600[a]*	*1730 (0.180)*	*330*	*180*
Total:	25 264	3052 (0.12)	844	582
4. 12 Jul. 87 to 21 Aug. 87	2 000[b]	620 (0.31)	125[+]	50[+]

[a] *C. capitata.*
[b] *D. dorsalis.*

species for genetic sexing purposes. The detailed procedures for handling eggs and microinjection techniques are as described by McInnis et al. in Ref. [8].

Following microinjection of p elements into embryos, the stepwise procedure over several successive generations (G_0 to G_3) is as follows (the terms neo and HI LAB represent neomycin (geneticin) and the standard, susceptible Hawaii Laboratory strain, respectively):

G_0: Injected eggs normal G_0 adults × HI LAB G_1 eggs;
G_1: G_1 eggs neo diet G_1 survivors inbreed G_2 eggs;
G_2: G_2 eggs neo diet G_2 survivors (all male *or* male and female).

This procedure continues, repeating the G_2 protocol until the particular line either dies out or looks good and is expanded in numbers for further testing.

By 21 Aug. 1988 we had microinjected over 25 000 medfly embryos in the G_0 generation (Table III). The percentage of punctured eggs that hatched improved gradually until March 1987, then rose dramatically owing principally to a subtle change in reducing pre-puncture egg desiccation. Average egg hatch rose from about 5% to about 20% after March 1987. Variation in egg hatch rate continued to vary widely, however: between 10% and 45% for separate cohorts. As noted in Table III, over 800 G_0 pupae have been collected and over 500 G_0 adults emerged. Of the latter, many survived to be outcrossed with HI LAB flies of the opposite sex and then to be egged. Nearly all of those egged produced hatched larvae and about one quarter of these yielded some pupae on the selective neomycin diet. The average yield was about 2% in the G_1 generation. Larval development was typically delayed one to

three days compared to larvae on normal diet, and adult emergence was considerably reduced from normal rates (about 95% down to about 50%).

Of the lines reaching the G_1 adult stage, most survived to be mated and egged for the G_2 generation. Of these, about 50% yielded pupae — a value twice that for the G_1 generation and four times that for controls. Pupal yield also rose dramatically to a level five times that of controls, on the average. Apparently, some increased tolerance to neomycin was transmitted from the G_1 survivors to their progeny. Similar improvements were realized in the numbers of G_2 lines providing adults and in emergence rates. In the G_3 generation, or the third cycle on selective media, some further improvement was realized in the proportion of egged G_2 adults reaching the pupal stage.

Several lines (approx. 10) which had survived three cycles on neomycin diet were then expanded for two cycles on a normal diet and evaluated in standard neomycin dose tests. The latter indicated at least severalfold better survival of these lines at each neomycin dose than the controls. Then, beginning in May 1987, we evaluated these lines for genomic transformation in high molecular weight DNA. No evidence for transformation has been found, suggesting that the resistance/tolerance we had observed was either already inherent in the medfly strains, or that the p elements were present as extrachromosomal, low molecular weight DNA plasmids. We have since observed that all G_3 selected lines have eventually decreased their tolerance to neomycin, sometimes very rapidly, i.e. in one generation. This behaviour tends to rule out natural inherent resistance in the medfly as a possible explanation. These available data suggest the possibility that the neomycin resistance may be transmitted extrachromosomally, then lost accidentally over time.

Since Jan. 1988 we have attempted to demonstrate transient expression of the pUChsneo structural gene for resistance to neomycin (geneticin) in the medfly. Transient expression, or biological activity of the non-integrated foreign gene, would be evident from significantly greater survival of the microinjected embryos reared on diet containing neomycin compared with that of non-injected embryos. Therefore, the transient expression bioassay involves treating some of the G_0 individuals on diet containing a weak concentration (100 ppm generally) of neomycin (Table IV). Tests consisted of two cohorts (50 eggs each) of microinjected eggs and two cohorts of normal eggs. Half of the injected and uninjected eggs were set on normal diet, and half on 100 ppm diet. In addition, some cohorts of each type received heat shocks and some did not. As can be seen from Table IV, punctured embryos survived significantly better on 100 ppm diet than unpunctured eggs, especially if they received the normal series of three heat shocks during the larval period. After correction for controls on normal diet, pupal yield of punctured eggs was 27.20 times that of unpunctured eggs when heat shocks were applied. The results for 500 ppm neomycin in diet indicate no significant difference between punctured and unpunctured eggs. Therefore, transient expression of the pUChsneo gene improves larval survival to normal levels when the neomycin dose is 100 ppm, but has no effect at 500 ppm.

TABLE IV. TRANSIENT EXPRESSION OF pUChsneo NEOMYCIN GENE IN *Ceratitis capitata* (Honolulu, HI 1988)

Treatment	No. of cohorts	Total eggs	Average pupal yield	No. of pupae/No. of hatched eggs: proportion of control
I. Punc.[1],100 ppm Neo:[2]				
With heat shock	4	200	0.57**[3]	1.36 (27.20)[4]
Without heat shock	7	350	0.36*	0.71 (1.73)
II. Punc., normal:				
With heat shock	5	250	0.42 n.s.	—
Without heat shock	6	300	0.51 n.s.	—
III. Unpunc., 100 ppm Neo:				
With heat shock	5	250	0.02	0.05
Without heat shock	7	350	0.20	0.41
IV. Unpunc., normal:				
With heat shock	5	250	0.45	—
Without heat shock	7	350	0.49	—
V. 500 ppm with heat shock:				
A. Punc. + Neo	7	350	0.03	0.10 (0.83)
B. Punc., normal	7	350	0.30	—
C. Unpunc. + Neo	7	350	0.05	0.12
D. Unpunc., normal	7	350	0.40	—

[1] Punc.: punctured (microinjected) embryos.

[2] 100 ppm Neo: 100 ppm (wt/wt) of geneticin (G418 Sigma) in larval diet.

[3] Results of χ^2 test, DF = 1, comparing pupal yield: punctured versus unpunctured control.

[4] Ratio of relative pupal yields for punctured versus unpunctured eggs after correction for normal diet controls.

* $P < 0.05$; ** $P < 0.01$; n.s. = not significant.

These results indicate that the presence of the foreign gene, pUChsneo, in medfly larvae can be activated (especially by heat shock) as it would in *Drosophila*, allowing for increased survival at low to moderate levels of neomycin. This focuses the overall problem of p element failure in tephritids squarely on the inability of the enzyme transposase to catalyse the integration of pUChsneo into medfly chromosomes. O'Brochta and Handler [9] have developed an in vivo excision assay to measure the

ability of the crucial transposase enzyme to function in a foreign host species. To date, they have not found evidence for p element transposase activity outside the family Drosophilidae. Of course, should their results be generalized across a wide range of species, the value of p elements for genetic engineering of insect pests will be very limited at best. Recently, we have gathered preliminary evidence for positive transposase activity using the same excision assay technique in the oriental fruit fly, *Dacus dorsalis*. More data are needed to confirm this unprecedented result, especially because false positives can occasionally occur. Meanwhile, we are exploring alternatives to p element mediated transformation, including a search for potential transposable vectors in tephritids themselves.

REFERENCES

[1] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*, a suitable case for genetic sexing, Genetica **58** (1982) 229–37.

[2] RUBIN, G.M., SPRADLING, A.C., Genetic transformation of *Drosophila* with transposable element vectors, Science **218** (1982) 348–53.

[3] STELLER, H., PIRROTTA, V., A transposable P-vector that confers selectable G418 resistance to *Drosophila* larvae, EMBO J. **4** (1985) 167–171.

[4] Report of Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through the Manipulation of Radiation-Induced Conditional Lethals and Other Genetic Measures, Vienna, July 1985, obtainable from the Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[5] McDONALD, P.T., McINNIS, D.O., *Ceratitis capitata* (Diptera: Tephritidae): oviposition behavior and fruit punctures by irradiated and untreated females in laboratory and field cages, J. Econ. Entomol. **78** (1985) 790–793.

[6] McINNIS, D.O., WONG, T.T.Y., TAM, S.Y.T., Mediterranean fruit fly (Diptera: Tephritidae): suppression efficiencies of unisexual and bisexual sterilized release populations in field cages, Ann. Entomol. Soc. Am. **79** (1986) 931–937.

[7] SOUTHERN, D.I., Cytogenetic observations in *Ceratitis capitata*, Experientia **32** (1975) 20–22.

[8] McINNIS, D.O., et al., "The Mediterranean fruit fly: progress in developing a genetic sexing strain using genetic engineering methodology", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) 251.

[9] O'BROCHTA, D.A., HANDLER, A.M., Mobility of p-elements in Drosophilids and Non-Drosophilids, Proc. Natl. Acad. Sci. U.S.A., in press.

ALCOHOLS AS DISCRIMINATING AGENTS FOR GENETIC SEXING IN THE MEDITERRANEAN FLUIT FLY, *Ceratitis capitata* (Wied.)

M.E. RIVA FRANCOS
Departamento Fisiología y Aplicaciones de la
Energía Nuclear,
Instituto Nacional de Investigaciones Agrarias (INIA),
Madrid, Spain

Abstract

ALCOHOLS AS DISCRIMINATING AGENTS FOR GENETIC SEXING IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

The locus of the alcohol dehydrogenase (ADH) has been used to develop a genetic sexing mechanism in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). Previous work (1982–1984) has led to the isolation of a translocation linking a null mutant of this locus to the Y chromosome of the males. This strain, T-128, together with others showing different ADH electrophoretic patterns, have been assayed for their resistance to alcohols, such as allyl-alcohol, pentynol, ethanol and 2-propanol. The strains carrying the T-128 translocation show a differential, sex dependent survival to some of these alcohols. Part of this work is still in progress. The mutagenic ethyl methanesulphate (EMS) is being used to induce new ADH null mutants using the strain T-128 as a marker. Several hundred females have been treated with 0.04% EMS and then outcrossed to T-128 males. Their progeny is put through selective larval medium (0.08% allyl-alcohol) and the surviving F_1 individuals and subsequent F_2 are being analysed. Population studies have shown that the genetic sexing strain, T-128, is a double translocation with complete linkage between the Adh^N allele (chromosome 2), and the Y chromosome, and incomplete linkage of the Y with the wild type allele of the apricot eye locus (ap^+) of chromosome 4.

1. INTRODUCTION

One of the most important goals in the research related to the sterile insect technique (SIT) for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), has been the development of a genetic sexing system for this species. Such a system, when applicable at an early developmental stage of the life cycle, would represent considerable economic advantages in a mass rearing facility, which is a major expense in the use of SIT for any species. The advantages and the necessity of such systems for sex separation have been pointed out by several authors [1–7].

The medfly ($2n = 12$; XX females, XY males) belongs to the order Cyclorraphidae, where crossing over is restricted to the female [8]. This allows the induction of stable translocations linking specific genes to the male chromosome.

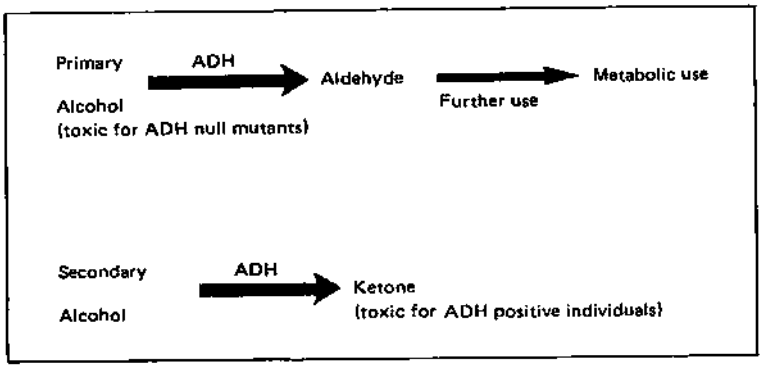


FIG.1. Relationship between presence or absence of ADH and alcohol toxicity.

Such male linked translocations can be used to develop genetic sexing strains if the Y linked gene is a morphological marker or a conditional lethal [9]. Recombination has been observed to occur at low rates in medfly males and to be slightly increased by the presence of chromosomal rearrangements [10]. Even a very low rate of recombination in the males could lead to breakdown of the translocation linkage and loss of genetic sexing qualities of the strain. Genetic sexing strains that seem very stable under laboratory conditions have already been produced in the medfly, such as those based on pupal colour [2, 11, 12] or sensitivity to purine [13] or alcohols [14]. It is relatively easy to induce Y autosome translocations in the medfly by irradiation [6], but finding or inducing conditional lethals is certainly much harder [15, 16].

We have chosen the alcohol dehydrogenase (ADH) locus to develop a genetic sexing system in the medfly, based on the model demonstrated for *Drosophila* [5]. In that species it is well known that individuals producing an active ADH enzyme can catalyse both the transformation of ethanol to acetaldehyde and the latter's conversion to acetate [17]. Individuals lacking an active form of this enzyme cannot perform these steps and are poisoned by environmental ethanol [18]. On the other hand, secondary alcohols are converted by ADH into ketones that are usually toxic [19]. Individuals producing active ADH perform this conversion and are poisoned by the ketones, whereas those lacking ADH activity show greater resistance to this type of alcohol (Fig. 1).

A translocation linking an ADH null mutant to the Y chromosome was isolated [14], first to serve as a marker for the identification and isolation of newly induced ADH null mutations [20] and as a shelter for the existing null mutation which is not viable as a homozygote [15], and, secondly, as a possible alternative to the genetic

sexing system based on resistance to ethanol. This translocation showed genetic sexing properties when exposed to allyl-alcohol,which acts similarly to secondary alcohols [21, 22] and kills preferentially ADH positive individuals. The T-128 males show a very decreased ADH activity. This fact, and the observation that it is probably a double translocation, have made T-128 the main object of our study, which can be divided in three principal parts:

(1) Induction of new ADH null mutants;
(2) Resistance of T-128 and other strains to various alcohols;
(3) Study of some life parameters of the genetic sexing line T-128 and other strains kept in our laboratory.

2. MATERIALS AND METHODS

2.1. Rearing conditions

General rearing conditions are as follows:

— Temperature: 22–27°C.
— Relative humidity: 50–60%.
— Adult food is a mixture of protein hydrolysate and sugar in varying proportions from 1:4 to 1:6.
— Strains are maintained in populations ranging from 200 to 900 individuals in cylindrical horizontal cages (20 cm × 13 cm).
— For single pairs, families or small numbers (up to 15 females × 15 males) small cubic cages (9 cm × 9 cm × 9 cm) are used.
— Eggs are collected in water and seeded every two days onto larval medium. Normally two or three egg collections are used for maintaining the lines.
— Larval diet: 320 g carrot powder; 120 g brewer's yeast; 110 mL 1N hydrochloric acid; 12.5 mL formaldehyde 4%; 15 mL propionic acid; and 1800 mL distilled water.
— Pupation takes place in bran.

2.2. Strains

SS: Strain originated from the mass rearing facility of the FAO/IAEA Laboratory at Seibersdorf. It is homozygous wild type for pupal and eye colour and homozygous for the S allele of the ADH locus.

wp ap F: Triple marker strain, homozygous for the following alleles: white pupae (*wp*, chromosome 5) apricot eye (*ap*, chromosome 4) and fast ADH (F, chromosome 2).

T-128^S: Translocation strain linking *Adh*^{N} (a null allele of ADH) to the Y chromosome, i.e. genotypically: male Y-N/S wp^+/wp^+; ap^+/ap^+; female XX SS wp^+/wp^+; ap^+/ap^+.

T-128^F: The same translocation as above but genotipically: male XY-N/F, female XX FF. It is maintained with wild type males and apricot eye females (*ap* males and ap^+ females are also produced in this strain but are removed each generation).

2.3. Electrophoresis

Servalyt prepacked gels (pH3–10) are used. These gels use the principle of isoelectrofocusing. Samples are prepared from single adult flies or pupa, homogenized in 100–200 mL distilled water. Sample papers are soaked in the homogenate and placed in the gels near the anode. The gels are run at 4°C for a minimum of 150 min; the first 30 min is a prefocusing run without samples, at 1700 V, 7–10 mA and 4.5 W. They are stained by incubation at 37°C for 30–45 min with 100 mL of 0.04M triphosphate buffer, pH8.6, 25 mg NAD^+, 20 mg NBT, 2 mg PMS and 5 mL 2-propanol.

2.4. Chemical mutagenesis

Two mutagens were used: formaldehyde and ethyl methanesulphonate (EMS). The formaldehyde was added to the larval medium in concentrations of 0.4% and 0.8% vol./vol. of the liquid components of the diet. The strain used was SS, and surviving males were outcrossed to the appropriate females. The F_1 larvae were reared in selective medium containing allyl-alcohol. The surviving males were backcrossed to parental females and the surviving females to T-128 males.

EMS was used with adult SS females according to the technique described by Busch-Petersen et al. [23]. They were starved for 24 h just after emergence and then treated with 0.04% or 0.08% EMS in 10% sugar water for 24 h. The EMS solution was removed, the flies were provided with normal food and water for three days and then mated to T-128^F males. F_1 eggs were placed on a medium containing 0.088% allyl-alcohol or on the regular diet as controls. The quantity of eggs treated was estimated by volume (1 mL = 20 000 eggs). Eggs of other strains were also occasionally put on the selective medium. Egg hatch, larval and pupal survival could then be counted directly.

2.5. Resistance to alcohols

The alcohols 1-pentyn-3-ol, 2-propanol and ethanol were incorporated in the larval diet. The two latter alcohols were tested in five concentrations. Eggs of the tested medfly strains were placed on black filter paper on top of the alcohol-

containing diet in a sealed Petri dish. Egg hatch was recorded following 7 days of incubation, and pupating larvae were collected and recorded.

Allyl-alcohol was incorporated in the larval diet as described above in the EMS section, but has also been tested in the egg bubbling flasks. A specified volume of eggs was placed in distilled water at 30°C for 48–72 h. One mL of allyl-alcohol was then added to every 500 mL of water in the bubbling flasks. These were sealed with parafilm for 2 h. Eggs were then washed twice with distilled water and seeded onto the regular larval diet. The number of resultant pupae and the adult sex ratio were then recorded.

2.6. Life parameters

The strains used in this work are monitored regularly for some life parameters and for control of their genetic characteristics.

T-128 was first maintained by alternately outcrossing the males to FF females one generation and to SS females the next [24]. However, as this is time consuming for routine maintenance, it was decided to maintain it as two separate strains (see above): $T\text{-}128^S$ and $T\text{-}128^F$. This latter strain comes from outcrossing T-128 males in generation F_{35} to *wp* ap^F females, and backcrossing the male progeny (Y–N/F, F_{36}) to *wp* ap^F females. From generation F_{37} it was inbred using wild type males and apricot eye females (see Section 3 below).

Both T-128 lines are checked every generation by outcrossing a sample of the males to females carrying the other electrophoretic ADH positive allele, i.e. $T\text{-}128^S$ males × FF females (always from the *wp ap F* strain).

3. RESULTS

3.1. Induction of new ADH nulls

The only available ADH null mutant is homozygous lethal [15], and the viability of homozygous null females is essential for genetic sexing [5]. As X ray irradiation very often produces deletions [25], the homozygotes can be non-viable. Several methods have been tried to induce and isolate new ADH nulls, with the T-128 as a marker [20].

Two chemical mutagens have been used, formaldehyde and EMS [26, 27]. Formaldehyde was used twice for each concentration (0.4% and 0.8%) on larvae of the SS strain. Males surviving the treatment were crossed to suitable females and the F_1 flies reared on selective larval medium (0.082% or 0.085% allyl-alcohol). The results are shown in Table I. Further crosses with surviving males and females led us nowhere. The method as a whole was inefficient (the compound is mutagenic in males only [28]) and time consuming; therefore it was not continued.

TABLE I. TREATMENT OF SS LARVAE WITH 0.4% AND 0.8% FORMALDEHYDE: F_1 SURVIVAL THROUGH SELECTIVE MEDIUM WITH ALLYL-ALCOHOL

Mating Male	× Female	Formaldehyde (%)	Allyl-alcohol (%)	Eggs	Egg hatch (%)	Pupae	Males	Females
SS	SS	0.4	0.082	1805	1514 (83.88)	0	—	—
T-128	SS	0.4	0.082	1740	1376 (79.08)	24	21	0
SS	SS	0.4	0.085	2410	1771 (73.49)	34	18	7
T-128	SS	0.4	0.085	2748	1967 (71.58)	308	249	13
SS	FF	0.8	0.082	1881	1068 (56.78)	0	0	0
T-128	SS	0.8	0.082	1644	1078 (65.57)	29	22	0

TABLE II. SURVIVAL OF F_1 PROGENY OF MEDFLY FEMALES TREATED WITH 0.04% EMS

Strain	Larval medium + allyl-alcohol (%)	Eggs[a]	Larvae	Egg hatch (%)	Pupae	Larval survival (%)	Males	Females
EMS F_1 1st exp.	0.088	46 200[b]	—	—	1252	—	1045	91[c]
EMS F_1 1st exp.	0.088	616	421	68.3	54	12.8	29	12
EMS F_1 1st exp.	0	605	396	65.5	257	64.9	73	141
SS	0.088	553	538	97.3	51	9.5	27	27
wp ap F	0.088	120	36	30.0	1	2.8	0	1
EMS F_1 2nd exp	0.088	44 400[b]	—	—	2013	—	1796	178
EMS F_1 2nd exp.	0.088	391	299	76.5	40	13.4	17	13
EMS F_1 2nd exp.	0	412	320	77.7	228	71.3	63	116
T-128^F F_{55}	0.088	71	53	74.7	4	7.6	4	0
T-128^F unselected F_{55}	0.088	125	104	83.2	15	14.4	10	3
T-128^S F_{54}	0.088	96	84	87.5	13	15.5	7	3

[a] No. of eggs (well formed) counted directly on black filter paper.
[b] No. of eggs estimated by volume, including misshapen eggs.
[c] + 3 intersex.

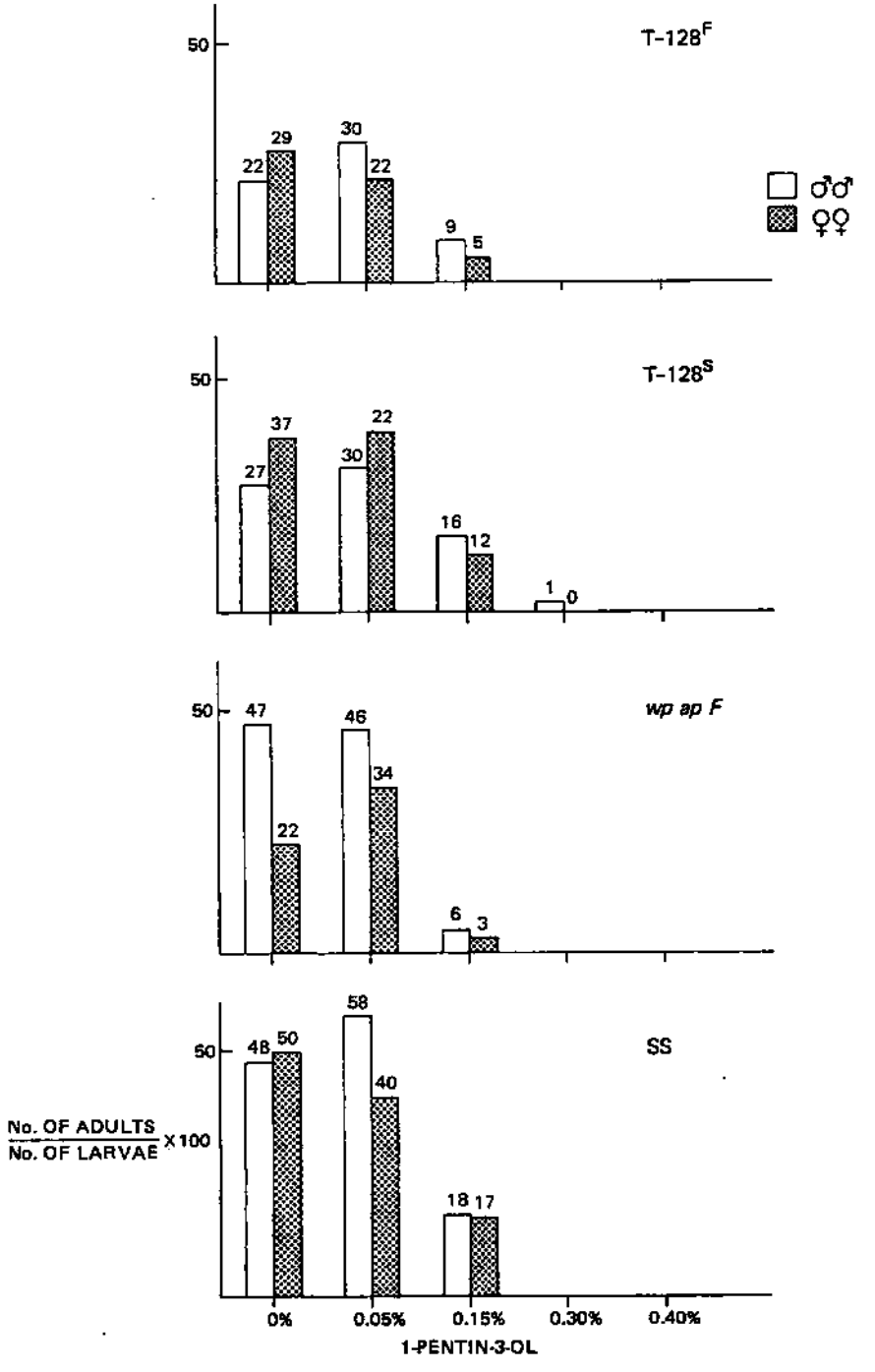


FIG. 2. Survival of males and females in medfly stocks after larvae have been treated with 1-pentyn-3-ol.

The possibility of using EMS directly by feeding adults [23] indicated a new approach to the chemical mutagenesis. We treated only females with 0.04% EMS, as described in Section 2, and then mated them to T-128 males, reared the progeny on 0.088% allyl-alcohol medium and analysed the female survivors. Two experiments have been conducted so far: 207 females were treated in the first and 180 in the second. The results are shown in Table II. The females and the T-128 males surviving the allyl-alcohol selective medium were crossed and the F_2 reared again on allyl-alcohol selective medium. The F_1 females and some males were then checked electrophoretically (a null mutation would produce a single ADH band in the females or no bands in the males). The F_2 progeny of these individuals is being studied.

3.2. Resistance to alcohols

3.2.1. 1-pentyn-3-ol

In *Drosophila*, this unsaturated tertiary alcohol selects ADH null individuals in the larval stage [29]. A preliminary experiment was performed to determine the range of concentrations which includes the discriminating dose for individuals with full and reduced ADH activity. Figure 2 shows the survival of larvae to adult males and females, and according to these results the range should be between 0.070% and 0.2%. These results agree very well with those of *Drosophila*, where individuals with reduced ADH activity (5% of wild type) show higher resistance than wild type, but flies with no detectable ADH activity (ADH null homozygotes) are virtually unaffected by concentrations of up to 0.2% pentynol. The lack of an ADH null homozygote strain in the medfly makes it impossible to obtain a clear cut discriminating dose.

3.2.2. 2-propanol

ADH has a known preference for secondary alcohol substrates in vitro, and 2-propanol is used as the classical substrate for ADH staining [30]. This property and the fact that it is not dangerous to handle, led us to test it for a possible differential response of males and females in T-128. The results are presented in Table III. No consistent sex discriminating effect was observed in any of the strains for the different concentrations as compared with the controls.

3.2.3. Ethanol

The genetic sexing properties of T-128 when exposed to allyl-alcohol, producing only males, should also apply in the opposite direction, i.e. preferential survival of females if exposed to a primary alcohol such as ethanol. The results of

TABLE III. SEX RATIO OF FOUR MEDFLY STRAINS AFTER TREATMENT WITH 2-PROPANOL IN THE LARVAL MEDIUM

Strain	2-propanol (%)	Males	Females	χ^2 (1:1)
SS	0.00	182	158	1.70
	0.25	206	178	2.00
	0.75	132	87	9.30***
	1.50	204	176	2.10
	2.00	183	144	4.70*
wp ap F	0.00	65	74	0.60
	0.25	53	64	0.50
	0.75	63	49	1.80
	1.50	63	64	0.01
	2.00	51	49	0.02
T-128^S	0.00	77	119	9.00***
	0.25	101	113	0.70
	0.75	97	147	10.30***
	1.50	109	138	3.40
	2.00	100	160	13.90***
T-128^F	0.00	101	120	1.60
	0.25	95	122	3.40
	0.75	81	109	4.10*
	1.50	90	113	2.60
	2.00	89	99	0.50

* Significant at 0.05 level.
*** Significant at 0.005 level.

the experiment confirm this hypothesis (Fig. 3). The differential survival is specially clear in T-128^S where the positive allele, *Adh*^{S}, is less active than *Adh*^{F}. The F band in the gels is always more intense and Y-NS males normally present a very faint band [24]. This different activity of the positive alleles has also been observed in *Drosophila* [31]. Table IV shows the linear regression coefficients for both sexes and for total larvae to adult survival in response to ethanol treatment. As expected (Fig. 3), there is a high negative correlation for *wp ap F* and T-128^F in all three cases and also for T-128^S males. SS, less affected by ethanol, has a low correlation for both sexes; T-128^S females show a high positive correlation.

Development is retarded by ethanol from one day in the lower concentrations (6% and 7%) to five days in 9%.

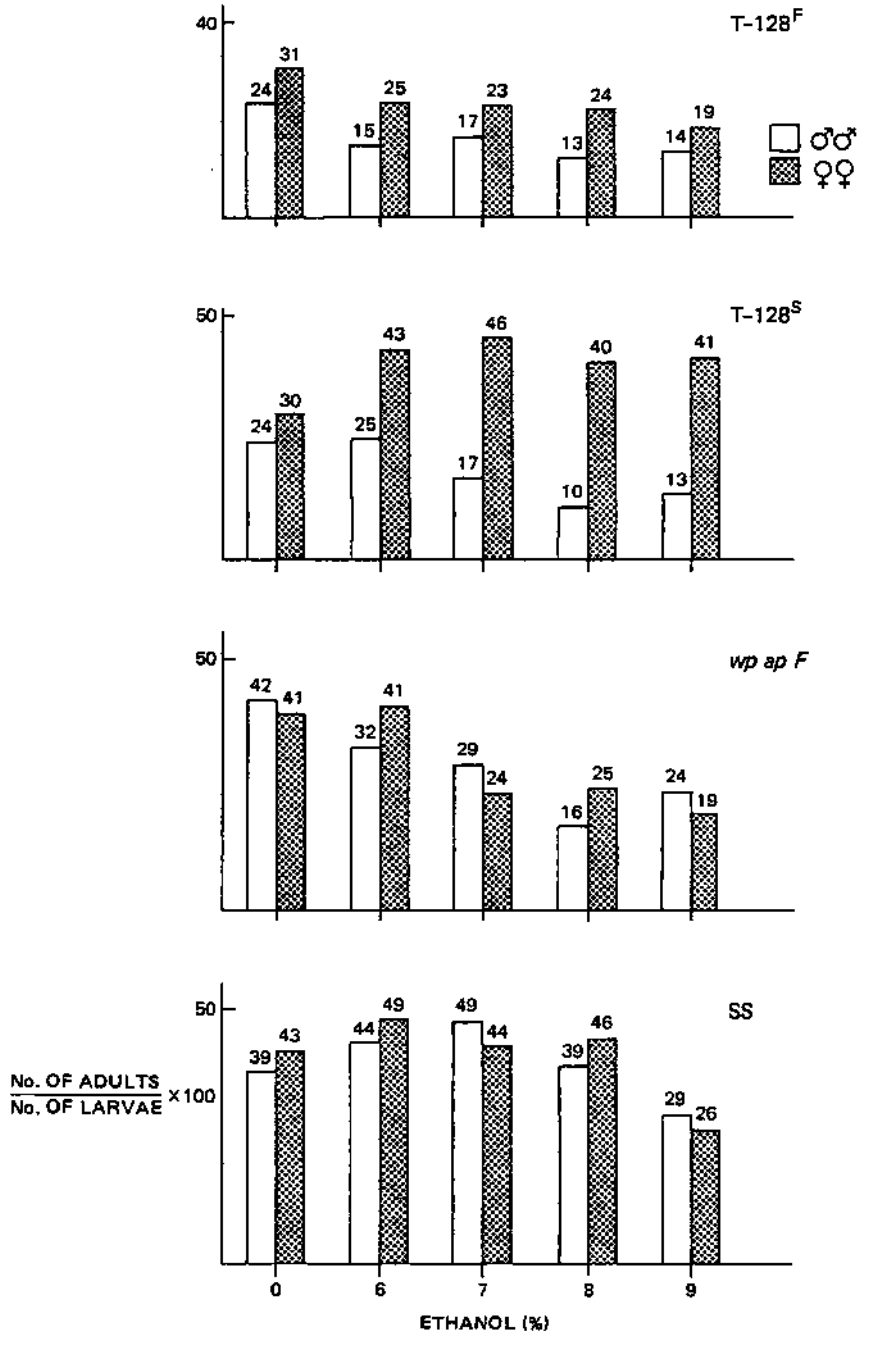


FIG. 3. Survival of males and females in four medfly strains after larvae have been treated with ethanol.

TABLE IV. LINEAR REGRESSION COEFFICIENT (r) FOR SURVIVAL OF ADULTS ON ETHANOL CONCENTRATION

Strain	r		
	Males	Females	Total adults
SS	−0.21	−0.36	−0.300
wp ap F	−0.88	−0.78	−0.880
T-128^{S}	−0.73	+0.81	−0.025
T-128^{F}	−0.96	−0.96	−0.990

Note: Survival is calculated as No. of adults × 100/No. of larvae.

TABLE V. MONITORING T-128 GENETIC SEXING CHARACTERISTICS WITH ALLYL-ALCOHOL IN THE LARVAL MEDIUM

Strain		Allyl-alcohol (%)	Eggs[a]	Pupae	Males	Females	χ^2 (1:1)
T-128	F_{34}	0.075	1 800	29	26	0	
T-128 × FF	F_{34}	0.075	7 400	143	128	0	
T-128	F_{34}	0.000	1 400	322	78	147	21.2***
T-128 × FF		0.000	3 400	683	162	255	20.7***
T-128	F_{37}	0.075	10 000	110	85	0	
T-128 × SS	F_{37}	0.075	14 000	126	106	0	
T-128^{S}	F_{38}	0.075	8 000	169	149	3	
SS		0.075	4 000	6	2	2	
wp ap F		0.075	1 400	0	0	0	
T-128^{S}	F_{44}	0.075	4 000	422	345	31	
T-128^{F}	F_{45}	0.075	3 000	46	45	0	
SS		0.075	4 000	282	164	114	9.0***
wp ap F		0.075	2 800	1	1	0	

[a] Estimated by volume.

*** Significant at 0.005 level.

TABLE VI. SEX RATIO OF T-128 MEDFLY STRAINS AFTER TREATMENT OF EGGS IN WATER WITH ALLYL-ALCOHOL

Max. age difference in eggs (h)	Time aerating (h)	Volume of eggs (mL)	Allyl-alcohol (mL)	Pupae	Males	Females	χ^2 (1:1)
3	68	0.20	1	180	52	62	0.87
21	48	0.20	1	212	33	55	5.50*
24	48	0.20	1	786	227	346	24.71**
21	48	0.18	0	493	114	173	12.13**

* Significant at 0.05 level.
** Significant at 0.01 level.

3.2.4. Allyl-alcohol

This alcohol has been tested extensively as a selective agent, favouring medfly individuals with low ADH activity [14, 15, 24, 32]. Some detailed studies to establish the discriminating dose between males and females have already been published [14, 24]. We used it as a selective agent in the larval medium when screening for new mutants (Table II) and as a control of T-128 stability (Table V). In this latter case a low percentage of females (8.25%) in line T-128^S occasionally survived the treatment. The SS strain also shows some survivors. The positive *Adh*^{S} allele is less active than *Adh*^{F}, and the SS and T-128^S strains also show better fitness parameters than *wp ap F* and T-128^F, respectively; these characteristics may explain the survival of some females of T-128^S and of both sexes in SS.

The requirements for a genetic sexing mechanism for mass rearing [4] are: (a) early development stage for eliminating females; (b) minimal changes required from present mass rearing technology; and (c) minimal contamination of equipment and personnel. With all these conditions in mind, an experiment was carried out to test allyl-alcohol on eggs (earliest development stage) while they are aerated (minimal changes) in flasks with water (minimum contamination). Unfortunately no effect on sex ratio was evident (Table VI). There is, in fact, the usual excess of females for this strain. This type of experiment should be carried out with eggs of a small age interval, preferably the same as in mass rearing. It would then be possible to calculate more accurately the time when most eggs have hatched and apply the treatment to mainly neonate larva. An alternative would be to dechorionate the eggs and check for sensitivity.

TABLE VII. SURVIVAL OF THE DIFFERENT LIFE STAGES OF FOUR MEDFLY STRAINS

Strain	No. of replicates	Egg hatch ± SE	Pupation ± SE	Pupal emergence ± SE	Overall survival (%)
SS	8	94.6 ± 1.4	86.8 ± 3.1	92.9 ± 11.1	76.3
wp ap F	8	57.3 ± 18.7	84.1 ± 6.2	97.2 ± 2.1	46.8
T-128^{S}	8	80.1 ± 8.2	72.8 ± 10.4	73.4 ± 5.7	42.8
T-128^{F}	8	67.9 ± 3.1	77.9 ± 9.1	75.6 ± 4.6	37.4

TABLE VIII. PUPAL EMERGENCE AND SEX RATIO IN FOUR MEDFLY STRAINS

Strain	Generation	Pupae	Pupal emergence (%)	Males	Females	χ^2 (1:1)
SS	Nov 86	1068	71.54	403	361	2.31
	Jan 87	1011	89.32	461	442	0.40
	Feb 87	871	91.05	403	390	0.20
	Apr 87	2861	90.74	1315	1281	0.50
	Sep 87	846	79.20	334	336	0.01
	Oct 87	1907	88.73	854	838	0.20
	Nov 87	1094	96.07	557	494	3.80
	Dec 88	1277	93.74	613	584	0.70
	Jan 88	1690	96.21	833	793	1.00
	Mar 88	1665	97.84	839	790	1.50
wp ap F	Nov 86	757	57.86	218	220	0.01
	Jan 87	3492	42.64	735	754	0.20
	Feb 87	2792	63.43	900	871	0.50
	May 87	1789	84.29	764	744	0.30
	Nov 87	1174	85.18	474	526	2.70
	Dec 87	740	62.57	242	221	1.00
	Jan 88	1301	94.70	625	607	0.30
	Mar 88	376	88.53	369	380	0.20
	Apr 88	1218	76.52	464	468	0.02

TABLE VIII. (cont.)

Strain	Generation	Pupae	Pupal emergence (%)	Males	Females	χ^2 (1:1)
T-128^S	F_{37}	1587	52.93	300	540	68.60***
(Jan 87–	F_{44}	1222	65.96	248	558	119.20***
May 88)	F_{45}	1481	80.82	530	667	15.90***
	F_{46}	3288	64.45	886	1233	56.80***
	F_{47}	560	56.96	127	192	13.20***
	F_{48}	794	67.13	218	315	17.70***
	F_{49}	738	80.76	267	329	6.50*
	F_{52}	405	75.75	222	312	15.20***
	F_{53}	1051	77.26	341	471	20.80***
T-128^F	F_{37}	3141	56.16	849	915	2.50
(Jan 87–	F_{38}	5187	54.56	1417	1413	0.01
June 88)	F_{40}	1026	67.74	299	396	13.50**
	F_{45}	612	82.68	165	341	61.20**
	F_{46}	3083	60.04	892	959	2.40
T-128^F	F_{47}	2865	50.79	744	723	0.30
(Jan 87–	F_{48}	1829	86.88	862	727	11.50***
June 88)	F_{49}	967	58.02	291	270	0.80
(cont.)	F_{50}	1320	65.30	322	540	55.10***
	F_{51}	2165	74.69	766	851	4.50*
	F_{52}	2425	64.74	666	904	36.10***
	F_{53}	1966	61.14	567	635	3.90*
	F_{54}	2813	63.24	811	968	13.90***
	F_{55}	602	78.41	228	244	0.50
T-128^F	F_{51}	745	10.60	39	40	0.01
unselected	F_{52}	2066	75.65	720	843	9.70**
(Feb 88–	F_{53}	2973	64.21	941	962	0.20
June 88)	F_{54}	1697	64.53	557	538	0.30
	F_{55}	3890	65.94	1160	1405	23.40***

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.005 level.

3.3. Life parameters

Survival of the various life stages for the four strains used in this work is presented in Table VII. The data are pooled from two generations, each with four replicates. The overall survival (46.8% and 42.8%) egg to adult of both T-128 strains suggests, as expected, that they carry a translocation and are semisterile. SS has an acceptable overall survival (76.3%) and is generally very easy to rear. Fitness of the triple marker strain *wp ap F* is very low, with no clear explanation for this except that each mutant is less fit than its respective wild type [2, 33, 34]. It has also been observed in previous work [15] that the FF strain was difficult to rear and in some way 'unstable'. Another characteristic of T-128 is its consistent production of an excess of females [14, 24] (Table VIII). SS and *wp ap F* have a 1:1 sex ratio, and both T-128 lines show an excess of females in most generations. As mentioned before, FF, a necessary strain for all the experiments [32], was difficult to maintain; FS phenotypes kept appearing in the strain (contamination) and if they were not soon detected the line would rapidly deteriorate. The triple marker strain *wp ap F* was constructed both for its marker qualities and as a shelter for FF as it is easier to detect contamination and to clean it if wild type individuals appear in the strain.

These considerations, as well as the loss of the FF(wp^+, ap^+) strain, contributed to leading us to consider maintaining T-128 as two or more lines (see Section 2); and at generation F_{35}, T-128 males which were Y-NS were outcrossed to *wp ap F* females and the male progeny, F_{36}, again backcrossed to *wp ap F* females. We expected to establish three lines for T-128:

— T-128^S: males XY-NS; females XX SS; wp^+/wp^+; ap^+/ap^+ both sexes.
— T-128^F: males XY-NF; wp^+/wp^+; ap^+/ap^+ and females XX/FF; wp^+/wp^+; ap^+/ap^+.
— T-128^F *wp ap*: males XY-NF; *wp/wp; ap/ap* and females FF; *wp/wp; ap/ap*.

TABLE IX. PHENOTYPIC SEGREGATION IN BACKCROSS OF T-128^F F_{36} (Y-NF; wp^+/wp; ap^+/ap) MALES TO *wp ap F* FEMALES

Generation	Sex	wp^+/ap^+	wp^+/ap	wp/ap^+	wp/ap	χ^2 (1:1:1:1)
F_{37}	Male	490	1	358	0	
	Female	126	391	79	319	294.9***

*** Significant at 0.005 level.

TABLE X. F_2 SEGREGATION FOR PUPAL AND EYE COLOUR, ADH TYPE AND SEX FROM VARIOUS CROSSES

Parental cross	F_2 phenotypes	Males		Females	
		Observed	Expected	Observed	Expected
wp ap F male × SS female;	*wp*$^+$ *ap*$^+$ SS	4	6.0	4	5.5
F_1: *wp ap F* male × F_1 female	*wp*$^+$ *ap*$^+$ FS	8	6.0	7	5.5
(χ^2 = 4.4; DF = 15 (not significant))	*wp*$^+$ *ap* SS	6	6.0	7	7.5
	wp$^+$ *ap* FS	6	6.0	8	7.5
	wp ap$^+$ SS	5	5.5	7	10.0
	wp ap$^+$ FS	6	5.5	13	10.0
	wp ap SS	7	6.5	9	7.0
	wp ap FS	6	6.5	5	7.0
wp ap F male × SS female;	*wp*$^+$ *ap*$^+$ SS	5	8.5	8	10.5
F_1 male × *wp ap F* female	*wp*$^+$ *ap*$^+$ FS	12	8.5	13	10.5
(χ^2 = 10.8; DF = 15 (not significant))	*wp*$^+$ *ap*$^+$ SS	10	9.0	4	6.5
	wp$^+$ *ap* FS	8	9.0	9	6.5
	wp ap$^+$ SS	3	3.0	4	4.0
	wp ap SS	3	3.0	4	4.0
	wp ap SS	2	5.0	1	2.0
	wp ap FS	8	5.0	3	2.0

TABLE X. (cont.)

Parental cross	F_2 phenotypes	Males		Females	
		Observed	Expected	Observed	Expected
SS male × *wp ap F* female;	*wp*$^+$ *ap*$^+$ SS	7	5.5	5	5.0
F_1: *wp ap F* male × F_1 female	*wp*$^+$ *ap*$^+$ FS	4	5.5	5	5.0
(χ^2 = 7.6; DF = 15 (not significant))	*wp*$^+$ *ap* SS	9	6.0	7	6.0
	wp$^+$ *ap* FS	3	6.0	5	6.0
	wp ap$^+$ SS	7	7.0	5	5.5
	wp ap$^+$ FS	7	7.0	6	5.5
	wp ap SS	4	7.0	4	5.5
	wp ap FS	10	7.0	7	5.5
SS male × *wp ap F* female;	*wp*$^+$ *ap*$^+$ SS	8	7.0	9	7.5
F_1: F_1 male × *wp ap F* female	*wp*$^+$ *ap*$^+$ FS	6	7.0	6	7.5
(χ^2 = 14.9; DF = 15 (not significant))	*wp*$^+$ *ap* SS	3	4.5	9	5.0
	wp$^+$ *ap* FS	6	4.5	1	5.0
	wp ap$^+$ SS	6	7.5	4	7.5
	wp ap$^+$ FS	9	7.5	11	7.5
	wp ap SS	6	7.0	3	5.0
	wp ap FS	8	7.0	7	5.0

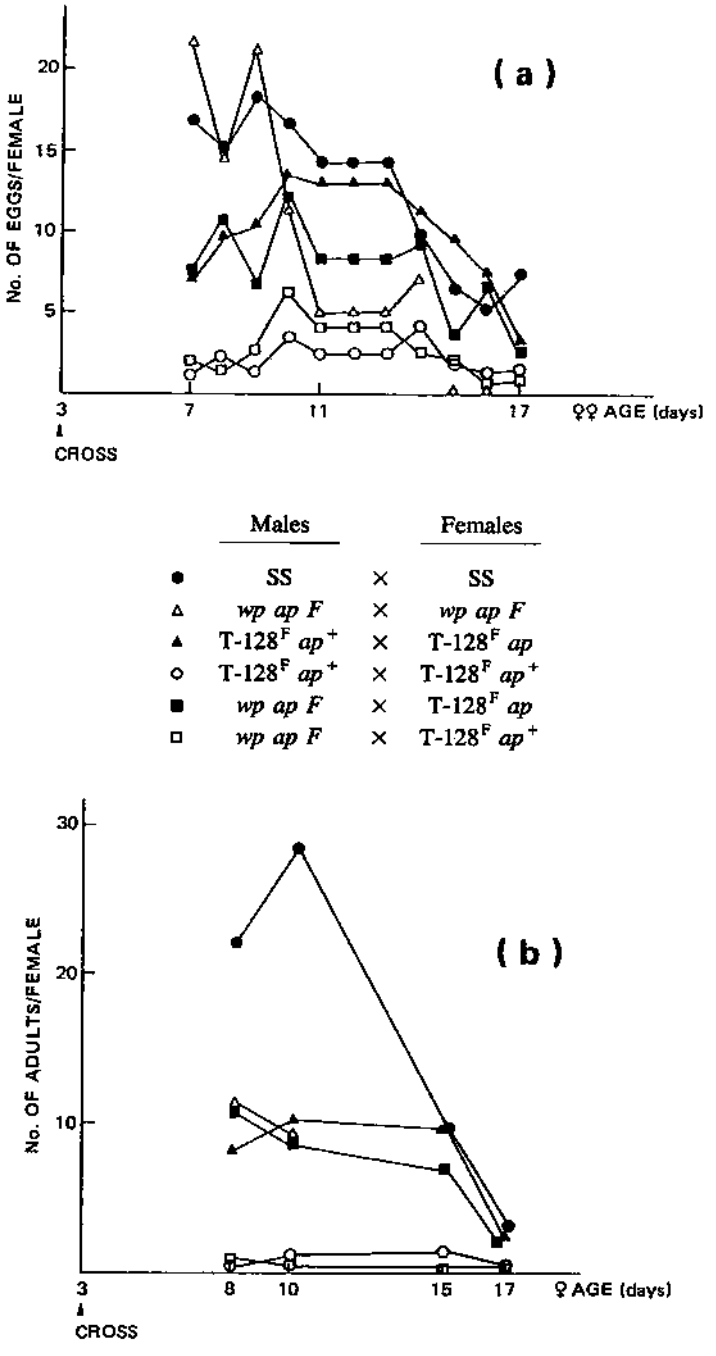


FIG. 4. Fecundity of T-128 females with wild type or apricot eyes. (a) Number of eggs/female per day; (b) number of adults/female per day.

This was not possible owing to the unexpected segregation observed (Table IX) in generation F_{37} after the above mating scheme had been carried out. The expected segregation was Mendelian for the F_2 of a backcross to double autosomal recessive individuals: one quarter of each phenotype wp^+/ap^+; wp^+/ap; wp/ap^+ and wp/ap in each sex. Only one male had apricot eyes (424.5 expected) and only 205 females had wild type eyes (457.5 expected). These results suggest a double translocation linking the Y chromosome to Adh^N and to ap^+ with incomplete linkage for this last trait. It had already been observed by other authors that T-128 carries a double translocation [35]. The strains SS and *wp ap F* were checked for the linkage of these three characters and sex. Table X(a)–(d) shows no linkage between these characters in those strains, as expected.

If T-128 is a double translocation the appearance of the deviant phenotypes, apricot eye males and wild type females, due to recombination or other phenomena [36, 37], can lead to the breakdown of genetic sexing properties.

A small scale experiment was carried out to check the fertility of both types of females, ap^+ and *ap*. Each type was crossed to the T-128^F males (ap^+) and to *wp ap F* males. Eggs were collected daily and fecundity, fertility and larval and pupal survival recorded. Figure 4(a) and (b) shows the daily egg and adult production for each type of cross. Both types have very low fecundity, and egg hatch is very low for the cross between *wp ap F* males × T-128^F ap^+ females. This could be explained if the T-128^F ap^+ females have a duplication or deficiency that can be compensated in some way with T-128 males but not when outcrossed to a strain that also has a low viability, like the *wp ap F* strain. Sex ratios and eye colour are presented in Table XI for each type of mating; the deviant wild type females produce the same number of apricot eye males and females.

TABLE XI. EYE COLOUR SEGREGATION IN PROGENY OF WILD TYPE AND APRICOT EYE T-128^F FEMALES (GENERATION F_{54})

Parental cross		No. of replications	Adult phenotype (F_{54})			
Male	Female		Male ap^+	Male *ap*	Female ap^+	Female *ap*
T-128^F ap^+	× T-128^F *ap*	4	372	0	150	335
T-128^F ap^+	× T-128^F ap^+	4	40	12	30	13
wp ap F	× T-128^F *ap*	4	0	512	0	410
wp ap F	× T-128^F ap^+	4	15	12	9	9

TABLE XII. DEVIANT PHENOTYPES IN SELECTED AND UNSELECTED T-128^F POPULATIONS ALONG GENERATIONS

Strain	Generation	Adult eye colour					
		ap$^+$ males	*ap* males	% *ap*	*ap*$^+$ females	*ap* females	% *ap*$^+$
T-128^F	37	848	1	0.12	205	710	22.4
	38	1416	1	0.07	348	1065	24.6
	40	299	0	0.00	144	252	36.4
	41	287	0	0.00	93	193	35.5
	45	160	5	3.03	22	319	6.5
	46	892	0	0.00	200	759	20.9
	47	744	0	0.00	139	584	19.2
	48	862	0	0.00	155	572	21.3
	49	291	0	0.00	40	230	14.8
	50	322	0	0.00	166	374	30.7
	51	763	3	0.39	260	591	30.6
	52	666	0	0.00	305	599	33.7
	53	562	5	0.89	152	483	23.9
	54	10	1	0.12	328	642	33.8
	55	228	0	0.00	82	162	33.6
T-128^F unselected	51	39	0	0.00	0	40	0.0
	52	713	0	0.00	238	595	28.6
	53	932	9	0.97	279	683	29.0
	54	553	4	0.72	170	368	31.6
	55	1156	4	0.35	431	974	30.7

Apricot eye T-128^F males had low fertility and short life span. When these males were dissected the testes were found to be very small or rudimentary.

According to our data, the contribution of these deviant phenotypes to subsequent generations is probably of no importance. Even so, the *Adh*^{N} allele seems tightly linked to the Y chromosome because it has been checked regularly for the 55 generations, and males always present one band and females three after the appropiate outcross (Y-NF males × SS females or Y-NS males × *wp ap F* females) [14, 24].

Another way in which the contribution of the deviant phenotypes to the next generation is measured is by monitoring a T-128^F line that is not cleaned out every generation: T-128 ^{F} unstable. The frequency of deviant phenotypes appearing along the generations in both T-128^F and T-128^F unstable populations are presented in Table XII. This work must continue because the unselected T-128^F population was initiated only five generations ago.

4. CONCLUSIONS

The original genetic sexing system based on the ADH locus required linking a positive ADH allele to the Y chromosome and isolating a null homozygous strain having no ADH activity. The first requirement was easy to produce, and five such translocations were induced and isolated [32] of which two are still available in our laboratory. The second requirement was not fulfilled and although null mutations were induced it was not possible to establish them as homozygous lines [15]. A different method was then tried in order to overcome this difficulty and isolate new ADH null mutants [20]: a translocation linking the only ADH null mutant available was isolated and showed genetic sexing properties [14, 24]. This strain, T-128, is available and is used as a marker to find new ADH null mutants with relative success. At the moment, six possible new mutations are being investigated in the laboratory. Even if they are not confirmed, the methodology is being refined and it should be possible to obtain more mutants in a relatively short time. Two difficulties must be taken into account. One is technical and is due to the not very efficient method for single pair mating in our laboratory. The second is the presence of a possible second ADH locus [38] that can interfere with the qualities of resistance/sensitivity to alcohols on which this system is based. However, after several tests with different alcohols, the T-128 strain shows the expected sexing qualities: females are more sensitive than males to secondary alcohols like 1-pentyn-3-ol or allyl-alcohol, and males are more affected by ethanol than females.

A third aspect of the work is the identification of T-128 as a double translocation with two possible genetic sexing characteristics: a conditional lethal, i.e. resistance to alcohols, and a morphological marker, apricot eye. Although the linkage of this last character is not complete it can be used as a marker for field

studies and, what is more important, to determine the effect of recombination and other phenomena such as meiotic segregation of duplication/deficiency gametes on the stability of translocations [36, 37, 39]. This work has been started and the line is monitored regularly.

Another very important aspect is the possibility of easily introducing new genetic material into this strain [24]. Males are the only carriers of mutations of the sexing trait (in this case Adh^N), and of chromosomal aberrations. These males can be outcrossed, without losing their genetic sexing properties, to any type of female provided they have full ADH activity. This is the type usually found in most populations, field or laboratory, because it is wild type. One case is the T-128^F, which has demonstrated that it is a double translocation, and, furthermore, the two autosomes involved are known: 2 for *Adh* and 4 for apricot eye.

In short, the following strains are currently available in our laboratory:

— T-128: a genetic sexing strain with the ADH locus Y-Adh^N;
— Two Y-Adh^Ftranslocations: T-1 and T-114 [32, 40];
— Two homozygous strains for ADH alleles: F and S, one of them also a double morphological marker: *wp ap F*;
— One genetic sexing strain with pupal colour: *wp-23*.

REFERENCES

[1] LACHANCE, L.E., "Genetic strategies affecting the success and economy of the sterile insect release method", Genetics in Relation to Insect Management (Proc. Conf. Bellagio, 1978), (HOY, M.A., McKELVEY, J.J., Eds), Rockefeller Foundation, New York (1979) 8–19.

[2] RÖSSLER, Y., Automated sexing of *Ceratitis capitata* (Dip.: Tephritidae): the development of strains with inherited, sex-limited pupal color dimorphism, Entomophaga **24** (1979) 411–416.

[3] Report of Consultants Meeting on Genetic Sexing Mechanisms for the Mediterranean Fruit Fly, *Ceratitis capitata*, Vienna, 1981, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[4] Report of Research Co-ordination Meeting on the Development of Sexing Mechanisms for the Mediterranean Fruit Fly, Vienna, 1983, obtainable from the Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[5] ROBINSON, A.S., VAN HEEMERT, C., Genetic sexing in *Drosophila melanogaster* using the alcohol dehydrogenase locus and a Y-linked translocation, Theor. Appl. Genet. **59** (1981) 23–24.

[6] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*, a suitable case for genetic sexing, Genetica **58** (1982) 229–237.

[7] WOOD, R.J., BUSCH-PETERSEN, E., "Possible genetic sexing mechanisms for *Ceratitis capitata* Wied.", Sterile Insect Technique and Radiation in Insect Control (Proc. Symp. Neuherberg, 1981), IAEA, Vienna (1982) 279–289.

[8] WAGONER, D.E., Linkage group karyotype correlation in the housefly determined by cytological analysis of X-ray induced translocations, Genetics **57** (1967) 724–739.

[9] ROBINSON, A.S., Sex ratio manipulation in relation to insect pest control, Annu. Rev. Genet. **17** (1983) 191–214.

[10] RÖSSLER, Y., Recombination in males and females of the Mediterranean fruit fly (Diptera: Tephritidae) with and without chromosomal aberrations, Ann. Entomol. Soc. Am. **75** (1982) 619–622.

[11] RÖSSLER, Y., "69-apricot" — a synthetic strain of the Mediterranean fruit fly, *Ceratitis capitata*, with sex limited pupal color and an eye color marker, Entomophaga **25** (1980) 276–281.

[12] ROBINSON, A.S., RIVA, M.E., "Male-linked translocations in *Ceratitis capitata* for genetic sexing and irradiation", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Athens, 1982), (CAVALLORO R., Ed.), Balkema, Rotterdam (1983) 190–196.

[13] SAUL, S.H., Genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae): conditional lethal translocations that preferentially eliminate females, Ann. Entomol. Soc. Am. **77** (1984) 280–283.

[14] ROBINSON, A.S., RIVA, M.E., ZAPATER, M., Genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata*, using the Adh locus, Theor. Appl. Genet. **72** (1986) 455–457.

[15] RIVA, M.E., ROBINSON, A.S., Induction of alcohol dehydrogenase null mutants in the Mediterranean fruit fly, *Ceratitis capitata*, Biochem. Genet. **24** (1986) 765–774.

[16] BUSCH-PETERSEN, E., SOUTHERN, D.I., Induced suppression of genetic recombination in females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), by translocation heterozygosity, Genetica **72** (1987) 161–169.

[17] HEINSTRA, P.W.H., et al., A dual function of alcohol dehydrogenase in *Drosophila*, *Genetica* **60** (1983) 129–137.

[18] GRELL, E.G., JACOBSON, K.B., MURPHY, J.B., Alterations of genetic material for analysis of alcohol dehydrogenase isozymes of *Drosophila melanogaster*, Ann. N.Y. Acad. Sci. **151** (1968) 331–455.

[19] DAVID, J., BOCQUET, C., Compared toxicities of different alcohols for two *Drosophila* sibling species: *D. melanogaster* and *D. simulans*, Comp. Biochem. Physiol. **54** (1976) 71–74.

[20] ROBINSON, A.S., RIVA, M.E., A simple method for the isolation of allelic series using male-linked translocations, Theor. Appl. Genet. **67** (1984) 305–306.

[21] MEGNET, R., Mutants partially deficient in alcohol dehydrogenase in *Schizosaccharomyces pombe*, Arch. Biochem. Biophys. **121** (1967) 194–201.

[22] FREELING, M., CHENG, D.S.K., Radiation induced alcohol dehydrogenase mutants in maize following allyl-alcohol selection of pollen, Genet. Res. **31** (1978) 107–129.

[23] BUSCH-PETERSEN, E., PYREK, A., RIPPEL, J., RUHM, E., Efficiency of different methods of EMS application for the induction of dominant lethals in germ cells of medfly, *Ceratitis capitata* (Wied.), males, Mutat. Res. **163** (1986) 247–254.

[24] RIVA, M.E., "The use of allyl alcohol as discriminating agent for genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata*", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOUS, A., Ed.), Elsevier, Amsterdam and New York (1987) 227–234.

[25] AARON, C.S., X-Ray induced mutations affecting the level of the enzyme alcohol dehydrogenase in *Drosophila melanogaster:* Frequency and genetic analysis of null-enzyme mutants, Mutat. Res. **63** (1979) 127–137.

[26] SAUL, S.H., Rosy-like mutant of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), and its potential for use in a genetic sexing program, Ann. Entomol. Soc. Am. **75** (1982) 480–483.

[27] LIFSCHITZ, E., "New mutants in *Ceratitis capitata*", Report on Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethal and Other Genetic Measures, Crete, 1986, obtainable from Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[28] AUERBACH, C., KILBEY, B.J., Mutation in eukaryotes, Annu. Rev. Genet. **5** (1971) 163–218.

[29] O'DONNELL, J., GERACE, L., LEISTER, F., SOFER, W., Chemical selection of mutants that affect alcohol dehydrogenase in *Drosophila* — II. Use of 1-pentyn-3-ol, Genetics **79** (1975) 73–83.

[30] DAVID, J.R., BOCQUET, C., ARENS, M.F., FOUILLET, P., Biological role of alcohol dehydrogenase in the tolerance of *Drosophila melanogaster* to aliphatic alcohols: utilization of an Adh null mutant, Biochem. Genet. **14** (1976) 989–997.

[31] OAKESHOTT, J.G., Selection at the alcohol dehydrogenase locus in the tolerance of *Drosophila melanogaster* imposed by environmental ethanol, Genet. Res. **26** (1976) 265–274.

[32] RIVA, M.E., ROBINSON, A.S., "Studies on the ADH locus in *Ceratitis capitata*", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Athens, 1982), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1983) 163–170.

[33] RÖSSLER, Y., The genetics of the Mediterranean fruit fly: a "white pupae" mutant, Ann. Entomol. Soc. Am. **72** (1979) 583–585.

[34] RÖSSLER, Y., Fitness of the "apricot eye" mutant of the Mediterranean fruit fly, *Ceratitis capitata*, Ann. Entomol. Soc. Am. **70** (1976) 544–548.

[35] WOOD, R.J., Dept of Zoology, Univ. of Manchester, personal communication.

[36] ROBINSON, A.S. Unexpected segregation ratios from male-linked translocations in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Genetica **62** (1984) 209–213.

[37] SAUL, S.H., Genetics of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), Agric. Zool. Rev. **1** (1986) 73–108.

[38] GASPERI, G., MALACRIDA, A.R., MILANI, R., "Protein variability and population genetics of *Ceratitis capitata*", Report on Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and other Genetic Measures, Crete, 1986, obtainable from the Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna.

[39] RÖSSLER, Y., 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, Ann. Entomol. Soc. Am. **78** (1985) 265–270.

[40] ZAPATER, M., ROBINSON, A.S., Sex chromosome aneuploidy in a male-linked translocation in *Ceratitis capitata*, Can. J. Genet. Cytol. **28** (1976) 161–167.

LABORATORY STUDIES ON INSECTICIDE RESISTANCE, ALCOHOL TOLERANCE AND SEX RATIO DISTORTION BY MEIOTIC DRIVE IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

R.J. WOOD
Department of Environmental Biology,
University of Manchester,
Manchester, United Kingdom

Abstract

LABORATORY STUDIES ON INSECTICIDE RESISTANCE, ALCOHOL TOLERANCE AND SEX RATIO DISTORTION BY MEIOTIC DRIVE IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.).

Three approaches to developing a genetic sexing technique for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), are discussed. Laboratory studies in late third instar larvae of the medfly revealed a potential for dieldrin resistance. A programme of sib selection produced the DiR strain, more than 60× resistant to dieldrin with cross-resistance to other cyclodienes, HCH, malathion and permethrin. Adults were not resistant. Crosses showed dieldrin resistance to be monofactorial, subject to a modifying effect from the genetic background on the expression of the homozygote. The 'backcrossing with selection' technique was used to separate dieldrin and malathion resistance but, in the process, resistance to both insecticides was lost after four to eight generations. Attempts to induce male linkage of the *R* gene by X irradiation were unsuccessful. Further genetic studies on resistance are recommended. With a view to producing an ethanol sensitive strain homozygous for an ADH null mutation (Adh^{-}/Adh^{-}), pentenol selection of late third instar larvae was carried out, combined with ethyl methane sulphonate (EMS) treatments of adults. This produced a maximum of 15× tolerance of pentenol but no associated change in ethanol tolerance. Electrophoresis (PAGE) showed that two major ADH systems were at their most active in late third instar larvae. A gene causing a male distorted sex ratio in the progeny of males carrying it was isolated after X irradiation. The expression of the gene, which appears to be an example of meiotic drive, was enhanced by reducing the ambient temperature of parent flies from 26°C±2.0 to 18°C±1.5 during days 2–5 of pupal development. Selection to increase the expression of the gene produced families with less than 20% females but sex ratio tended to revert towards normal in subsequent generations. A potential is seen for producing strains in which sex ratio can be regulated by temperature.

1. INTRODUCTION

The IAEA contract (2488/TC) under which this work on genetic sexing was carried out, ran for the full extent of the programme (1984–1988). The work had in fact been started earlier, shortly after the problem of genetic sexing had been

reviewed at a meeting organized by the International Organization for Biological Control, held in Sassari, Sardinia, in 1978 [1]. Inspiration came from the successful development of genetic sexing systems in mosquitoes by means of Y linked insecticide resistance genes [2]. The potential saving in costs for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), was calculated at US $30/million males [3]. The first attempt to produce a sexing system for the medfly, utilizing a Y linked pupal colour gene, was reported by Rössler [4]. In the following years, Robinson and Van Heemert [5] developed a system for *Drosophila melanogaster* based on a Y translocated *Adh* (alcohol dehydrogenous) gene. The scope of the problem was discussed by various authors [5–9].

Our own research started with studies on insecticide resistance [9–13] and continued with an investigation of ethanol and pentenol tolerance [9]. Problems with developing a genetic sexing system based on insecticide resistance or alcohol tolerance (discussed in this paper) led us more recently to investigate sex ratio distortion by meiotic drive, a subject on which we had experience with mosquitoes [14]. We came across sex ratio distortion in the medfly after X irradiating males for a different purpose [13]. Work on it is now in progress [14–16] and will be reported.

2. MATERIALS AND METHODS

2.1. Strains

SOUTHAMPTON:	Probably of Hawaiian origin, obtained from Dr. P. Howse, Southampton University, in 1978.
COSTA RICA:	From Costa Rica, supplied by Dr. E.F. Boller in 1978.
ITALY:	Collected in Italy and supplied by Boots Ltd in 1978.
FRANCE (F):	Collected in France and supplied by Boots Ltd in 1978.
DiR:	A substrain of SOUTHAMPTON with third instar larvae resistant to dieldrin, malathion and permethrin.
DiS:	A substrain of COSTA RICA with third instar larvae susceptible to dieldrin, malathion and permethrin.
T(Y-N)128:	A double translocation involving the Y chromosome and two autosomes with a null mutant of *Adh-1*^{S} holandrically inherited.

F/Pe: A substrain of FRANCE selected with pentenol.

F/Pe/EMS: A substrain of F/Pe selected with pentenol and also treated with EMS.

SEIBERSDORF: A strain from the FAO/IAEA Laboratories at Seibersdorf, near Vienna, Austria.

A425: A strain showing sex ratio distortion by meiotic drive descended from an X ray treated DiR male.

2.2. Insecticide tests

Late third instar larvae were exposed to insecticides (dieldrin, malathion, permethrin, etc.) by immersion in ethanolic dispersions in deionized water at 26°C ± 1.5, as described in detail in Ref. [11]. After exposure in batches of 50 for one hour, the larvae were washed and dried and allowed to pupate in fine sand, mortality being recorded 10 days later, at emergence. The resistance ratio at LC_{50} (median lethal concentration) was estimated with respect to an unselected control strain.

Adults were exposed by applying a solution of the insecticide in absolute alcohol to the dorsal thorax with a microsyringe and the LD_{50} was recorded two hours later, taking ability to walk as the criterion of 'survival'.

2.3. Pentenol tests

Late third instar larvae were exposed in aqueous solutions of 1-penten-3-ol at a range of concentrations for three hours at 28°C ± 1 in a fume cupboard. After exposure, larvae were washed and dried and allowed to pupate in fine sand. Mortality was assessed at emergence and the effective LC_{50} calculated (for details see Ref. [17]).

2.4. Ethanol tests

Tolerance to ethanol was assessed by immersing late third instar larvae in a 90% aqueous solution of ethanol (99.4% purity) at 26°C ± 1.5. Larvae were exposed for a range of times (1–6 hours). Controls in which larvae were immersed in deionized water under the same conditions were run for 3 and 6 hours. Larvae were treated and mortality assessed as in the pentenol tests. Tolerance was assessed according to the effective LT_{50} (median lethal time) (for details see Ref. [17]).

2.5. EMS treatment

The mutagen EMS (ethyl methanesulphonate) was supplied at a concentration of 0.07% in the adult drinking water (1% agar). 0.07% was the LC_5 for two day old adults (0.07 ± 0.01). The LC_{50} was 0.21 ± 0.01 (for further details see Ref. [18]).

2.6. Irradiation

The procedure was to irradiate either pupae aged seven days which were exposed to 1.4 krad X rays or young adults exposed at 4.5 or 5.0 krad (in each case at 120 rad/min). This was done with a PANTAK 300X X ray generator operating at 300 kV and 10 mA. A flattening filter gave uniformity of ±0.02 (further details are given in Ref. [13]).

3. DISCUSSION OF RESULTS

3.1. Resistance to insecticide

Selection with dieldrin was carried out on late third instar larvae of four strains. The reason for larvae being the object of study was the requirement to separate the sexes as early as possible in development. The third instar was used because of ease of collecting larvae of a standard age and size. Dieldrin was chosen because it was no longer used for field control.

ITALY and FRANCE did not respond to selection, but in SOUTHAMPTON resistance increased to 45× after 21 generations and in COSTA RICA to 25× after 26 generations. Increase in resistance was accompanied by evidence of segregation of a major *R* gene [9,11]. To achieve homozygosity it was necessary to apply single family sib selection [19], breeding from unexposed sibs within the most resistant F_2 progenies from single pair matings. By this means a highly dieldrin resistant strain (DiR) was isolated from SOUTHAMPTON and a susceptible strain (DiS) from COSTA RICA [11]. DiR and DiS differed in tolerance to dieldrin by about 60×. Crosses between these two strains demonstrated that resistance was due to a single major gene which segregated clearly in backcrosses (Fig. 1) although subject to modification by the genetic background [10, 11]. Tests with other insecticides revealed a wide spectrum of cross resistance in DiR, not only to chemically related compounds but also to malathion and permethrin [10].

With the isolation of DiR and DiS the way was open to try to produce a sexing system based on insecticide resistance comparable to the systems developed for mosquitoes [2]. It was proposed that the *R* gene would be translocated onto the Y chromosome by exposure to irradiation, producing a strain in which resistance would

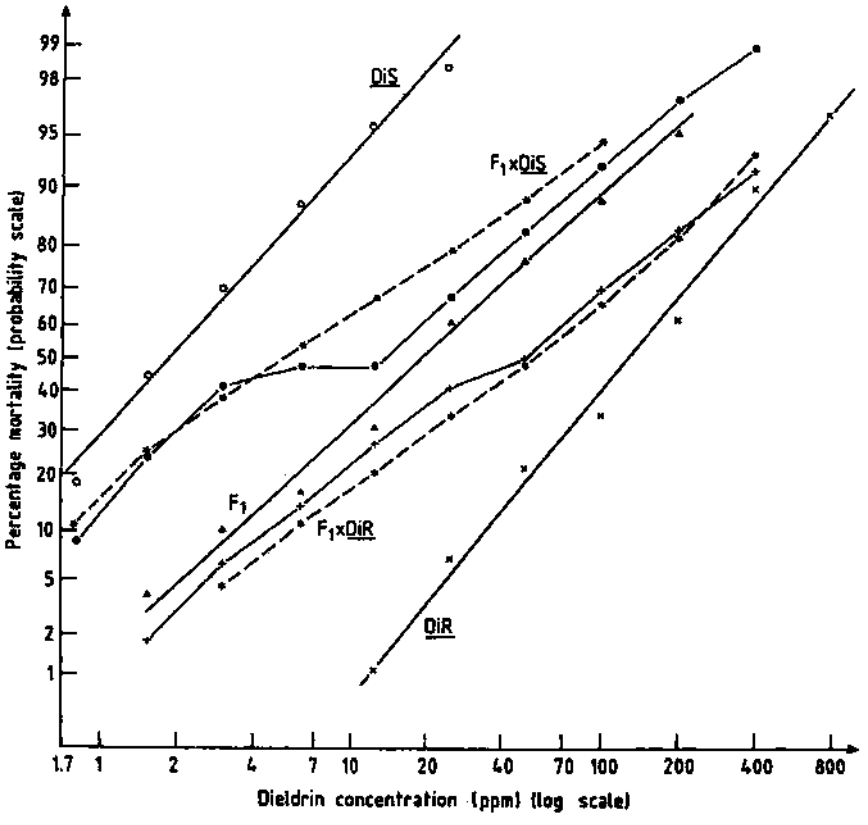


FIG. 1. Pattern of inheritance of the dieldrin resistance gene in medfly larvae showing overlap between SS, SR and RR genotypes (in DiS, F_1 and DiR, respectively) and a change in expression of these genotypes in the two backcrosses where segregation, indicated by inflections on I d-p lines, is clearer than expected (modified after Busch-Petersen and Wood [11]).

be holandrically inherited and thus exclusive to the male sex. Female larvae would then be killed by treatment with the insecticide (dieldrin) and resistant sterile males released [9]. The obvious danger was of releasing *fertile* resistant males by accident. This seemed less serious, however, when it was shown that DiR adults were fully susceptible to all insecticides tested [17] and would therefore respond normally to insecticidal control measures (bait sprays, cover sprays, male annihilation), all of which are directed against the adult stage.

The procedure for inducing a Y translocation of the *R* gene is shown in Fig. 2. It involved irradiation of DiR males (genotype RR), crossing them to DiS females (SS), backcrossing F_1 males (RS) to DiS females (SS) by single pair mating, and

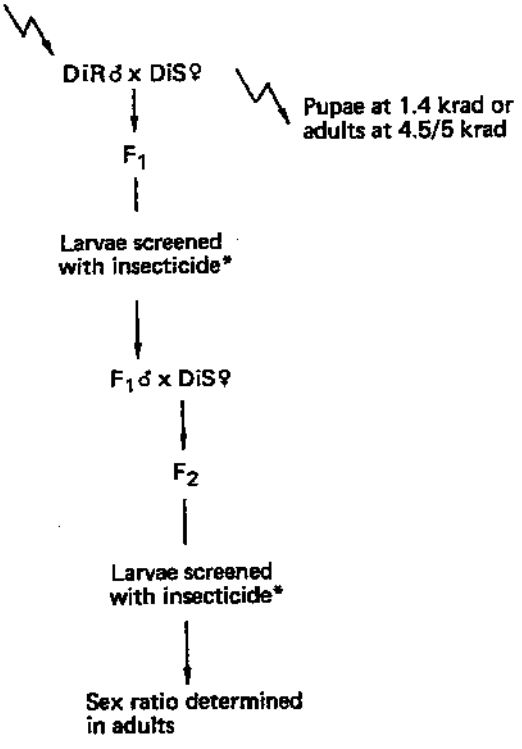


FIG. 2. Procedure designed to isolate a Y translocated R *gene. A star denotes discriminating dose of dieldrin, malathion or permethrin, separating RS and SS genotypes.*

exposing the progenies to a 'discriminating dose' of insecticide to separate RS from SS, after which the survivors were scored for sex. Any single pair progeny in which the survivors were all male or mainly male (while the unexposed sibs had a normal sex ratio) would indicate the possibility that a Y linked translocation of the *R* gene had been induced [9, 13]. The insecticide dose would be set at a level sufficient to kill all segregating SS genotypes but leave as high a proportion as possible of RS genotypes surviving (Fig. 1).

A total of 878 F_1 males from mass crosses between irradiated DiR males and DiS females were backcrossed in single pairs to DiS; 620 matings provided sufficient progeny to be screened with insecticide. In the early backcrosses, dieldrin was used at 40 mg/L but because of delayed mortality the dose was reduced to 10 mg/L. In the final experiments we switched to using malathion at 20 mg/L or permethrin at 100 mg/L. All backcross families with a significant excess of males were investigated in succeeding generations but none gave evidence of carrying a translocated *R* gene.

In seeking to understand why no family carried the desired translocation, several possibilities were explored [13] but the most likely cause is now considered to be modification of expression of the *R* gene (or cluster of linked genes) by the genetic background. Some influence of a background was evident in the crossing experiments of Busch-Petersen and Wood [10, 11], when backcross segregations were more clearly defined than expected (Fig. 1), reflecting a different expression of RS in the progeny of backcrosses to RR and SS. Further evidence of a background effect came from selection experiments to test whether malathion and dieldrin resistance could be separated. The procedure was to make repeated backcrosses of RS to SS with selection to remove SS segregants in each generation before the backcross was made. This technique of 'backcrossing with selection' is considered to be the best method of identifying major genes [20]. Backcrosses were made to DiS; selections were made either with dieldrin or malathion in different selected lines. In this way it proved possible to separate dieldrin and malathion resistance, leading to the conclusion that they are genetically different. The genes did not, however, behave as major factors because in both cases resistance was lost after four to eight generations of backcrossing. Dieldrin resistance proved less stable than malathion resistance, even under dieldrin selection (Saaid and Wood, unpublished).

3.2. Alcohol dehydrogenase

Robinson and Van Heemert [21] produced an effective genetic sexing system for *Drosophila* using ethanol. This was based on the *Adh* (alcohol dehydrogenase) gene. Females were killed by ethanol in the larval medium. A necessary first step for developing such a system in *Drosophila* or any other insect is the existence of pure tolerant (Adh^+/Adh^+) and pure sensitive (Adh^-/Adh^-) strains, the latter being homozygous for a null mutation.

Laboratory studies have shown that wild type strains of the medfly are tolerant of ethanol, both in the larval food and also as a vapour (adults) [10, 22]. Naturally occurring Adh^- alleles are not observed [23]. So the problem was to induce an Adh^- mutation and select it. The approach we adopted was to expose adult flies to the mutagen EMS and treat larvae with the secondary alcohol pentenol. Studies with *D. melanogaster* reported by Sofer and Hatkoff [24] showed that susceptibility to ethanol (Adh^-) is associated with tolerance of pentenol. This is because the enzyme ADH, which breaks down ethanol to relatively non-toxic products, breaks down pentenol to a highly toxic ketone. Thus, if selection leads to an increase in resistance to pentenol, and if this is due to loss of capacity to manufacture ADH, the result should be an increase in sensitivity to ethanol by selection of an Adh^- mutation. Four strains were selected with pentenol, at first without mutagen treatment. All four showed evidence of inherited variation in response to pentenol. FRANCE and COSTA RICA responded most strongly and were kept for further selection [9]. In FRANCE, selection was carried out for more than 60 generations, 19 of these being

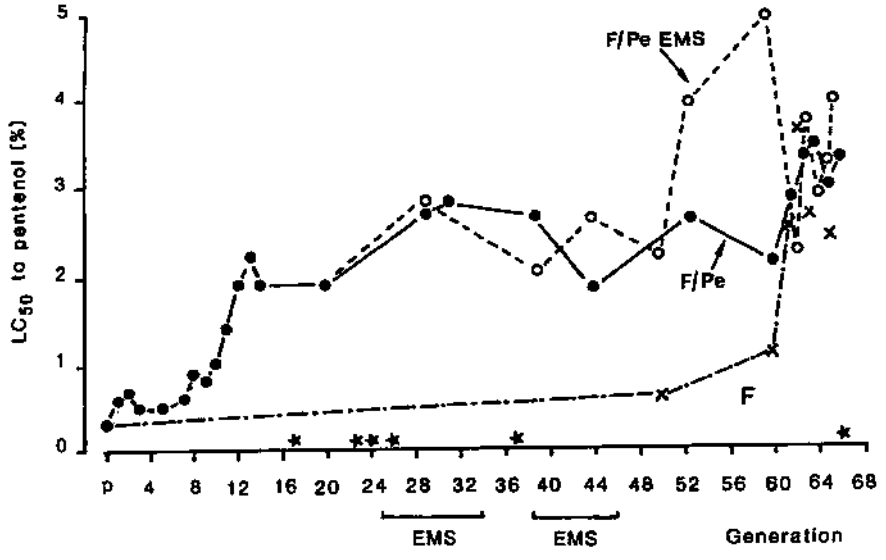


FIG. 3. The FRANCE strain selected with pentenol (F/Pe). The main selected line is compared with a subline (F/Pe/EMS) which was treated with EMS in the generations indicated and with a control strain (F). A star indicates generations in which selection was relaxed.

TABLE I. TOLERANCE TO A 90% SOLUTION OF 99.4% ETHANOL IN DEIONIZED WATER IN LATE THIRD INSTAR (JUMPING) LARVAE OF THE FRANCE STRAIN SELECTED WITH PENTENOL (AT THIRD INSTAR) (F/Pe) AND A SUBLINE TREATED WITH EMS (AT THE ADULT STAGE) (F/Pe/EMS) COMPARED WITH THE UNSELECTED BASED STRAIN (F) AS A CONTROL

Generation	Control	Selected strain	
	(F)	F/Pe	F/Pe/EMS
29	4.3 ± 0.5	5.5 ± 0.8	6.6 ± 0.5
53	3.2 ± 0.6	5.2 ± 1.2	3.8 ± 1.0
60	2.8 ± 0.7	1.2 ± 0.3	2.1 ± 0.3
61	4.3 ± 0.9	5.5 ± 1.7	4.8 ± 1.7

Note: Values are given for LT_{50} in hours with 95% limits.

exposed to EMS. The results are shown in Fig. 3, in which EMS treated (F/Pe EMS) and non-treated (F/Pe) sublines are compared with the basic strain (F) which was left unselected and untreated as a control. Periods of EMS treatment in generations 25–34 and 38–46 are indicated. Tolerance of pentenol increased with selection whether or not EMS was applied but the response to selection was greater by generation 60 in the EMS treated subline, reaching a maximum of 15× the original level in the base strain (ratio of LC_{50}s). Later, the two sublines merged again. Tolerance in the control strain was higher than expected when tested at generations 60 onwards, becoming indistinguishable from the selected sublines after generation 61 (Fig. 3). It seemed clear that we had somehow contaminated the lines and the experiment was stopped at this point.

Tests with ethanol on late third instar larvae of selected sublines and the control strain were carried out in generations 29, 53, 60 and 61. Values of LT_{50} (hours) after immersion in a 90% solution of 99.4% pure ethanol in deionized water are shown in Table I. In no case was there any change in tolerance of ethanol in comparison with the control line F, the variation between generations being quite as great as between lines.

An explanation for the failure to select for an *Adh*$^-$ mutation has been provided by subsequent electrophoresis studies on the ADH enzymes. It is now known that, in late third instar larvae of the medfly, two principal ADH systems are active, controlled by the major genes *Adh-1* located on chromosome 2 [25] and *Adh-2* so far unlocated. In designing the pentenol selection experiment we were overinfluenced by the situation in *D. melanogaster* where only one *Adh* locus is active. Van Heemert and Van den Brink [23] had discovered three electrophoretic zones of ADH activity in the medfly but they and also Riva and Robinson [26] reported on variation at a single *Adh* locus. The significance of the three zones was clarified by Milani et al. [27], using a combination of electrophoresis and linkage analysis. We have since shown that all three loci are at their most active in late third instar (jumping) larvae (Shahjahan and Wood, unpublished). The late third instar is therefore the least satisfactory stage to apply pentenol selection with any hope of isolating an *Adh* null, although from a bioassay point of view these larvae are ideal, being uniformly sized at a standard developmental stage, and easily collected and handled.

Before realizing the inherent disadvantage of using jumping larvae for alcohol bioassay, we applied the same technique to investigate the T(Y-N)128 strain of Robinson et al. [28], which had a null mutation of the *Adh-1*^{S} allele inherited only by the male. Robinson et al. [28] showed that treatment of the larval diet with allyl-alcohol killed off females of the T(Y-N)128 strain but allowed a proportion of males to survive. We exposed jumping larvae and were unable to repeat this experiment [29], presumably for the reason already given that the effect of the loss of *Adh-1* function was offset by activity of *Adh-2*. The procedure used to select the null, in order to maintain the male linked pattern of inheritance, was equally ineffective.

TABLE II. SEX RATIO IN THE T(Y-N)128 MAINTAINED IN THE LABORATORY WITHOUT SELECTION

Generation	Sample size	Females (%)	χ^2 (1:1)	p
1	284	82.1	116.6	<0.001
2	90	87.8	51.4	<0.001
6	133	65.4[a]	12.6	<0.001
6	106	65.0[b]	9.7	<0.01
9	67	64.2	5.4	<0.02
10	355	56.3	5.7	<0.02
11	519	50.1	0.002	n.s.[c]
12	371	51.5	0.3	n.s.
17	207	47.8	0.4	n.s.
18	1175	45.4	10.1	<0.01
21	1017	42.0	26.1	<0.001
22	929	40.0	36.8	<0.001
23	890	45.4	7.5	<0.01

[a] T(Y-N) male × South Africa female.
[b] T(Y-N) male × Seibersdorf female.
[c] n.s. = not significant.

Consequently the null was lost. This was accompanied by a marked increase in fertility and a change in sex ratio, from excess female to excess male (Table II).

We investigated the strain cytologically before and after loss of the null, and found that the males carried a double translocation, the first between chromosomes 2 and 5, the second between chromosomes 2 and Y. Dr. D.I. Southern's sketch of the translocation reported by Wood et al. [29] is reproduced in Fig. 4 in a modified form, with his permission. After the null had been lost, it was clear that the double translocation was still intact, with no evidence of wild type chromosome recombinants, although the proportion of translocation (i.e. male) genotypes had increased (Southern and Wood, unpublished). The apparent explanation for loss of the null was recombination without disruption of the double translocation. Recombination would allow the wild type allele to be inherited by a proportion of males and the null by a proportion of females in which it would quickly be lost because of selection against it.

It is clear from Fig. 4 that the null mutant (at the *Adh-1* locus on chromosome 2) might have been either on the 2^{5Y} chromosome or 5^2 chromosome. These two chromosomes are only viable when existing together in the same male gamete.

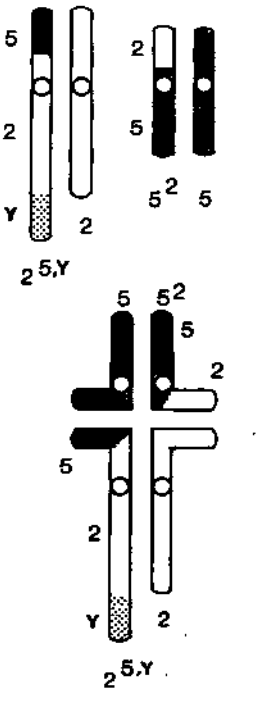


FIG. 4. The double translocation characterizing males of strain T(Y-N)128 (after a sketch by Dr. D.I. Southern). NB: The second autosome could be No. 4; chromosomes 4 and 5 are difficult to distinguish.

It is not necessary, therefore, for the null to be physically linked to the Y chromosome in order to be male linked. In fact the evidence is against the null being on 2^{5Y} because neither of the recombinants 2^5 or 2^Y has been observed cytologically. We suggest that the *Adh* locus is probably on the 5^2 chromosome, which places it distally on one arm of chromosome 2. Recombination would be relatively simple in this position and there would be every chance of the null being lost rapidly by natural selection. The evidence suggests that the loss was almost complete by generation 11 of laboratory culture. The reason the null was not lost in the hands of Riva is not clear. Robinson, who supplied us with the strain, had also experienced evidence of 'instability' and had tried to 'purify' the strain by outcrossing males selected with allyl-alcohol in the larval medium to Adh^F/Adh^F females (Robinson, personal communication, 18 Feb. 1985), as we also subsequently tried to do. Recombination was, it seems, occurring in the Wageningen and Manchester laboratories but not in Madrid. The difference has not been explained.

Two other possible explanations for the loss of the male/female difference in sensitivity to allyl-alcohol were examined. These were (a) selection for a mutagenic change in *Adh-2*, and (b) back mutation from *Adh-1*^{N} to *Adh-1*^{S}. Both were rejected after examination by electrophoresis. The T(Y-N)128 and SEIBERSDORF strains showed essentially the same pattern of esterase bands except for some small differences in the position of bands and in the presence of minor bands in T(Y-N)128, absent in SEIBERSDORF. The major *Adh-1*^{F} bands were identical in the two strains. *Adh-1*^{S} was not present (Shahjahan and Wood, unpublished).

3.3. Sex ratio distortion by meiotic drive

Work on sex ratio distortion due to meiotic drive came about as an adjunct to research directed at producing a male linked *R* gene. All F_2 families of the scheme shown in Fig. 2 were assessed for sex ratio and any showing a significant excess of males were kept for further study [13]. One of these, descended from a male irradiated as a seven day old pupa with 1.2 krad X rays, gave rise to a strain A425 which has continued to show an excess of males for more than 60 generations [16]. The sex ratio distortion is weak, having stabilized at about 45% females, but can be enhanced by selection down to less than 20% females. So far, however, it has not been possible to stabilize any selected strain at this level.

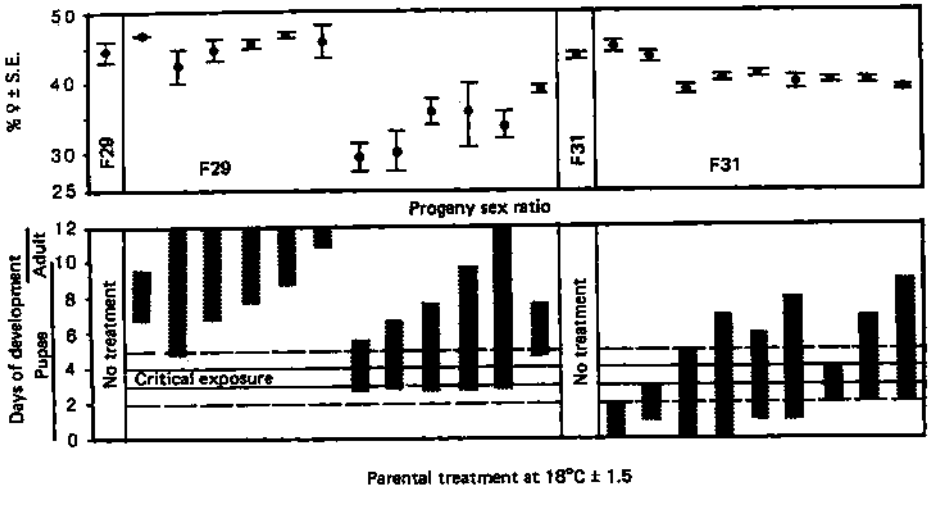


FIG. 5. Sex ratio in the progeny of individuals of strain C10 which had been maintained for various periods of pupal development at 18°C ± 1.5, the remainder of development having been at 26°C ± 2.

Recent studies have shown that the male producing factor (*MP*) found in strain A425 can be inherited only through the males, although both sexes are capable of transmitting resistance or sensitivity to it. The *MP* factor is therefore believed to be on the Y chromosome whereas resistance/sensitivity is X linked or autosomal. No chromosome abnormality can be observed. The sex ratio distortion appears to be due to a change in the normal 1:1 ratio of X:Y at meiosis. The Y chromosome shows the phenomenon of meiotic drive; the X chromosome varies in sensitivity to it. We have yet to determine the way the meiotic drive operates but we have discovered one of its properties — sensitivity to temperature. There is a critical period during pupal development (day 4) when reducing the temperature from the normal 26°C ± 2.0 to 18°C ± 1.5 increases the sex ratio distortion significantly in the subsequent generation (Fig. 5).

Studies are now proceeding in order to select a strain with a considerably distorted sex ratio which can be increased as required by appropriate temperature treatment.

4. CONCLUSIONS

4.1. Insecticide resistance

Construction of a genetic sexing technique based on male linked insecticide resistance proved to be less straightforward than expected. Three main conditions need to be met if an *R* gene is to be useful for this purpose:

(a) It should ideally relate to an insecticide not of commercial interest;
(b) It must be expressed in larvae but preferably not in adults;
(c) It must segregate with consistent expression and full penetrance whatever the genetic background.

The first two conditions were met by the gene we attempted to use but the third was not. The question now is how to isolate a gene which fulfils all these conditions.

One possibility is to look for a field selected gene because such a gene is more likely to be free from background effects. The problem is that a gene arising in the field is likely to protect the adult against insecticides of practical interest. The spectre of releasing fertile resistant males by accident is inevitably raised as a warning of the potential risk.

An alternative approach is to carry out further work on larval resistance in laboratory strains. The DiR strain itself might have potential if the dieldrin *R* gene could be separated from the gene(s) giving resistance to malathion and permethrin. More needs to be known about the genetics of resistance, using markers to map the *R* genes.

4.2. Alcohol dehydrogenase

The complicated picture revealed in *C. capitata* demonstrated the imprudence of assuming the situation in *D. melanogaster* to be the norm. With present knowledge of differential activity of *Adh-1* and *Adh-2* during the life cycle, there is more chance of isolating useful null mutations than when Robinson and Riva did their pioneering work. It should now be possible to get a null mutation into more stable linkage with the male than was achieved in T(Y-N)128. A simple translocation is likely to cause less problems with fertility. The T(Y-N)128 strain has taught us that male linkage does not necessarily mean physical linkage to the Y chromosome. Such 'linkage' due to the balance of chromosome combinations is likely to be inherently unstable and is to be distinguished from true Y linkage and avoided. Making this distinction should help to speed the way towards an effective sexing mechanism based on a Y linked *Adh* gene. The programme to isolate a homozygous null strain by complementation, recommended by Robinson and Riva [30], is basically sound and seems well worth trying if we can get a stable substitute for strain T(Y-N)128.

4.3. Sex ratio distortion by meiotic drive

The big question in relation to the MP factor is how to isolate it in the absence of resistant X chromosomes which, when present, are quickly selected. Studies on the genetic basis of variation in sensitivity, using genetic and cytological markers, are clearly important. If it proves to be polygenic, it is probably a lost cause from the genetic sexing point of view. It might, however, be due to one or two major genes as in the *MD* system of *Aedes aegypti* [14]. It is therefore important to investigate. The temperature sensitivity is also a significant aspect to investigate further, particularly by cytogenetic techniques, to discover precisely the stage of meiosis which is affected.

ACKNOWLEDGEMENTS

Grateful thanks are due to our colleagues E. Busch-Petersen, D. Southern, S. Saaid, R. Shahjahan and others who co-operated in this research. Thanks go also to those who supplied us with medfly strains.

REFERENCES

[1] WOOD, R.J., Genetic sexing techniques: introduction, IOBC/WPRS Bull. 2 1 (1979) 101.

[2] CURTIS, C.F., AKIJAMA, J., DAVIDSON, G., A genetic sexing system in *Anopheles gambiae* species A., Mosq. News **36** (1976) 492.

[3] LACHANCE, L.E., "Genetic strategies affecting the success and economy of the sterile insect release method", Genetics in Relation to Insect Management (Proc. Conf. Bellagio, 1978), Rockefeller Foundation, New York (1979) 8.

[4] RÖSSLER, Y., Automated sexing of the Mediterranean fruit fly: the development of strains with inherited sex-limited pupal colour dimorphism, Entomophaga **24** (1979) 411.

[5] ROBINSON, A.S., VAN HEEMERT, C., Genetic sexing in *Drosophila melanogaster* using the alcohol dehydrogenase locus and a Y-linked translocation, Theor. Appl. Genet. **59** (1981) 23.

[6] WOOD, R.J., MARCHINI, L.C., BUSCH-PETERSEN, E., HARRIS, D.J., "Genetic studies of tephritid flies in relation to their control", Fruit Fly Problems (Proc. Symp. Kyoto and Naha, 1980), Natl Inst. Agricultural Sciences, Yatabe, Japan (1980) 47.

[7] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*, a suitable case for genetic sexing, Genetica **58** (1982) 229.

[8] RÖSSLER, Y., "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 (1982) 291.

[9] WOOD, R.J., BUSCH-PETERSEN, E., "Possible genetic sexing mechanisms for *Ceratitis capitata* Wied.", ibid, p. 279.

[10] BUSCH-PETERSEN, E., WOOD, R.J., "Insecticide resistance as a prospective candidate for the genetic sexing of the Mediterranean fruit fly *Ceratitis capitata* (Wied.)", Fruit Flies of Economic Importance (Proc. CEC/IOBC Symp. Athens, 1982), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1983) 182.

[11] BUSCH-PETERSEN, E., WOOD, R.J., The isolation and inheritance of dieldrin resistance in the Mediterrean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), Bull. Entom. Res. **76** (1986) 567.

[12] WOOD, R.J., "Control strategies to reduce the chance of resistance with special reference to tephritid fruit flies", Pest Control (NATO ASI Series G, Vol. 11), (MANGEL, M., CAREY, J.R., PLANT, R.E., Eds), Springer-Verlag, Berlin (1986) 399.

[13] WOOD, R.J., SAAID, S.S., SHAHJAHAN, R.M., SOUTHERN, D.I., "Excess male production in lines of the Mediterrean fruit fly *Ceratitis capitata* (Wied.) isolated after X irradiation followed by outcrossing", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 179.

[14] WOOD, R.J., OUDA, N.A., The genetic basis of resistance and sensitivity to the meiotic drive gene *D* in the mosquito *Aedes aegypti* L., Genetica **72** (1987) 69.

[15] WOOD, R.J., "Temperature-dependent sex ratio distortion after X-irradiation of *Ceratitis capitata* males", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Rome, 1987), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1988) in press.

[16] WOOD, R.J., SHAHJAHAN, R.M., "Temperature dependent sex ratio distortion after X-irradiation of medfly males", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Symp. Vienna, 1987), IAEA, Vienna (1988) in press.

[17] SAAID, R.A.S., Genetic and Toxicological Studies in the Mediterranean Fruit fly *Ceratitis capitata* in Relation to the Development of a Genetic Sexing Technique, unpublished PhD Thesis, Univ. of Manchester, UK (1986).

[18] BUSCH-PETERSEN, E., Genetical Studies on Insecticide Resistance and Alcohol Tolerance in the Mediterranean Fruit Fly *Ceratitis capitata* (Wied.), unpublished PhD Thesis, Univ. of Manchester, UK (1983).

[19] BENNET, J., A comparison of selective methods and a test of the preadaptive hypothesis, Heredity **15** (1960) 65.

[20] WRIGHT, S., The genetics of quantitative variability: quantitative inheritance, isolation of major units, Agricultural Research Council pamphlet, ARC, London (1952).

[21] ROBINSON, A.S., VAN HEEMERT, C., Genetic sexing in *Drosophila melanogaster* using the alcohol dehydrogenase locus and a Y-linked translocation, Theor. Appl. Genet. **59** (1980) 23.

[22] KEISER, I., MIYASHITA, D.H., SCHNEIDER, E.L., HARRIS, E.J., CHAMBERS, D.L., Survival of oriental fruit flies, melon flies and Mediterranean fruit flies after immersion in alcohol or acetone as larvae, J. Econ. Entomol. **67** (1974) 303.

[23] VAN HEEMERT, C., VAN DEN BRINK, W.J., Alcohol dehydrogenase polymorphism in the Mediterrean fruit fly *Ceratitis capitata*, Entomol. Exp. Appl. **28** (1980) 229.

[24] SOFER, W., HATKOFF, M., Chemical selection of alcohol dehydrogenase negative mutants in *Drosophila*, Genetics **72** (1972) 545.

[25] ZAPATER, M., ROBINSON, A.S., Sex chromosome aneuploidy in a male-linked translocation in *Ceratitis capitata*, Can. J. Genet. Cytol. **28** (1986) 161.

[26] RIVA, M.E, ROBINSON, A.S., "Studies on the ADH locus in *Ceratitis capitata*", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Athens, 1982), (CAVALLORO, R., Ed.), Balkema, Rotterdam (1983) 163.

[27] MILANI, R., GASPERI, G., MALACRIDA, A.R., "Recent progress and perspectives in the genetical studies of *Ceratitis capitata*", Report of Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and Other Genetic Measures, Vienna, July 1985, available from FAO/IAEA Joint Division, International Atomic Energy Agency.

[28] ROBINSON, A.S., RIVA, M.E., ZAPATER, N.,Genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata* using the alcohol dehydrogenase locus,Theor. Appl. Genet. **72** (1986) 455.

[29] WOOD, R.J., SOUTHERN, D.I., SAAID, S.S., PROUDLOVE, G.S.,"Progress towards developing a genetic sexing mechanism for the Mediterranean fruit fly *Ceratitis capitata* (Wied.)", Report of Research Co-ordination Meeting on the Development of Sexing Mechanisms in Fruit Flies through Manipulation of Radiation-Induced Conditional Lethals and Other Genetic Measures, Vienna, July 1985, available from FAO/IAEA Joint Division, International Atomic Energy Agency, Vienna.

[30] ROBINSON, A.S., RIVA, M.E., A simple method for the isolation of allelic series using male-linked translocations, Theor. Appl. Genet. **67** (1984) 305.

GENES FOR GENETIC SEXING IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), AND THE MOSQUITO, *Anopheles stephensi*

A.S. ROBINSON*
Research Institute ITAL,
Wageningen, Netherlands

Abstract

GENES FOR GENETIC SEXING IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.), AND THE MOSQUITO, *Anopheles stephensi*.

A system was developed utilizing alcohol dehydrogenase (ADH) that produced only male medflies. High sterility in the line plus male mortality from the treatment made the system unsuitable for genetic sexing on a large scale. A strain of *Anopheles stephensi* was developed in which females could be killed by dieldrin, but males survived. A large field cage release of medfly sexed by colour in the pupal stage demonstrated the advantages of male-only releases. The pupal colour sexing strain broke down under mass rearing.

1. INTRODUCTION

The results presented here cover the period 1981–1987. The research was carried out initially with research contracts but from 1984 onwards in the form of research agreements. This division was necessary as the Research Institute ITAL would no longer carry out work on fruit flies after 1984. The research agreements were based on closely related work on the mosquito *Anopheles stephensi*. The aim of the work has been to develop genetic sexing systems for these two species based on two principles: sex killing and sex separation.

1.1. Sex killing

In this system either the males are made resistant to a particular environmental constraint or the females are made susceptible. If the constraint can be applied at an early developmental stage, this is the most efficient technique.

---

* Present address: Research Centre of Crete, IMBB, P.O. Box 1527, Heraklion, Crete, Greece.

1.1.1. Alcohol dehydrogenase (ADH)

In *Drosophila* it is known that individuals producing an active alcohol dehydrogenase enzyme (ADH) can metabolize ethanol to acetaldehyde and the latter's conversion to acetate [1]. Individuals with no active enzyme cannot perform these steps and are poisoned by environmental ethanol [2]. Using *Drosophila melanogaster* as a model, a genetic sexing strain was synthesized in which an active ADH allele was linked by a translocation to the male sex, the females being ADH null and thus sensitive to environmental ethanol [3].

To develop this system in the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), translocations linking an active ADH allele were induced and isolated and cytologically confirmed. The absence of sophisticated genetic marker strains for the isolation of null mutants means that the identification of a single mutant can be achieved only through the electrophoretic screening of many F_1 individuals following mutagenic treatment [4]. In an attempt to increase the efficiency of this method, the F_1 larvae were reared in a selective medium containing the secondary alcohol, allyl-alcohol. This alcohol preferentially selects for individuals having reduced ADH activity [5]. Individuals having the active enzyme convert this secondary alcohol into a toxic ketone. Using a combination of a selective larval medium and electrophoretic screening, several ADH mutants were identified and characterized.

1.1.2. Resistance to dieldrin

By linking an insecticide resistance gene to the male-determining chromosome many genetic sexing systems have been developed in mosquitoes [6]. By focusing on dieldrin resistance in *Anopheles stephensi*, a genetic sexing line was developed in this species and it was used in large cage experiments.

1.2. Sex separation

Here sexing is based on a recognizable phenotypic difference between males and females. In the medfly gene, systems were available which could be used: pupal colour mutants. By linking a particular gene to the male-determining chromosome it was possible to produce a strain in which females emerged from white pupae and males emerged from brown pupae.

2. RESULTS

2.1. Alcohol dehydrogenase (ADH)

The approach adopted was as follows:

— Identification of mutants at the ADH locus;
— Translocation of an active ADH allele to the male-determining chromosome;
— Induction and isolation of ADH null mutants.

Using conventional electrophoretic screening of many medfly populations, a slow and a fast allele were found at the *Adh-1* locus. Both alleles showed a single major band, the heterozygote having three bands. Following irradiation, a series of translocations was induced in which one of the alleles was translocated to the male-determining chromosome [7]. Cytological analysis enabled the *Adh-1* locus to be assigned to chromosome 2 [8]. During this cytological analysis important data relating to sex determination in this species became available. It was shown that, in contrast to *Drosophila*, the Y chromosome carries the male-determining factor and, further, that the male determinant is on the long arm of the Y.

The induction and isolation of null mutants proved to be extremely difficult and frustrating owing to the absence of the necessary genetic marker strains. The scheme used to isolate null mutants is shown in Fig. 1. Following irradiation and treatment of F_1 larvae by a selective medium, null heterozygotes were identified by the fact that they only showed one band on the electrophoresis gel whereas the wild type F_1 individuals had three bands. The F_1 male (FN) was outcrossed to SS females and the F_2 male progeny were outcrossed individually to FF females. Following oviposition the F_1 males were checked, single banded males (SN) identified and progeny retained. This mating scheme enabled the mutant to be maintained as heterozygotes in half the population (Table I).

Following inbreeding of the mutant lines, no individuals lacking ADH activity were found and it was concluded that all the mutants were homozygous inviable. Complementation studies yielded non-viable combinations [9].

Maintaining the mutant lines was laborious, so a scheme was devised in which the null mutation was translocated to the male-determining chromosome. The line could then be maintained without selection and it could be used for genetic sexing in the following way. Males carrying this translocation are heterozygous null and females have two active alleles so that in the presence of larval medium containing allyl-alcohol which preferentially selects for individuals having low ADH, the males should survive and the females should die. By finely adjusting the concentration of the alcohol in the medium it was possible to obtain only male survivors (Table II). However, the high sterility in the line plus the fact that a large number of males were being killed by the treatment makes the line unsuitable for genetic sexing on a large scale [10].

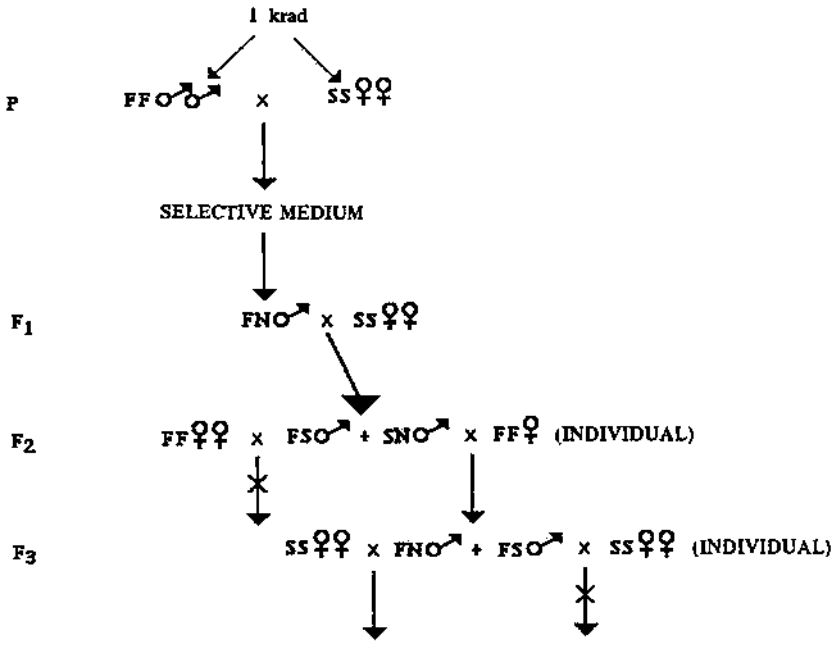


FIG. 1. Mating scheme for isolation of ADH null mutants.

TABLE I. SEGREGATION DATA FOR FOUR ADH NULL MUTANTS

Mutant	No. of FS individuals	No. of heterozygous ADH null	χ^2 (1:1) (not significant)
N_1	101	79	2.685
N_2	92	88	0.088
N_3	79	77	0.025
N_4	65	60	0.200

Conclusions

There are two major difficulties in using ADH for genetic sexing in medflies. The first is the fact that a second ADH locus has been identified and the second is the difficulty of isolating rare null mutants in the absence of genetic marker strains.

TABLE II. EFFECT OF ALLYL-ALCOHOL ON LARVAL SURVIVAL OF TWO MEDFLY STRAINS

Strain	Allyl-alcohol concentration	No. of eggs	No. of larvae	No. of pupae	No. of adults	
					Males	Females
T(Y-*Adh*^{b})	0.0	482	418	282	75	112
	0.08	500	407	67	52	0
	0.092	494	346	5	5	0
Wild type	0.0	778	615	546	241	286
	0.08	1060	791	4	2	0
	0.092	1090	798	0	0	0

It is not clear at the moment if an ADH null strain at *Adh-1* could be used for genetic sexing if the strain was wild type at *Adh-2*. My feeling is that it would, considering the fact that a genetic sexing line, albeit very inefficient, could be synthesized using a heterozygous null mutant at *Adh-1*.

The translocation strain with the null mutation is extremely valuable as it can be used efficiently to isolate new ADH null mutants [11]. This aspect should have top priority if further work is carried out on ADH.

2.2. Dieldrin

In *A. stephensi*, dieldrin resistance is coded for by a gene located on linkage group 3 [12], and DDT resistance is controlled by a single gene also on chromosome 3. These two resistance genes were combined into a single strain, which was used as a target for the induction of male linked translocations. Males of the DDT and dieldrin resistant line were irradiated with 5 krad of X rays and crossed to susceptible females. Backcross progeny descending from semisterile families were retained and reared to F_2 adults. The F_2 adults were inbred and large numbers of F_3 fourth instar larvae were exposed to discriminating doses of both insecticides, 5 ppm DDT and 0.15 ppm dieldrin for 24 hours. The surviving larvae were checked for sex, and families showing a strong sex ratio distortion in favour of males were retained. In total, 216 semisterile F_3 families were checked, resulting from 2430 F_1 ovipositions. Several lines were retained, amongst them T(Y-DI)35, which shows extremely close linkage between the male-determining chromosome and dieldrin resistance. The line was inbred at the F_4 and the F_5 larvae exposed to 0.15 ppm

TABLE III. EFFECT OF DIELDRIN ON FOURTH INSTAR LARVAE OF LINE T(Y-D1)35 IN *A. stephensi*

Generation	Dieldrin dose (ppm)	Total No. exposed	Total (%) died	Surviving pupae		Recombination (%)
				Males	Females	
F_5	0.15	50	23 (46.0)	27	0	0.0
F_6	0.15	350	216 (61.7)	133	1	0.746
F_7	0.10	600	363 (60.5)	236	1	0.422
F_8	0.10	2467	1254 (50.8)	1210	3	0.247
F_{10}	0.15	2005	1265 (63.1)	738	2	0.270
F_{11}	0.10	2017	1149 (56.7)	864	4	0.461
	Total	7489	4270 (56.9)	3208	11	0.342

dieldrin; only male pupae survived the treatment (see Table III). The line was retested at generations 6, 7, 8, 10 and 11. A total of 7489 larvae were exposed to discriminating doses of dieldrin and 3219 survived. From these only 11 female pupae were produced (none of which emerged) and 3208 male pupae (Table III). The line has now been reared to the 18th generation in very large numbers without any sign of loss of the linkage between dieldrin resistance and the male sex. The fertility of the translocation was about 25% and cytological studies showed that a complex translocation was involved in which the Y chromosome was broken twice, with one break in each arm. There was a break in chromosome 3L and one in chromosome 2. Polytene analysis revealed that the break in chromosome 3L was at map reference 43/44 (using the polytene map of Sharma et al. [13]). Therefore the dieldrin locus must be very close to this position [14].

The treatment of this line with dieldrin to effect genetic sexing took place for 24 hours, and four instar larvae were used. A serious problem associated with this method was reduced emergence of the male pupae, so an alternative insecticide treatment was developed: the cone method, in which a cone of paper with a hole in the top is placed over a bowl containing pupae. When the mosquitoes emerge they rest on the walls of the cage and the females pick up a lethal dose of insecticide whereas the males survive with no reduction in their competitiveness. The females do emerge from the cone but die within 24 hours. The results of using the cone method can be seen in Table IV. This system was used to make large scale releases of males into the laboratory cage.

TABLE IV. EFFECT OF DIELDRIN ON NEWLY EMERGED *A. stephensi* USING THE CONE METHOD

Strain	No. of pupae		No. of mosquitoes			
	Tested	Dead	Males	Females	Males	Females
Susceptible	2510	0	0	8	1234	1268
Resistant	1060	93	450	487	11	19
Genetic sexing	1324	35	639	4	6	640

Conclusions

The availability of a well analysed genetic resistance mechanism was of great benefit in this study and, as in many mosquito species, a large enough screen produced the necessary translocation.

The dieldrin resistance was extremely tightly linked and subsequent inbreeding for many generations did not reveal any breakdown in the strain.

2.3. Pupal colour

The first genetic sexing strain in the medfly was produced by linking the wild type allele of black pupae to the male-determining chromosome [15]. However, as the discrimination between the black pupae (females) and the brown pupae (males) was not always satisfactory it was decided to use another pupal colour mutant, *white*.

Following irradiation, six lines were isolated which appeared suitable for genetic sexing [16]. Three of these were chosen for further study and many fitness parameters were measured. The lines showed good stability under laboratory rearing conditions. However, two of the lines showed unusual segregation ratios in that, instead of a 1:1 ratio of white pupae to wild type pupae, there were 1.5 times as many white pupae (Table V). It was proposed that the excess white pupae were due to the survival, to that stage, of duplication deficiency zygotes. This hypothesis was supported by the fact that one third of the white pupae did not emerge so that the final sex ratio was 1:1 [17].

The line showing no distortion in pupal colour segregation (T-101) was chosen to initiate field cage studies, using male-only releases. The question being asked was: what is the biological effectiveness of a release of males-only compared to a release involving males and females? The strain was transferred to the FAO/IAEA Entomology Laboratory at Seibersdorf where it was mass reared and the pupae air freighted to Procida Island, Italy, for field cage studies.

TABLE V. SEGREGATION OF PUPAL COLOUR GENOTYPES IN THREE MALE LINKED TRANSLOCATIONS

	No. of brown pupae	No. (%) of males emerged	No. of white pupae	No. (%) of females emerged
T-23	1549	1334 (86)	2380	1113 (47)
	1	:	1.5	
T-49	3008	2388 (79)	4749	1977 (42)
	1	:	1.6	
T-101	4980	4118 (83)	4824	3274 (68)
	1	:	0.9	

In large walk-in field cages containing two citrus trees a series of experiments was carried out in which mating propensity data were collected when either males alone were released or males and females. The target population consisted of wild type flies collected locally as pupae from infested fruit.

It was clearly shown that removal of females prior to release greatly increased the penetrance of the released males into the wild population and thereby the amount of sterility in the population. There appeared to be no serious mating barrier between the released males and the wild females [18].

The results were considered as giving some indication that, in addition to the economic saving to be made by using a genetic sexing strain, there are very important biological advantages if only males are released.

Of some concern during these experiments was the breakdown of the strain under a mass rearing regime (Table VI) [19]. This was unexpected, as under normal laboratory rearing this breakdown had not occurred. The two most obvious causes of strain breakdown are contamination, recombination or a combination of the two. Dealing first with recombination where equal frequencies of males emerging from white pupae and females emerging from brown pupae are expected, the observed breakdown in no way suggested that recombination alone was responsible for the breakdown. Contamination is a more complex problem as different types of contamination can occur: virgin females, males or mated females. All these types of contamination were simulated using different levels of contamination but in no case could a satisfactory match be found between the observed breakdown and any of the simulated strategies.

The fact that the proportion of white pupae in the strain rapidly declined suggested that under mass rearing conditions the white pupae genotype suffered

TABLE VI. EFFECT OF MASS REARING ON THE STABILITY OF LINE T-101

Generation	Brown pupae (%)	Males in white pupae (%)	Females in brown pupae (%)
P	59.8	0.1	0.1
1	53.7	0.5	4.4
2	62.2	0.2	8.1
3	61.8	2.0	12.5
4	63.2	8.0	20.2
5	67.5	14.2	28.2
6	73.3	15.2	30.2
7	76.5	11.6	39.9
8	77.7	18.7	39.7
9	86.8	18.4	44.5

some viability reductions. By simulating reduced viability of the white pupae genotype in combination with a low level of recombination, the breakdown of the strain could be mirrored. It was therefore concluded that under normal scale laboratory rearing with a more relaxed rearing scheme there were no viability differences between the genotypes and the strain remained stable.

Transfer of the strain to the more stringent conditions of mass rearing revealed the reduced viability of the white pupae genotype.

Conclusions

The results of this work on pupal sexing can be summed up as follows. Firstly, if a suitably large scale radiation experiment should be carried out, a strain could be isolated which would show sufficient stability for it to be mass reared and used as a genetic sexing line. Secondly, the increased biological effectiveness of an all male release would enable a genetic sexing line based on pupal colour to provide an economically attractive alternative to conventional sterile insect technique releases. Thirdly, upscaling the rearing of a genetic sexing line is a more complex process than was at first considered. In addition to the problem of contamination, the possible effects of mass rearing on any viability differences amongst the genotypes can seriously jeopardize the stability of a genetic sexing line.

3. WHERE TO NOW?

With the benefit of hindsight several suggestions can be offered for future work.

Pupal sexing is a viable and practical means of sexing medflies. The simplicity of the approach (not to mention the availability of the relevant gene systems), speaks for itself.

The use of other systems involving the isolation of rare mutants should only be started when the essential balancer stocks are available. This would suggest that, in addition to funding the direct approaches to genetic sexing, the IAEA should consider funding more basic studies.

Field studies using the available pupal sexing strains must be expanded in order to quantify more exactly the merits of an all male release.

ACKNOWLEDGEMENTS

This work would not have been possible without the significant contribution of M.E. Riva and M. Zapater. Gijs Schelling provided excellent technical assistance. The medfly work was supported by the IAEA and the mosquito work by several Dutch ministries.

REFERENCES

[1] HEINSTRA, P.W.G., et al., A dual function of alcohol dehydrogenase in *Drosophila*, Genetica **60** (1983) 129.

[2] GRELL, S.G., JACOBSON, K.B., MURPHY, J.B., Alterations of genetic material for analysis of alcohol dehydrogenase isozymes of *Drosophila melanogaster*, Ann. N.Y. Acad. Sci. **151** (1968) 441.

[3] ROBINSON, A.S., VAN HEEMERT, C., Genetic sexing in *Drosophila melanogaster* using the alcohol dehydrogenase locus and a Y-linked translocation, Theor. Appl. Genet. **59** (1980) 23.

[4] DICKINSON, W.J., SULLIVAN, D.T., Gene Enzyme Systems in *Drosophila*, Springer, Berlin–Heidelberg–New York (1975).

[5] FREELING, M., CHENG, D.S.K., Radiation induced alcohol dehydrogenase mutants in maize following allyl alcohol selection of pollen, Genet. Res. **31** (1978) 107.

[6] ROBINSON, A.S., Sex-ratio manipulation in relation to insect pest control, Ann. Rev. Genet. **17** (1983) 191.

[7] RIVA, M.E., ROBINSON, A.S., "Studies on the ADH locus in *Ceratitis capitata*", Fruit Flies of Economic Importance (Proc. CEC/IOBC Int. Symp. Athens, 1982), Balkema, Rotterdam (1983) 163–171.

[8] ZAPATER, M., ROBINSON, A.S., Sex chromosome aneuploidy in a male-linked translocation in *Ceratitis capitata*, Can. J. Genet. Cytol. **28** (1986) 161.

[9] RIVA, M.E., ROBINSON, A.S., Induction of alcohol dehydrogenase null mutants in the Mediterranean fruit fly, *Ceratitis capitata*, Biochem. Genet. **24** (1986) 765.

[10] ROBINSON, A.S., RIVA, M.E., ZAPATER, M., Genetic sexing in the Mediterranean fruit fly, *Ceratitis capitata*, using the alcohol dehydrogenase locus, Theor. Appl. Genet. **72** (1986) 445.

[11] ROBINSON, A.S., RIVA, M.E., A simple method for the isolation of allelic series using male-linked translocations, Theor. Appl. Genet. **67** (1984) 305–306.

[12] AKHTAR, K., SAKAI, R.K., BAKER, R.H., Linkage group III in the malaria vector, *Anopheles stephensi*, J. Hered. **73** (1982) 473.

[13] SHARMA, G.P., CHANDRY, S., CHARDRY, A., Studies on the salivary gland chromosomes of an isolated pupulation on *A. stephensi*, Cytobios **20** (1977) 35.

[14] ROBINSON, A.S., PHAM VAN LAP, Cytological, linkage and insecticide studies on a genetic sexing line in *Anopheles stephensi*, Heredity **58** (1987) 95.

[15] RÖSSLER, Y., Automatic sexing of the Mediterranean fruitfly: the development of strains with inherited, sex-limited pupal colour dimorphism, Entomophaga **24** (1979) 411.

[16] ROBINSON, A.S., VAN HEEMERT, C., *Ceratitis capitata*: a suitable case for genetic sexing, Genetica **58** (1982) 229.

[17] ROBINSON, A.S., Unexpected segregation ratios from male-linked translocations in the Mediterranean fruitfly, *Ceratitis capitata*, Genetica **62** (1984) 209.

[18] ROBINSON, A.S., CIRIO, U., HOOPER, G.H.S., CAPARELLA, M., Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, *Ceratitis capitata*, Entomol. Exp. Appl. **41** (1986) 23.

[19] HOOPER, G.H.S., ROBINSON, A.S., MARCHAND, R.P., "Behaviour of a genetic sexing strain of Mediterranean fruit fly, *Ceratitis capitata*, during large scale rearing", Fruit Flies (Proc. 2nd Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier, Amsterdam and New York (1987) 349–362.

GENETIC SEXING STRAINS FOR FOUR SPECIES OF INSECTS

J.A. SEAWRIGHT
Insects Affecting Man and Animals Laboratory,
Agricultural Research Service,
United States Department of Agriculture,
Gainesville, Florida,
United States of America

Abstract

GENETIC SEXING STRAINS FOR FOUR SPECIES OF INSECTS.

Genetic sexing strains were assembled by using classical genetic and cytogenetic techniques for four medically important species of insects. Male linked reciprocal translocations were used to impose pseudolinkage of sex and selectable genes (shown in parentheses) for *Anopheles albimanus* (propoxur resistance), *Anopheles quadrimaculatus* sp. A (malathion resistance), *Stomoxys calcitrans* (dieldrin resistance, malathion resistance, and black pupa), and *Musca domestica* (black pupa). These strains would be of great value in implementation of the sterile insect technique for control of these species because the females can either be killed in the egg stage (in the case of insecticide resistance as the selectable gene) or they can be separated from the males and thus excluded from releases.

1. INTRODUCTION

Genetic methods for the control of pest insects usually involve the release of millions of radiosterilized males produced in large mass rearing factories. Good examples of genetic control methods are the large operational programmes being conducted for the eradication of the screw-worm fly, *Cochliomyia hominovorax*, and the Mediterranean fruit fly (medfly), *Ceratitis capitata*. Release of the females of phytophagous species can result in serious crop damage, and hematophagous species can contribute to the transmission of diseases. The females are also generally neutral in so far as the efficacy of genetic control methods is concerned, but mass rearing of the sexes requires the same expenditure of resources. For these reasons, it is desirable to avoid having to rear and release the females during the execution of the sterile insect technique (SIT). The obvious need to release only sterile males in an SIT programme led us to initiate research projects on the assembly of genetic sexing strains designed so that the females could be killed, either in the egg stage or as neonate larvae.

Our work in the area of genetic sexing has dealt strictly with anopheline mosquitoes and the stable fly, *Stomoxys calcitrans*. Therefore, we were concerned about the use of SIT for hematophagous disease vectors, in which the females inflict bites and transmit disease. The impetus for our research stems from the successful use of sterile males in operational pilot studies on the genetic control of *Anopheles albimanus* in El Salvador [1] and the stable fly on the island of St. Croix [2]. We were also interested in assembling genetic sexing systems for pest species for which the basic genetic information was limited, in order to demonstrate that these specialty strains could be bred by using classical genetic and cytogenetic techniques without a heavy investment in basic research. Part of our work was oriented towards training scientists from developing countries, so we also included colour mutants as tools for demonstration systems. In this report the results are summarized of our experimentation on assembling genetic sexing strains of *Anopheles albimanus*, *A. quadrimaculatus*, *Stomoxys calcitrans* (stable fly), and *Musca domestica* (house fly). Selectable genes were either pesticide resistance or pupal colour mutants. Radiation was used to break and rearrange the chromosomes so that the selectable gene was linked to the male (Y) chromosome.

2. MATERIALS AND METHODS

2.1. Species and selectable genes

The species and selectable genes used to assemble genetic sexing strains were:

Species	Selectable gene
A. albimanus	Propoxur resistance (pr^R)
A. quadrimaculatus sp. A	Malathion resistance (mal^R)
S. calcitrans	Dieldrin resistance (dl^R)
S. calcitrans	Malathion resistance (mal^R)
S. calcitrans	Black pupa (*bp*)
M. domestica	Black pupa (*bp*)

The mode of inheritance of each of the resistance mechanisms had been studied and was known to be under the control of a single dominant gene. The dieldrin resistance in *S. calcitrans* was sex linked with modifiers on the autosomes, and the other resistance traits were autosomal. The black pupa traits in both species were known to be recessive and autosomal.

2.2. Induction of translocations

Since the males of anopheline mosquitoes, stable fly, and house fly are heterogametic for sex determination, a reciprocal translocation between the Y chromosome and the autosomal linkage group containing the gene responsible for either the pesticide resistance or the wild type allele of black pupae (bp^+) provided the basis for a sexing system. For inducing an appropriate male linked translocation, adult males (less than 24 hours old) were exposed to an appropriate dose of gamma rays from a ^{137}Cs unit. These irradiated males were crossed to females that were homozygous recessive for either pesticide susceptibility or the *bp* mutant, and the resulting F_1 males were backcrossed to homozygous recessive females. The crossing scheme to detect Y linked translocations was similar to that of Kaiser et al. [3]. For the systems based on resistance, the backcross families were bioassayed with the pesticide. Linkage between sex and resistance was taken as an indication that a reciprocal translocation had been induced. In the systems based on black pupa, linkage between sex and the wild type colour was taken as evidence of a male linked translocation. For *A. albimanus*, male linked translocations were already available from previous work [3], and a simple irradiation and cross scheme was devised to induce inversions that would suppress crossing over between the translocation breakpoints and the pr^r gene.

2.3. Cytological confirmation of translocations and inversions

For the two anopheline species, meiotic chromosomes from adult testes and salivary gland polytene chromosomes were prepared as described by Kaiser et al. [3] and Mitchell and Seawright [4] and used for cytological confirmation of translocations and inversions. Stable fly and house fly were treated with colchicine, and preparations of the chromosomes from adult testes were made according to Willis et al. [5] and Walder and Seawright [6], respectively.

2.4. Techniques for mass treatment of eggs with pesticide

Determination of a discriminating treatment that would kill the susceptible type but not the resistant type was accomplished by floating the eggs of the mosquitoes on aqueous solutions and coating the eggs of the stable fly with various concentrations of the respective pesticide.

3. RESULTS

No unforeseen difficulties were encountered in assembling the genetic sexing strains, and it was relatively easy to induce translocations and/or inversions that

established pseudolinkage between the selectable gene and sex. Except for the critical decisions on pursuing certain pesticide resistance mechanisms and cytological analysis, technicians and students did most of the work with only a minimum of supervision.

3.1. Anopheles albimanus

A strain, *In(2R)[T(Y;2R)3]1*, designated MACHO, of *A. albimanus* had been assembled [3] and subsequently used for mass rearing sterile males during a pilot study in El Salvador [7]. Females of the MACHO strain could be killed by floating the eggs on an aqueous solution of 20 ppm propoxur for 30 minutes. Discrimination between the hybrid and susceptible types could be made in all four life stages. In the MACHO strain, pseudolinkage of the propoxur resistance gene to sex was effected by means of a male linked translocation and an inversion. The translocation was isolated first and the inversion was induced by irradiation of males of the translocation strain. The inversion was necessary because, in contrast to the higher dipterans (e.g. house fly and stable fly), meiotic crossing over is the normal state for both sexes of anopheline mosquitoes, so we expected to obtain strains for which the pseudolinkage would be incomplete. The only problem with the MACHO stock was the production of 0.2% recombinant, resistant females. Although precautions had to be taken to monitor the breakdown of the pseudolinkage during mass rearing, this level of recombination was an inconvenience rather than an impediment in producing sterile males.

In an effort to assemble a stock with more stability, we irradiated males of another translocation strain, *T(Y;2R)6*, which had less crossing over than the translocation used to make MACHO. Six new translocation inversion stocks were isolated, but none of these strains was better, and only one of the new aberration complexes was as good as MACHO. A subsequent genetic analysis, using mutant markers which became available after the initial work with isolation of genetic sexing strains, showed that the inverted segments of 2R in these strains were too large. Therefore, a low rate (0.2%) of double recombination within the inversion switched the propoxur resistance gene to the normal 2R and hence to the female gametes (details were published [8]). More recently, we have used our more extensive knowledge of the genetic map of 2R to synthesize a balanced strain with overlapping inversions that completely fixes propoxur in the inversion complex. This development means that if there is ever a need for stable sexing strains of this mosquito, it should be possible to make overlapping inversions first and then induce a translocation inside one of the inversions.

3.2. Anopheles quadrimaculatus

A translocation strain, *T(Y;3R)1*, was assembled that had very tight pseudolinkage between sex and malathion resistance. *T(Y;3R)1* bred true through several

generations. Six other stocks showed a lesser degree of pseudolinkage, with recombinant, resistant females being present. A cytological analysis of the polytene chromosomes divulged that in *T(Y;3R)1* the translocation breakpoint on the autosome was inside a small, naturally occurring paracentric inversion on chromosome 3. The malathion resistance locus was advantageously packaged by nature for genetic sexing, and no viable resistant females were present in an analysis of 10 000 mosquitoes (over 12 generations) of this strain. Mass treatment of eggs for 24 hours with an aqueous solution of 200 ppm of malathion was adequate to kill all the susceptible females. Shorter time intervals with higher concentrations of insecticide could also be used for the treatment of eggs, but, for convenience and safety of personnel, we fixed the treatment time at 24 hours to reduce the insecticide concentration. A 24 hour treatment was used on an operational scale for mass rearing sterile males of *A. albimanus* [7]. The resistance trait was manifest in all life stages (i.e. egg, larva, pupa and adult), so the females could if necessary be selectively eliminated at any point of the life cycle. Details of this genetic sexing system were published [9].

3.3. Stable fly

3.3.1. Dieldrin resistance

The strain based on dieldrin resistance for the stable fly was the easiest to assemble because the resistance gene was already sex linked. Since the male of the stable fly is heterogametic and has achiasmatic meiosis, a simple cross of a resistant male to a susceptible female, followed by a backcross of the hybrid male progeny to susceptible females, established a strain, designated STUD, that bred true. It was impossible to obtain mortality of the susceptible female embryos by treatment with dieldrin in aqueous solution, probably because the insecticide did not pass through the egg chorion. Therefore, mass treatment of the STUD strain with dieldrin was accomplished by placing eggs on a strip of filter paper impregnated with the insecticide, and the newly hatched larvae crawled across the pesticide residue to reach the rearing media. The strain was inefficient, in that a dosage of dieldrin that killed all the females also killed 30% of the males. We were therefore concerned about the possibility of debilitating effects of the dieldrin treatment on the surviving males. A series of laboratory and field cage tests was conducted to measure the mating competitiveness of males of this strain after they had been treated with dieldrin and sterilized by gamma radiation. Although the competitiveness estimates were variable, the overall (total) estimate indicated an acceptable level of competitiveness. Complete details of the characteristics of STUD and the competitiveness tests were published [10, 11].

3.3.2. Malathion resistance

A dominant malathion resistance gene, present in several different field populations collected in the United States of America, served as the basis for the best genetic sexing strains assembled for the stable fly. The resistance was very intense (×500), and separation of the hybrid and susceptible types was easily achieved. (Another dominant malathion resistance gene that conferred a less intense resistance was also detected in a population from Mauritius.) Several strains, differing in the translocation breakpoints, have been isolated. The susceptible females can be killed by coating the eggs of these strains with malathion. Complete details of this system and the characteristics of the *T(1:2)5* translocation were published [12].

3.3.3. Black pupa

A recessive mutant, black pupa (*bp*), was used for assembly of a genetic sexing system that allowed the separation of the sexes by means of a seed sorting machine. Such a system would be useful for an integrated pest management programme involving the release of parasites and sterile insects, because the females could be used for parasite production. Details of this system were published [5, 13].

3.4. House fly: black pupa

As part of a training programme for an IAEA Fellow, a genetic sexing system, based on a black pupa mutant phenotypically identical to the *bp* trait in the stable fly, was assembled for the house fly. Details of this work were published [6].

4. DISCUSSION

The results of our work indicate that genetic sexing systems can be easily assembled by means of conventional genetic and cytogenetic techniques. An X-Y (or other heterogametic male) sex determination system is a prerequisite for the assembly of strains based on male linked translocations. In the development of these useful strains, the critical step is the isolation of a suitable selectable (conditional) marker, e.g. pesticide resistance due to a single, dominant gene. If a good pesticide resistance gene is not available or is undesirable owing to of the possibility of accidental release of the resistant type, then there are other types of conditional traits (e.g. heat sensitive lethals) that could be considered. The induction of effective translocations and/or inversions is simply a matter of setting up a good protocol and staying with it until the desired strain is found.

For assembling genetic sexing strains, an in depth study of basic genetic information on a species is helpful, but not necessary. Except for the house fly, all the

strains discussed above were made with scanty genetic information. None of the work was attempted until sound methods were available for examining the chromosomes of each species, and the capability of cytological confirmation of chromosome aberrations should be considered as an important part of this type of work. In the case of *A. albimanus*, crossing over in the male prevented the establishment of a completely true breeding strain, and this is a case where more complete genetic information would have been useful. For example, with the current, extensive genetic map and existing mutants for *A. albimanus*, it should be relatively easy to make a translocation inversion complex that would fix the resistance gene in the male.

After the synthesis of genetic sexing strains through chromosomal manipulations, 'genetic leakage' of undesirable types that cause a breakdown of the ability to separate the sexes is a critical problem. Knowing the level of stability of a strain is of paramount importance. In regard to leakage, we must consider all the usually minor genetic events, e.g. low rates of premeiotic or meiotic crossing over in males that are normally achiasmate (such as the male stable fly), survival of deficiency duplication types under the favourable conditions of laboratory rearing, inadvertent inclusion of other resistance or tolerance mechanisms, etc. Perhaps the best way to handle leakage lies in trying to anticipate problems before they occur, so that once a problem arises it can be identified quickly and a strategy formulated to overcome the fault. Minor problems with stability can become magnified during mass rearing because millions of insects will be reared under ideal conditions. The amount of basic information available on a species will dictate the course of action to some extent.

Recent advances in molecular techniques open new avenues for the synthesis of genetic sexing strains, and indeed for the development of genetic control systems that will be quite different from SIT, but the full utilization of recombinant DNA methods for genetic engineering of operational control methods is still futuristic. At least for the near future, conventional use of SIT will be the basis for most genetic control efforts. Until new systems based on molecular manipulations come on line it is possible to use a combination of the old and the new, i.e. use germline transformation to insert a conditional mechanism and use classical techniques to establish pseudolinkage and to control recombination. In that context, natural or induced paracentric inversions will be valuable for maintaining specific gene arrangements.

REFERENCES

[1] LOFGREN, C.S., et al., Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador — III. Field methods and population control, Am. J. Trop. Med. Hyg. **23** (1974) 288.

[2] PATTERSON, R.S., LABRECQUE, G.C., WILLIAMS, D.F., WEIDHAAS, D.E., Control of the stable fly, *Stomoxys calcitrans*, on St. Croix, US Virgin Islands, using

integrated pest management measures — III. Field techniques and population control, J. Med. Entomol. **18** (1981) 203.

[3] KAISER, P.E., SEAWRIGHT, J.A., DAME, D.A., JOSLYN, D.J., Development of a genetic sexing system for *Anopheles albimanus*, J. Econ. Entomol. **71** (1978) 766.

[4] MITCHELL, S.E., SEAWRIGHT, J.A., Chromosome-linkage group correlation in *Anopheles quadrimaculatus* (Say), J. Hered. **75** (1984) 341.

[5] WILLIS, N.L., HILBURN, L.R., SEAWRIGHT, J.A., Black pupa, a recessive mutant on chromosome 3 of the stable fly, *Stomoxys calcitrans*, J. Hered. **74** (1983) 114.

[6] WALDER, J.M.M., SEAWRIGHT, J.A., Genetic method for the separation of males and females of the house fly, *Musca domestica* (Diptera:Muscidae), J. Econ. Entomol. **78** (1985) 1030.

[7] BAILEY, D.L., LOWE, R.E., DAME, D.A., SEAWRIGHT, J.A., Mass rearing the genetically altered Macho strain of *Anopheles albimanus* Wiedemann, Am. J. Trop. Med. Hyg. **29** (1980) 141.

[8] SEAWRIGHT, J.A., KAISER, P.E., SUGUNA, S.G., FOCKS, D.A., Genetic sexing strains of *Anopheles albimanus*, Mosq. News **41** (1981) 107.

[9] KIM, S.S., SEAWRIGHT, J.A., KAISER, P.E., A genetic sexing strain of *Anopheles quadrimaculatus*, species A, J. Am. Mosq. Cont. Assoc. **3** (1987) 50.

[10] WILLIS, N.L., SMITTLE, B.J., SEAWRIGHT, J.A., A genetic sexing strain for *Stomoxys calcitrans* (L.), Proc. Fla. Anti-Mosq. Assoc. **51** (1980) 52.

[11] SMITTLE, B.J., SEAWRIGHT, J.A., WILLIS, N.L., Mating competitiveness of irradiated stable flies, *Stomoxys calcitrans* (Diptera:Muscidae), from a genetic sexing strain, J. Med. Entomol. **21** (1984) 179.

[12] SEAWRIGHT, J.A., BIRKY, B.K., SMITTLE, B.J., Use of a genetic technique for separating the sexes of the stable fly (Diptera:Muscidae), J. Econ. Entomol. **79** (1986) 1413.

[13] SMITTLE, B.J., SEAWRIGHT, J.A., BIRKY, B.K., Use of a commercial color sorter to separate sexes of stable fly (Diptera:Muscidae) pupae from a genetic sexing strain, J. Econ. Entomol. **79** (1986) 877.

Part III

RECOMMENDATIONS, ANNEX and LIST OF PARTICIPANTS

RECOMMENDATIONS

In 1980 a Consultants Meeting was organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture to examine the practicability of assembling genetic sexing strains for use in sterile insect technique (SIT) programmes for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). The assessment of the potential benefits and suitability of *C. capitata* as a target for genetic manipulation led to positive recommendations for encouraging an expanded research effort. As a result the Joint Division established a research programme aimed at providing support on ongoing research efforts and stimulating work in new and promising areas. The Joint Division also started its own research programme at the IAEA Laboratories, Seibersdorf.

During the last eight years the research programme has been successful from several standpoints. The number of scientists working actively on the genetics of *C. capitata* has grown and a considerable effort is now being made by international groups. The major accomplishments include:

- An extensive genetic linkage map of mutants and biochemical markers;
- Standards for cytogenetic studies that include maps and polytene chromosomes from salivary glands and trichogen cells and descriptions of meiotic and mitotic chromosomes;
- Critical information on sex determination and sex distortion phenomena;
- Increased understanding of the origin and expansion of *C. capitata* through population genetic analysis;
- Genetic sexing strains based on pupal colour mutants, null alleles of alcohol dehydrogenase, and null mutants of xanthine dehydrogenase;
- Laboratory and field evaluations of several candidate pupal colour strains, including mass rearing; and
- An accumulation of basic information on recombinant DNA techniques that will be valuable in future work on genetic sexing through the manipulation of genes.

Although genetic sexing strains have been assembled, they are of limited utility, either because they are not efficient biologically or because sex separation is carried out too late in development to avoid the cost of rearing females. Therefore, in the near future, an intensive effort will be continued by the scientists involved in this critical research programme. The consultants made the following recommendations:

(1) The Joint FAO/IAEA Division should continue to support and co-ordinate the research to develop genetic sexing systems for the Mediterranean fruit fly, *Ceratitis capitata*.

(2) Because of the need to quantify the efficiency of releasing males only, high priority should be given to the field evaluation of existing pupal colour sexing strains. The quality and stability of these strains under mass rearing should be

measured prior to the execution of pilot release studies. Appropriate protocols designed to track critical biological parameters should be included.

(3) High priority must be given to the isolation of new *Adh* null mutants, using the existing translocation strain. This will only be successful in the short term if sufficient technical help is available. The available genetic sexing line should be transferred to Vienna to assess its behaviour under semi-mass rearing conditions. The presence of *2-Adh* genes is a complication and it is essential that the biochemical, genetic and cytological characterization of the strain be assessed.

(4) Development of genetic sexing strains by using molecular techniques should be encouraged. Good progress has been made with the manipulation of genes during the past two years, and the future prospects for this approach are good. Emphasis in the near future should be on genetic transformation.

(5) Techniques now available for the preparation of polytene chromosomes from the salivary glands and trichogen cells should be used to map in situ clones of genes from *C. capitata*. The salivary map should be adopted as the standard, and the trichogen cell chromosomes should be numbered according to homology. In addition to the genetic maps constructed by in situ hybridization methods, genetic maps of visible mutants and electrophoretic loci are highly recommended. Continuation of the research on formal genetics is essential since the availability of numerous biochemical markers will greatly facilitate evaluation of the quality of released flies. Included in the formal genetic support technology is the provision for genetic stock centres which will permit the maintenance of both biochemical and mutant stocks.

(6) The research at Seibersdorf on EMS induced *tsl* mutants should be continued for at least one more year. If 'balancer' inversions become available, these valuable tools should be included in the experimental protocols. If success in the form of stable mutants is not achieved, the resources in the programme could be redirected to address other genetic studies of a basic nature.

(7) The continuation of classical genetic studies on mutants, with emphasis on sex-determining genes and sex distortion by meiotic drive, should be encouraged. The establishment of a solid base of information on the inheritance of mutants involved with sex will be invaluable for research of a molecular nature.

(8) It is highly desirable to have a functioning laboratory in East Africa to provide medflies from genetically heterogeneous populations to other laboratories. Therefore, the work in Kenya (T. Mukiama, Nairobi), should be encouraged and supported financially. Access to populations with a high degree of natural variability is essential to geneticists.

(9) The Agency should continue to issue and distribute the Medfly Genetics Information Circular. This document has been well received and is useful to researchers everywhere.

Annex

THIRD MEDFLY GENETICS INFORMATION CIRCULAR*

INTRODUCTION

This third edition of the Medfly Genetics Information Circular (MGIC) supersedes the previous circulars of 1987 and 1985. Since the appearance of the second circular all chromosomes have been marked with visible mutants (Table 1). As a complete correlation has now been established between chromosomes (No. 1–6) and linkage groups (A–E), it is suggested that chromosome numbers in future be used instead of linkage groups.

Table 1 has been edited to include all assigned mutations and biochemical loci irrespective of their publication in refereed journals.

Four new mutations have been found but have not yet been assigned. These are: stout bristles, bulgy eyes, depressed thorax (Lab. 08) and dark body (Lab. 05).

Two additional genetic sexing strains have been constructed, putting the total number of chromosomal aberrations to 17 available lines (Table 2).

Two more laboratories (15 and 16) have been added to the list of medfly genetic laboratories.

A complete polytene chromosome map of *C. capitata* has been compiled by Dr. A. Zacharopoulou (Lab. 13) using salivary glands, and has been correlated to the mitotic chromosomes as follows:

Mitotic chromosomes	Polytene	(Linkage group)
1		X
2	1– 20 (2)	D
3	21– 40 (3)	C
4	41– 60 (4)	A
5	61– 80 (5)	B
6	81–100 (6)	E (yet to be confirmed)

This information was presented at the RCM in Colymbari, 1988, and will be included, together with the other presentations, in a publication by the IAEA. The vitollogenin genes of the medfly have been mapped on the 5th chromosome (Section 72 A of the salivary gland chromosomes) using in situ hybridization (Report by Dr. Zacharopoulou in collaboration with Dr. Rina, IMBB, University of Crete).

---

* Issued by the Insect and Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, in December 1988.

The conclusions of the RCM have been inserted at the end of the circular.

We have done our utmost to provide a complete list of published post-1986 literature on medfly genetics. However, in order to facilitate a complete updating of the MGIC at regular intervals, we kindly request you to send us a reprint of relevant future publications. These should be mailed to Dr. D.A. Lindquist, Head, Insect & Pest Control Section, Joint FAO/IAEA Division, International Atomic Energy Agency, Wagramerstrasse 5, P.O. Box 200, A-1400 Vienna, Austria, as soon as they appear in print. It is our hope that this will enable us to provide an even better service to you. Any comments concerning contents, omissions or layout of the MGIC would also be welcome.

In response to our latest request for information several of you suggested that we include a complete list of *all* literature on medfly genetics. We have considered this suggestion very carefully and shall attempt to produce such a list for inclusion in the next (fourth) edition of the MGIC. In order that this list really does become complete, we kindly request you to supply a reprint of any publication on medfly genetics, which was not included in the first three editions of the MGIC, no matter how old or obscure it may be.

We wish you all very much success in your future involvement with medfly genetics and look forward to hearing of the progress of each and all of you.

Table 1

LIST OF ASSIGNED MUTATIONS IN *C. Capitata*

Symbol	Description	Chromosome	Lab.	Ref.
r	red eye	1	9	1
Acon-1	Aconitase 1	2	3	
Adh-1	Alcohol dehydrogenase 1	2	3	2
Adh-2	Alcohol dehydrogenase 2	2	3	
Aox	Aldehyde oxidase	2	3	
Est-6	Esterase 6 (adult zone III)	2	3	
LspI	Larval serum protein I	2	3	
Mdh-1	Malate dehydrogenase 1	2	3	
Mdh-2	Malate dehydrogenase 2	2	3	
Me	Malic enzyme	2	3	
Mpi	Mannose phos. isomerase	2	3	
ry	rosy eye, purine susceptible	2	9	3
Xdh	Xanthine dehydrogenase	2	3	
dp	dark pupa	3	8	4
ew	eroded wings, missing distal part	3	8	
Got-2	Glutamate oxalacetate transaminase 2	3	3	
ap	apricot eye	4	8	4
B	Bar eye	4	8	5
dc	double chaetae	4	8	4
Est-1	Esterase 1 (adult zone I)	4	3	6
Est-2	Esterase 2 (adult zone II)	4	3	6
ñ-Est	ñ-esterase	4	3	
Hk-2	Hexokinase 2	4	3	6
l	lethal	4	3	7
lt	light eye	4	9	8
Pgi	Phosphoglucoisomerase	4	3	6
Sd-1	Sex-distorter 1	4	3	6
sk	sparkling eye	4	9	9
Sp	Spotty abdomen	4	8	
Sr	Sergeant abdomen	4	8	
Cy	Curly wings	5	8	5
Fh	Fumerate hydratase	5	3	6
h	harpoon chaetae	5	8	
Had	Hydroxiacid dehydrogenase	5	3	6
Hk-1	Hexokinase 1	5	3	
LspIII	Larval serum protein III	5	3	
or	orange-red eye	5	8	10
Pgd	Phosphogluconate hydrogenase	5	3	6
Pr	Purple eye	5	9	8
rb	ruby eye	5	8	

Table 1. (cont.)

Symbol	Description	Chromosome	Lab.	Ref.
ro	rough eye	5	8	
wp	white pupa	5	8	11
Zw	Glucose-6-phosphate-dehydrogenase (Zwischenferment)	5	3	6
bo	brown-orange eye	(6)*	8	5
Got-1	Glutamate oxalacetate transaminase 1	(6)*	3	
Gox	Glucose oxidase	(6)*	3	
gr	garnet eye	(6)*	8	5
Idh	Isocitrate dehydrogenase	(6)*	3	
LspII	Larval serum protein II	(6)*	3	
Pgm	Phosphoglucomutase	(6)*	3	
Sd-2	Sex-distorter 2	(6)*	3	

* These loci show free assortment with a number of loci located on chromosomes 1–5; the implications are thus that they are located on chromosome 6; however, direct evidence has yet to be produced.

Table 2

CHROMOSOME ABERRATIONS IN THE MEDFLY

Aberration	Lab.	Publ.	Ref.
T:(4,5)19	5	1987	12
T:(2,4)30B	5	1987	12
T:(2,4)Y(5)30C	5	1987	12
T:(4,6)109	5	1987	12
T:(4,6)147	5	1987	12
T:(2,4)30/55	5	1987	12
T:(4,6)55/109	5	1987	12
T:(Y,5)101	7/5	1984	13
T:(Y,2,4,5)?	7/5		
T:(Y,5)30C	5	1988	14
T:(Y,3)69	8	1979	15
T:(Y,3)127	8	1979	15
T:(Y,3)69ap	8	1980	16
T:(Y,4)4	8	1985	17
T:(Y,5)122	14		
T:(Y,3,5)11	14		
T:(Y,2,4)128	12/3		

References for Tables 1 and 2

[1] SAUL, S.H., Diagnosing the heterogamic sex in the Mediterranean fruit fly, (Diptera: Tephritidae): The first sex linked gene, Ann. Entomol. Soc. Am. **78** (1985) 198–200.

[2] ANONYMOUS, Report on Consultants Meeting on a Genetic Sexing Mechanism for the Mediterranean fruit fly, *Ceratitis capitata*, FAO/IAEA, Vienna, Austria (1980) 1–160.

[3] SAUL, S.H., Rosy-like mutant of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), and its potential for use in a genetic sexing programme, Ann. Entomol. Soc. Am. **75** (1982) 480–483.

[4] ROESSLER, Y., KOLTIN, Y., The genetics of the Mediterranean fruit fly, *Ceratitis capitata*: Three morphological mutations, Ann. Entomol. Soc. Am. **69** (1976) 604–608.

[5] ROESSLER, Y., ROSENTHAL, H., The genetics of the Mediterranean fruit fly: Eye-color, eye-shape and wing mutation, Ann. Entomol. Soc. Am. **81** (1988) 350–355.

[6] MALACRIDA, A.R., GASPERI, G., BARUFFI, L., BISCALDI, G.F., MILANI, R., "Updating of the genetics of *Ceratitis capitata* Wied.", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Int. Symp. Vienna, 1987), IAEA, Vienna, Austria (1988) 221–227.

[7] GASPERI, G., MALACRIDA, A.R., TOSETTI, M., MILANI, R., "Enzyme variability: A tool for investigating the genome organization and the population structure of *Ceratitis capitata*", Fruit Flies of Economic Importance 84 (Proc. CEC/IOBC Int. Symp. Hamburg, F.R.G., 1984), (CAVALLORO, R., Ed.), A.A. Balkema, Rotterdam (1986) 153–161.

[8] SAUL, S.H., Two new eye color mutants in the Mediterranean fruit fly, *Ceratitis capitata*, Proc. Hawaii Entomol. Soc. **25** (1985) 125–130.

[9] SAUL, S.H., ROESSLER, Y., Sparkling eye trait and the dc locus in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Ann. Entomol. Soc. Am. **77** (1984) 561–563.

[10] ROESSLER, Y., Recombination in males and females of the Mediterranean fruit fly (Diptera: Tephritidae) with and without chromosomal aberrations, Ann. Entomol. Soc. Am. **75** (1982) 619–622.

[11] ROESSLER, Y., The genetics of the Mediterranean fruit fly: A "White pupae" mutant, Ann. Entomol. Soc. Am. **72** (1979) 583–585.

[12] BUSCH-PETERSEN, E., SOUTHERN, D.I., Induced suppression of genetic recombination in females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), by translocation heterozygosity, Genetica **72** (1987) 161–169.

[13] ROBINSON, A.S., Unexpected segregation ratios from male-linked translocations in the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), Genetica **62** (1984) 209–213.

[14] BUSCH-PETERSEN, E., RIPFEL, J., PYREK, A., KAFU, A., "Isolation and mass-rearing of a pupal genetic sexing strain of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Int. Symp. Vienna, 1987), IAEA, Vienna, Austria (1988) 211–219.

[15] ROESSLER, Y., Automated sexing of the Mediterranean fruit fly: The development of strains with inherited, sex-limited pupal color dimorphism, Entomophaga **24** (1979) 411–414.

[16] ROESSLER, Y., "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** (1980) 275–281.

[17] ROESSLER, Y., 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, Ann. Entomol. Soc. Am. **78** (1985) 265–270.

List of Medfly Genetics Laboratories

1. Dr. J.P. Carante, Institut Univ. de Technologie, Dept. Biologie Appliquée, 43, blvd du 11 Novembre 1918, 69922 Villeurbanne, Cédex, France.
2. Dr. S. Cavicchi, Istituto di Genetica dell'Università, Bologna, Italy.
3. Dr. G. Gasperi, Dr. A. Malacrida & Prof. R. Milani, Univ. di Pavia, Dept. Biologia Animale, Piazza Botta 9-10. 27100 Pavia, Italy.
4. Prof. E. Lifschitz & J.L. Cladera, Dept. de Genética, Instituto Nacional de Tecnología Agraria, C.C. 25 (1712) Castelar, Republica Argentina.
5. Dr. E. Busch-Petersen, Insect & Pest Control Section, International Atomic Energy Agency, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria.
6. Dr. D.O. McInnis, Tropical Fruit & Vegetable Research Lab., USDA-ARS, P.O. Box 2280, Honolulu, Hawaii, USA.
7. Dr. A.S. Robinson, Dept. of Biology, University of Crete, 71409 Iraklion, Crete, Greece.
8. Dr. Y. Rössler, The "Israel Cohen" Institute for Biological Control, Citrus Agrotechnical Services, 27 K.K.L. Str., Rehovot 76345, Israel.
9. Dr. S.H. Saul, Dept. of Entomology, Univ. of Hawaii at Manoa, 3050 Maile Way, Room 310, Honolulu, Hawaii 96833, U.S.A.
10. Dr. R.J. Wood & Dr. D.I. Southern, Dept. of Environmental Biology, School of Biological Sciences, Williamson Building, Univ. of Manchester, Manchester M13 9PL, U.K.
11. Dr. D. Bedo, CSIRO, Black Mountain, Canberra City, A.C.T. 2600, Australia.
12. Dr. M.E. Riva, Instituto Nacional de Investigaciones Agrarias, Dept. Protección Vegetal, Crrtra. de la Coruna km 7, Apdo. 8111, 28040 Madrid, Spain.
13. Dr. A. Zacharopoulou, Dept. of Biology, Div. of Genetics, Cell and Developmental Biology, University of Patras, Patras, Greece.
14. Dr. M. Zapater, Catedra de Genética, Facultad de Agronomía, Universidad de Agronomía, Av. San Martin 4453, 1417 Buenos Aires, Argentina.
15. Dr. M. Loukas, Dept. of Genetics, Agricultural College of Athens, Iera Odos 75, Votanikos, Athens 11855, Greece.
16. Dr. J.M.M. Walder, Head, Entomology Section, Centro de Energia Nuclear na Agricultura, Universidade de Sao Paulo, Caixa Postal 96, 13.400 Piracicaba, Sao Paulo, Brazil.
17. Dr. D.S. Haymer, Dept. of Genetics, John A. Burns School of Medicine, University of Hawaii at Monao, 1960 East-West Road, Honolulu, Hawaii 96822, U.S.A.

References Published Subsequent to the Second Medfly Genetics Information Circular 1987

[1] BEDO, D.G., Polytene chromosome mapping in *Ceratitis capitata* (Diptera: Tephritidae), Genome **29** (1987) 598–611.

[2] BEDO, D.G., ZACHAROPOULOU, A., Inter-tissue variability of polytene chromosome banding patterns, Trends in Genetics **4** (1988) 90–991.

[3] BUSCH-PETERSEN, E., "Development of induced sex-separation mechanisms in *Ceratitis capitata* (Wied.): EMS tolerance and suppression of female recombination", Fruit Flies (Proc. Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier Science Publ., Amsterdam (1987) 209–216.

[4] BUSCH-PETERSEN, E., SOUTHERN, D.I., Induced suppression of genetic recombination in females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), by translocation heterozygosity, Genetica **72** (1987) 161–169.

[5] BUSCH-PETERSEN, E., RIPFEL, J., PYREK, A., KAFU, A., "Isolation and mass-rearing of a pupal genetic strain of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)", Modern Insect Control: Nuclear Techniques and Biotechnology (Proc. Int. Symp. Vienna, 1987), IAEA, Vienna, Austria (1988) 211–219.

[6] ECONOMOPOULOS, A.P. (Ed.), Fruit Flies, Proceedings of the Second International Symposium, 16–21 Sept. 1986, Colymbari, Crete, Greece, Elsevier Science Publ., Amsterdam (1987) 590 p.

[7] GASPERI, G., MALACRIDA, A.R., MILANI, R., "Protein variability and population genetics of *Ceratitis capitata*", Fruit Flies (Proc. Int. Symp. Colymbari, Crete, 1986) (ECONOMOPOULOS, A.P., Ed.), Elsevier Science Publ., Amsterdam (1987) 149–157.

[8] HAKIM, A.M., AWAD, A.I., "Studies on the Mediterranean fruit fly dark pupae, inheritance, biology and ecological factors", Fruit Flies (Proc. Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOUS, A.P., Ed.), Elsevier Science Publ., Amsterdam (1987) 125–133.

[9] HARRIS, D.J., WOOD, R.J., BAILEY, S.E.R., Two-way selection for mating activity in the Mediterranean fruit fly, *Ceratitis capitata*, Entomol. exp. appl. **47** (1988) 239–248.

[10] HOOPER, G.H.S., ROBINSON, A.S., MARCHAND, R.P., "Behaviour of a genetic sexing strain of Mediterranean fruit fly, *Ceratitis capitata*, during large-scale rearing", Fruit Flies (Proc. Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier Science Publ., Amsterdam (1987) 349–362.

[11] KOURTI, A., LOUKAS, M., "Population genetics of medfly: Enzyme polymorphism and routes of dispersion of the fly", Fruit Flies (Proc. Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier Science Publ., Amsterdam (1987) 167 (abstract only).

[12] KOURTI, A., LOUKAS, M., MICHELAKIS, S., "Genetic variation in medfly populations: is there any oviposition preference pattern associated with the taxonomic status of the fruit or fruit-size of the host plant?", Fruit Flies (Proc. Int. Symp. Colymbari, Crete, 1986), (ECONOMOPOULOS, A.P., Ed.), Elsevier Science Publ., Amsterdam. 1987) 175 (abstract only).

LIST OF PARTICIPANTS

Economopoulos, A.P.	FAO/IAEA Entomology Unit, IAEA Seibersdorf Laboratory, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
Gasperi, G.	Dipartimento di Biologia Animale, Università di Pavia, Piazza Botta 9, I-27100 Pavia, Italy
Lindquist, D.A. *(Scientific Secretary)*	Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria
Loukas, M.	Department of Genetics, Agricultural University of Athens, Iera Odos 75, Votanicos, Athens, Greece
Malacrida, A.R.	Dipartimento di Biologia Animale, Università di Pavia, Piazza Botta 9, I-27100 Pavia, Italy
McInnis, D.O.	Tropical Fruit and Vegetable Research Laboratory, Agricultural Research Service, United States Department of Agriculture, P.O. Box 2280, Honolulu, HI 96804, United States of America
Mukiama, T.K.	Department of Botany, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

Riva Francos, M.E.
Departamento Fisiología y Aplicaciones
de la Energía Nuclear,
Instituto Nacional de Investigaciones Agrarias (INIA),
Carretera de la Coruña, km 7,
Apartado 8111,
E-28080 Madrid, Spain

Robinson, A.S.
Research Institute ITAL,
P.O. Box 48,
NL-6700AA Wageningen, Netherlands

Present address:
Research Centre of Crete, IMBB,
P.O. Box 1527,
Heraklion, Crete, Greece

Rössler, Y.
"Israel Cohen" Institute for Biological Control,
27, K.K.L. St.,
Rehovot 76345, Israel

Seawright, J.A.
Insects Affecting Man and Animals Laboratory,
Agricultural Research Service,
United States Department of Agriculture,
P.O. Box 14565,
Gainesville, FL 32604, United States of America

Walder, J.M.M.
Centro de Energia Nuclear na Agricultura,
Universidade de São Paulo,
C.P. 96,
13400 Piracicaba, São Paulo, Brazil

Wood, R.J.
Department of Environmental Biology,
University of Manchester,
Manchester M13 9PL, United Kingdom

Zacharopoulou, A.
Division of Genetics, Cell and Development Biology,
Department of Biology,
University of Patras,
Patras, Greece

Zapater, M.
Facultad de Agronomía,
Universidad de Buenos Aires,
Av. San Martin 4453,
1417 Buenos Aires, Argentina

HOW TO ORDER IAEA PUBLICATIONS

An exclusive sales agent for IAEA publications, to whom all orders and inquiries should be addressed, has been appointed in the following country:

UNITED STATES OF AMERICA — UNIPUB, 4611-F Assembly Drive, Lanham, MD 20706-4391

In the following countries IAEA publications may be purchased from the sales agents or booksellers listed or through major local booksellers. Payment can be made in local currency or with UNESCO coupons.

ARGENTINA	Comisión Nacional de Energía Atómica, Avenida del Libertador 8250, RA-1429 Buenos Aires
AUSTRALIA	Hunter Publications, 58 A Gipps Street, Collingwood, Victoria 3066
BELGIUM	Service Courrier UNESCO, 202, Avenue du Roi, B-1060 Brussels
CHILE	Comisión Chilena de Energía Nuclear, Venta de Publicaciones, Amunategui 95, Casilla 188-D, Santiago
CHINA	IAEA Publications in Chinese: China Nuclear Energy Industry Corporation, Translation Section, P.O. Box 2103, Beijing IAEA Publications other than in Chinese: China National Publications Import & Export Corporation, Deutsche Abteilung, P.O. Box 88, Beijing
CZECHOSLOVAKIA	S.N.T.L., Mikulandska 4, CS-116 86 Prague 1 Alfa, Publishers, Hurbanovo námestie 3, CS-815 89 Bratislava
FRANCE	Office International de Documentation et Librairie, 48, rue Gay-Lussac, F-75240 Paris Cedex 05
HUNGARY	Kultura, Hungarian Foreign Trading Company, P.O. Box 149, H-1389 Budapest 62
INDIA	Oxford Book and Stationery Co., 17, Park Street, Calcutta-700 016 Oxford Book and Stationery Co., Scindia House, New Delhi-110 001
ISRAEL	Heiliger & Co. Ltd. 23 Keren Hayesod Street, Jerusalem 94188
ITALY	Libreria Scientifica, Dott. Lucio de Biasio "aeiou", Via Meravigli 16, I-20123 Milan
JAPAN	Maruzen Company, Ltd, P.O. Box 5050, 100-31 Tokyo International
PAKISTAN	Mirza Book Agency, 65, Shahrah Quaid-e-Azam, P.O. Box 729, Lahore 3
POLAND	Ars Polona-Ruch, Centrala Handlu Zagranicznego, Krakowskie Przedmiescie 7, PL-00-068 Warsaw
ROMANIA	Ilexim, P.O. Box 136-137, Bucharest
SOUTH AFRICA	Van Schaik Bookstore (Pty) Ltd, P.O. Box 724, Pretoria 0001
SPAIN	Díaz de Santos, Lagasca 95, E-28006 Madrid Díaz de Santos, Balmes 417, E-08022 Barcelona
SWEDEN	AB Fritzes Kungl. Hovbokhandel, Fredsgatan 2, P.O. Box 16356, S-103 27 Stockholm
UNITED KINGDOM	Her Majesty's Stationery Office, Publications Centre, Agency Section, 51 Nine Elms Lane, London SW8 5DR
USSR	Mezhdunarodnaya Kniga, Smolenskaya-Sennaya 32-34, Moscow G-200
YUGOSLAVIA	Jugoslovenska Knjiga, Terazije 27, P.O. Box 36, YU-11001 Belgrade

---

Orders from countries where sales agents have not yet been appointed and requests for information should be addressed directly to:

Division of Publications
International Atomic Energy Agency
Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria

INTERNATIONAL
ATOMIC ENERGY AGENCY
VIENNA, 1990