ANNUAL REPORT 1996

ENTOMOLOGY UNIT

FAO/IAEA
AGRICULTURE AND
BIOTECHNOLOGY
LABORATORIES,
SEIBERSDORF

CONTENTS

1. INTRODUCTION

This is the second annual report of the Entomology Unit collated under the present format and it will be the last. The annual report 1997 will be written to reflect the projects, tasks and milestones as worked out in great detail during Programme of Work and Budget 1997-1998 sub-programme discussions. The tasks and milestones will provide a logical framework within which the work can be reported but the nature of the scientific process guarantees that new ideas and developments will also be included. The report focuses on the development and the transfer of technology as described by sub-programme D.4 Insect and Pest Control. Special attention was also given in the reporting period to Systems for Tsetse Automation Rearing and Releases (STARR)

1.1. TECHNOLOGY TRANSFER

The Units activities in 1996 have continued to be dominated by technology transfer for both tsetse and the medfly, *Ceratitis capitata*, supplemented by significant progress in R and D for both species. The successful transfer of medfly genetic sexing strains (GSS) to large operational programmes in Argentina and Guatemala and their increased effectiveness in the suppression of medfly field populations has clearly vindicated the substantial investment made in the development of these strains. Many basic studies on medfly genetics and cytology have been essential in order that these strains could be isolated and studied and this knowledge is now being used to interpret the behaviour of the strains under operational mass-rearing conditions. The development and testing of a "filter system" to manage the behaviour of GSS in mass-rearing facilities has been a major achievement of the Unit. In tsetse, the transfer of improved rearing systems from Seibersdorf to the Tsetse and Trypanosomosis Research Institute (TTRI) at Tanga led to a rapid expansion in the size of the *Glossina austeni* colony. This resulted in an increased production of sterile males for release on Zanzibar and the subsequent drastic decline in the wild *G. austeni* population. From September 1996 no further shipments of flies were sent from Seibersdorf; the cessation of shipments to Tanga was way ahead of schedule. The take-over by the TTRI was also much facilitated by excellent fellowship training received by the staff during their stay in the Unit. The cessation of shipments of flies liberated staff resources in the tsetse group that were partially re-directed into tsetse R and D and to increased support for medfly genetic sexing and mass-rearing. The provision of 16,000 L of fresh frozen irradiated blood to the facility in Tanga has also been successful with the result that several other organisations are now receiving this type of diet from Seibersdorf.

1.2. TSETSE AUTOMATION (STARR)

The work on tsetse automation has to be seen in a wide context as it includes areas in which "automation" per se is of little relevance but where enormous gains can be made in the efficiency of mass-rearing, e.g. by allowing flies to emerge directly into production cages at the right density and sex ratio. The improvements that have already been introduced to the Tanga facility will be inadequate for future large scale

projects on mainland Africa, simply up-scaling what is currently being done, will not be successful. During 1996 a prototype tsetse production unit (TPU) was evaluated with mixed results. The prototype was successful in holding many fly cages, bringing them automatically to a feeding area and collecting pupae centrally. However, the cage design was inappropriate leading to many fly deaths with the result that it has not been possible to maintain a self-sustaining colony on the machine in 1996. Progress in other areas has been substantial and the group is well on the way to developing a system that will allow flies of the right sex and number to emerge directly into production cages. This will remove the final chilling procedure of the current rearing system. The chilling procedure required that every fly in the colony had to chilled, sexed and returned to the cage. Progress has also been made on the evaluation of freeze dried blood as a diet for tsetse. The laboratory was also able to recruit a consultant for one year to work on the automation project.

1.3. RESEARCH AND DEVELOPMENT

Major breakthroughs have been made in the improvement of the stability of genetic sexing strains, one relates to the current strains and has been implemented and the second to the development of future strains. Current sexing strains when mass-reared in operational facilities do show a slow loss of integrity with time which would necessitate regular, if infrequent, strain replacement. A “filter system” has been conceived, tested and transferred which removes the need for strain replacement and which will greatly increase the acceptability of genetic sexing strains. In order to remove the problem of loss of integrity at source, chromosomal inversions have now been isolated in medfly for the first time. These, when introduced the current genetic sexing strains, will lead to much improved stability.

In preparation for the implementation of a Co-ordinated Research Programme (CRP) on tsetse genetics, preliminary research has been carried out on the use of random amplified polymorphic DNA (RAPD’s) and mtDNA for population analysis. This type of analysis will be essential to delineate populations on mainland Africa and to decide on the degree of isolation of target populations. This is a new field with very few researchers confronting a large problem so preliminary work has focused on an analysis of *G. pallidipes* from the Southern rift valley in Ethiopia. This area is under serious consideration for a large scale SIT programme in the near future and the work will be reported in the 1997 report.

1.4. FELLOWSHIP TRAINING

During 1996, 45 man months of training was provided, all on an individual basis. We feel that this is the best way to meet the needs of the fellows with the resources available. The amount of training is less than last year and is a reflection of the successful technology transfer mentioned above which now permits fellows to train on specific aspects of medfly sexing strains in several facilities. The same is true in tsetse where training in tsetse mass-rearing can now be given in Tanga. This trend will continue with only fellows requiring specific training, that cannot be given elsewhere, being trained in Seibersdorf

1.5. INFRASTRUCTURE AND STAFF

The critical shortage of office space as documented in the last report has not changed and is unlikely to do so in the near future. However conditions of work for staff in the rearing areas have been considerably improved. In tsetse a new air filtration system has been installed with immediate positive results in the air quality and in medfly, problems associated with the preparation of larval diet have been somewhat improved although a complete solution will not be available until the move into the new building in the spring 1997. Worker safety under insect mass rearing conditions is an extremely important issue and funds have to be made available to make the necessary, sometimes expensive, changes required. No major pieces of equipment were procured in 1996, however, as planned, the computers of all staff have been upgraded and a colour laser printer purchased which is also available on the network to other users at Seibersdorf.

The 1996 organigram can be seen on page 6. The cessation of the shipments of tsetse flies to Tanga with the consequent downsizing of the *G. austeni* colony in Seibersdorf resulted in the re-assignment of two positions from tsetse, one to medfly rearing and the other to genetic sexing. The two APO's who left the staff in 1996 both went to relatively permanent positions with one going to Guatemala where he will be a key figure in the mass-rearing of the medfly genetic sexing strains.

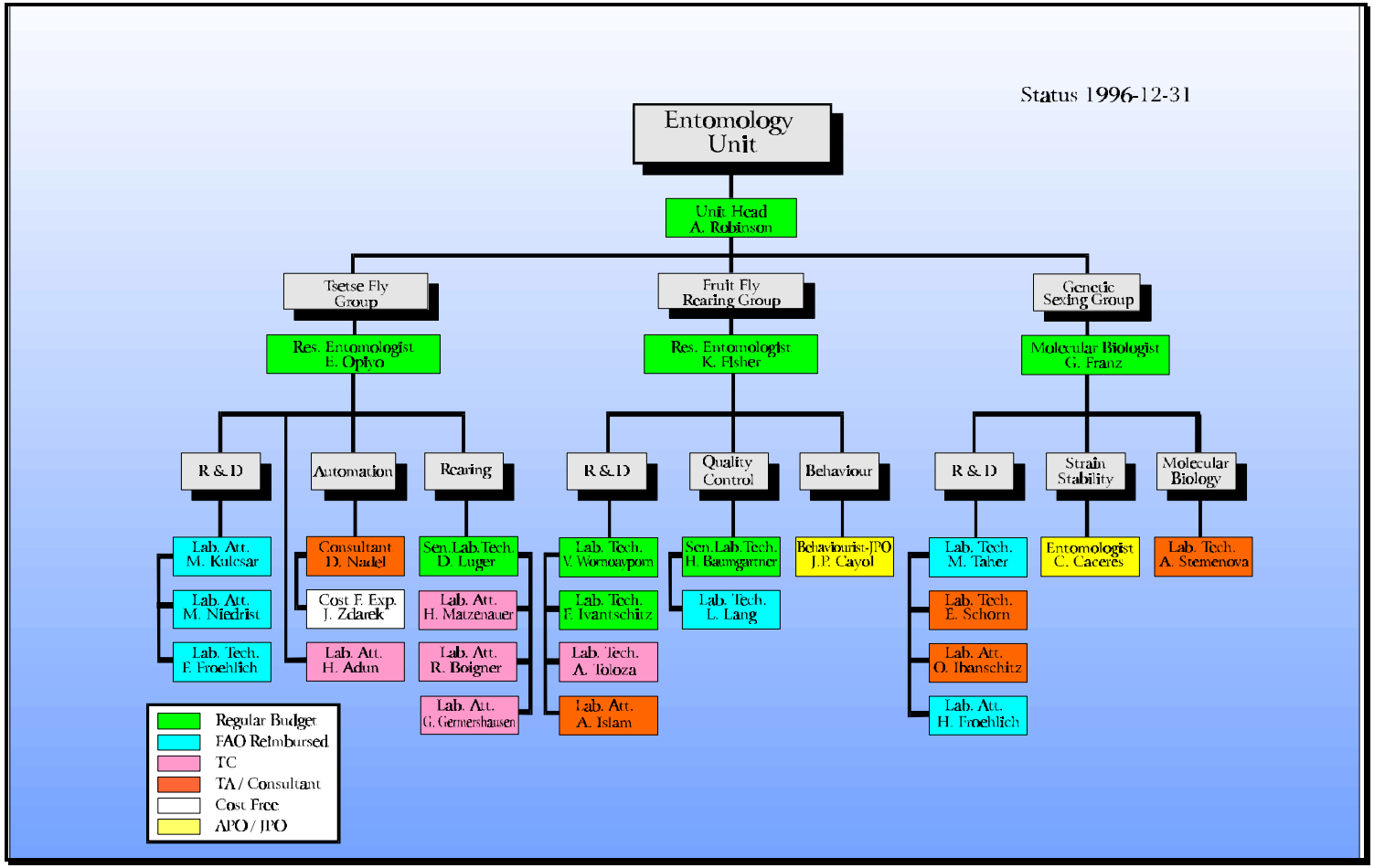
Several staff act as a scientific secretary for ongoing CRP's and are also technical officers for TC projects and there is also an increase in the request for staff to make contributions at International meetings. All these responsibilities, on top of expert missions, make it essential that planning is optimal to ensure that the laboratory tasks and deadlines are not neglected.

1.6. CONSULTANTS

Consultants are a very efficient way to stimulate R and D as they bring fresh ideas and techniques into the laboratory, they are generally recruited to focus on a particular problem. The following short term consultants spent time in the Unit

Atkinson, P.	Australia	(1996-05-28 - 1996-06-05)	medley transformation
O'Brochta, D.	U.S.A.	(1996-05-28 - 1996-06-05)	medfly transformation
Cockburn, A.F.	U.S.A.	(1996-09-30 - 1996-11-29)	tsetse genetics
Cosenzo, E.	Argentina	(1996-10-14 - 1996-10-17)	genetic sexing
Gomez Riera, P.	Argentina	(1996-10-14 - 1996-10-17)	genetic sexing

1.7. ORGANOGRAM



2. MEDFLY GROUP

2.1. PRODUCTION

During 1996, medfly mass-rearing research continued to implement successful transfers of genetic sexing strains (GSS) and associated technology into Argentina, Chile, Greece, Guatemala and Madeira. Results from Argentina and Guatemala demonstrate that the GSS are indeed working efficiently in the field. Seibersdorf has played a vital role in supplying, supporting and improving this technology throughout 1996.

Three GSS formed the basis of the research in Seibersdorf: SEIB 6-96 (a white pupae (*wp*) strain), VIENNA 6-94 and VIENNA 42/TOL 94 (both temperature sensitive lethal (*tsl*) strains). SEIB 6-96 male pupae (brown) must be mechanically separated from female pupae (white). In contrast, *tsl* strains produce males by killing the temperature sensitive females during embryogenesis, by raising the incubation temperature to 34°C. At standard incubation temperatures (23°C) both sexes survive.

Mass rearing research at Seibersdorf has not only facilitated international transfer of these strains, but has also provided a detailed study of their performance under mass rearing conditions. In particular, quantitative characterisation of the quality and production parameters of the strains, further optimisation of high temperature treatments of VIENNA 42/TOL 94 and a biological filter capable of controlling genetic recombination in the strains have been studied.

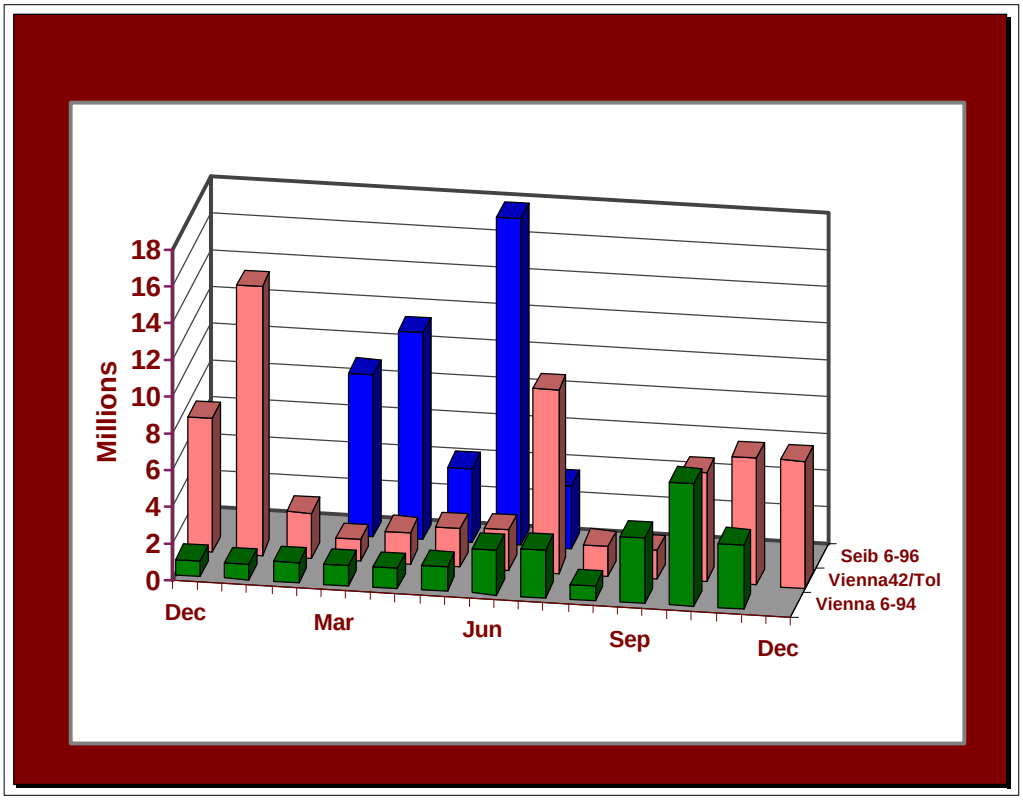
2.1.1. Mass Production of Genetic Sexing Strains.

2.1.1.1. Monthly Production

The simultaneous mass production of three GSS is unique to Seibersdorf, since in most facilities efforts are concentrated on a single strain. The Seibersdorf facility achieved a high level of production of all these GSS, sufficient to recognise and define characteristics of these strains under mass rearing conditions. This is an important aspect of the development of the technology since without such data, operational facilities will not be able to apply the correct quality assurance procedures to their product.

Figure 1 shows that there were months when more than 20 million pupae of GSS were produced. Each strain was maintained without contamination and this was confirmed by a mitochondrial-DNA analysis. This analysis was necessary as it is impossible to differentiate some of the strains purely on their phenotype. Production during August was depressed by a bacterial disease which resulted in changes to diet formulation (discussed below).

Figure 1. GSS Production 1996, Seibersdorf



2.1.1.2. Characteristics of Genetic Sexing Strains.

The characteristics of the 3 GSS are different, although *tsl* strains share some similarities. By far the most exceptional aspect of the rearing common to all GSS is the part played by thermal regulation during rearing. This is particularly important in the production of *tsl*, where temperature sensitive females are affected by the metabolic heat generated during rearing. However, research has shown that for all strains, thermal regulation influences the survival of adjacent-1 individuals. Adjacent-1 individuals carry an unbalanced chromosome set and they normally do not complete their development. However in certain GSS they can survive to a particular stage and they can therefore compromise production efficiency. Adjacent-1 individuals impact upon quality control and it is becoming clear that rearing management strategies must regulate the production of adjacent-1 individuals in a specific manner.

To demonstrate some of the differences in characteristics of the GSS, these elements of quality assurance, used to measure, assess and monitor the development, growth and stability of the insects, are compared below.

- the emergence of flies from pupae
- their subsequent ability to fly
- the efficiency of production at various stages

One of the principal differences between these strains is the pattern of emergence of flies from puparia. SEIB 6-96 develops more quickly than the *tsl* strains and all pupae are collected over a 3 day period, compared with 5 or 7 days for VIENNA 6-94 and VIENNA 42/TOL 94 respectively (Table 1). The emergence of females from white

pupae is similar for each of the strains (there are no adjacent-1 females), but emergence of males from brown pupae is progressively reduced, due to the presence of adjacent-1 individuals which have very poor survival and therefore their emergence is poor (if they emerge at all). Their development as larvae and pupae is also retarded, and they appear in the later pupal collections. Management of thermal regulation during production is becoming a key issue, since it can change the overall quality of successive pupae collections.

Table 1: Female and Male Emergence (%) for Different Larval Collections.

% Emergence						
	SEIB 6-96		**VIENNA 6-94**		**VIENNA 42/TOL**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Day 1	90.5	93.0	80.0	76.2	74.7	74.5
Day 2	72.3	91.1	55.0	72.6	68.1	72.1
Day 3	37.0	86.4	32.1	74.3	70.4	58.9
Day 4	-	-	16.0	69.8	69.5	46.4
Day 5	-	-	6.5	62.8	64.7	12.8
Day 6					64.2	10.1
Day 7					59.2	2.1

Flight ability is a measure of the proportion of emerged flies that are pre-disposed to dispersal, and only takes into account those flies that could fly if they wanted to, an important parameter in releasing sterile flies. The characteristic flight ability for all strains studied to date is that the majority of emerged males of both strains are able to fly away and disperse (Table 2) and that the value for this parameter is common to all strains. This means that when a male emerges, it has an equal chance of flight, regardless of the strain**.**

Table 2: Flight Ability Index for Different Larval Collections.

Flight Ability Index						
	SEIB 6-96		**VIENNA 6-94**		**VIENNA 42/TOL**	
	Males	Females	Males	Females	Males	Females
Day 1	85.7	88.6	86.1	52.0	89.8	73.0
Day 2	83.3	86.1	81.6	57.2	88.9	73.1
Day 3	78.1	84.9	80.6	70.3	91.3	63.9
Day 4	-	-	74.8	69.5	87.9	77.5
Day 5	-	-	60.8	51.3	85.0	89.3
Day 6					86.5	
Day 7					90.2	

The efficiency of mass production is of economic importance as well as qualitative importance. Table 3 illustrates that all these GSS produce a similar number of pupae from a given number of eggs (i.e. they have similar egg-pupae efficiency) and that the total number of flying individuals (males and females) are also similar at the end of

production. Again this represents a good common character that we can apply to the GSS generically and it allows good decision making within and between facilities that are mass rearing GSS. The fact that the white pupae strain produces more females is relatively immaterial as females are only used in mass rearing and not in the field.

Table 3: Relative Production Efficiencies of Three GSS.

	Efficiencies of Production					
	SEIB 6-96		VIENNA 6-94		VIENNA 42/TOL	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Efficiency 1 (% egg to pupae)	43.8		41.6		40.9	
Efficiency 2 (% Pupae to flyers)	70.5	48.8	59.5	41.6	59.9	46.0
Production Efficiency(Total flyers)	34.5	35.0	21.3	36.7	25.0	34.0
Flyers, regardless of gender	69.5		58.0		59.0	

Diet composition is critical to the efficiency of medfly mass production. During 1996, the Seibersdorf facility studied this aspect in relation to disease control on diets. The Seibersdorf diet became infested with a bacteria that caused rapid heat production during the late larval development stage. It is likely that Drosophila introduced the disease. During August the situation became critical, with production efficiency dropping to nearly zero for 2 weeks. Table 4 shows the decline in production efficiency, and clearly shows why monitoring of this trait is worthwhile. In this case efficiency is defined as the proportion eggs that become pupae.

Table 4: Monthly Production Efficiencies.

Monthly Production Efficiencies			
Month	**SEIB 6-96**	**VIENNA 6-94**	**VIENNA 42/TOL**
Dec		26.3%	35.4%
Jan		23.6%	38.3%
Feb		24.8%	39.1%
Mar	37.1%	30.8%	30.7%
Apr	36.8%	25.8%	21.3%
May	38.0%	30.4%	22.2%
Jun	28.0%	32.5%	28.0%
Jul	38.1%	42.1%	33.8%
Aug		18.0%	21.9%
Sep		40.8%	17.8%
Oct		45.2%	31.1%
Nov		40.8%	40.2%
Dec			40.7%

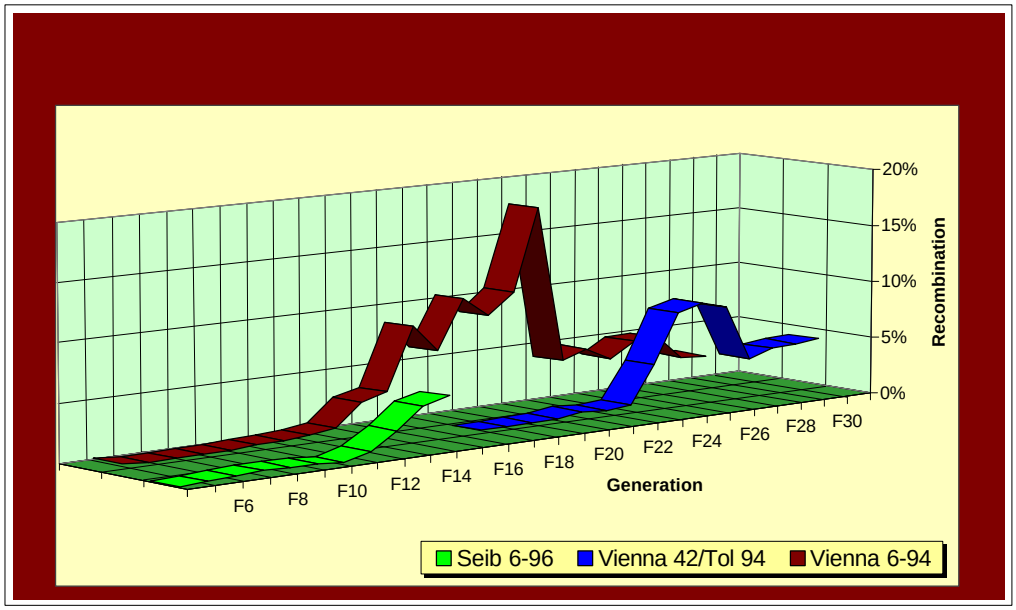
In order to overcome the disease, three modifications to diet were introduced. Firstly, the diet pH was reduced to change the biochemical nature of the diet making it more hostile to the problem bacteria or fungi. Secondly, a new diet based on Nipagin™ was developed, so that it could be rotated with the low pH diet, preventing the disease from adapting to the pH changes. Thirdly, the sodium benzoate concentration at low pH, known to produce a fine balance between disease control and larval lethality, was

also reduced. The results of the changes (Table 4) can easily be seen from September, when the VIENNA 6-94 strain responded immediately to the changes by increased production efficiency. However, it took another 2 months before the VIENNA 42/TOL 94 strain adapted to the new environment, possibly pointing to a difference between strains. It should be noted that VIENNA 42/TOL-94 is a special case in GSS, since it is based upon out-crossed genetic material from Guatemala.

2.1.1.3. Recombination

An important aspect of GSS is genetic recombination, which leads to the build up of females that cannot be separated from the males which are to be released. Recombination in current GSS is very low ordinarily, however under the selection pressures of mass rearing the appearance of recombinants is encouraged. Although there is a tendency for recombinants to build up during mass rearing, it can be seen (Fig. 2) that this process can occur over a long period, making the strains relatively stable. Since it takes about 1 month for each generation to be completed, it takes 18-24 months before high levels of recombinants are found. This is certainly manageable, especially if recombinant filtering technology is employed (see below).

Figure 2. Long Term Recombination in Three GSS



2.1.2. Improvements in Genetic Sexing Strain Technology

2.1.2.1. Recombinant Filter

The existence of recombinants in GSS is the product of genetic recombination, a phenomenon present in almost all living organisms. However, it becomes more obvious when special features such as sex-linked pupal colour or temperature sensitivity are gradually lost. This seems to occur in large scale mass rearing at a faster

rate than in small scale mass rearing and is probably a function of the greater selection pressure present in large scale mass rearing.

In response to this problem, which has occurred in Guatemala and Argentina, a **Recombinant Filter** was conceived and developed in Seibersdorf to control the build up of recombinants. The principle of the recombinant filter is to maintain a small standby line of a GSS, with a very low percentage of recombinants, which can at regular intervals refresh the mainstream of production with new material.

This principal, although developed for genetic sexing strains, is also applicable to bisexual strain production. The filter allows for simple strain replacement, pre-adaptation to required parameters, low stress rearing, control over recombination in mass rearing, the selection of desirable traits, adaptation to new and existing technologies and the addition and testing of new genetic material at any time without effecting production. The recombinant filter in its entirety consists of 6 components (Figure 3):

- genetic stocks
- clean stream
- initiation stream
- injection stream
- colony stream
- release stream

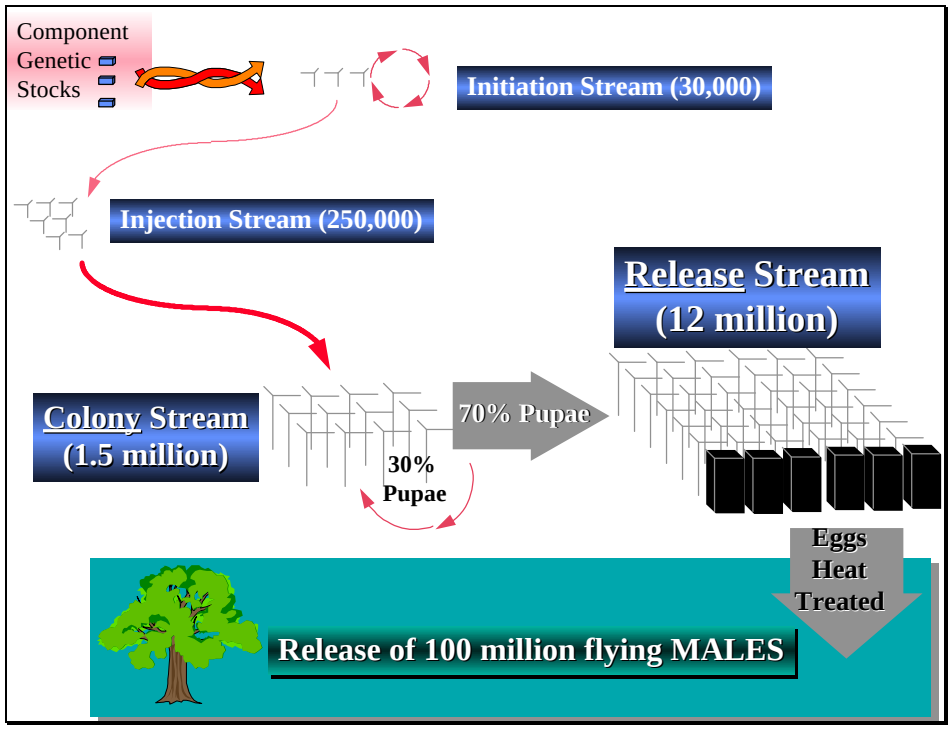
There are however only 3 fundamental parts to the recombinant filter:

> The ***clean stream,*** a small colony of the strain maintained free of recombinant individuals and under minimal stress conditions (diurnal lighting, low densities, positive male competitiveness, optimal temperature and lighting, etc.). It provides 'clean' material for the ***colony stream***, when recombination in the colony stream reaches a threshold value, say 2%.
>
> The ***colony stream*** is really the centre of the filter (Figure 3). It runs collaterally with the release stream and interfaces with it, producing stock for itself and for a much larger colony called the ***release stream***. The colony stream is a middle-sized, low stress colony and is the only place that recombinants can accumulate.
>
> The ***release stream*** produces the large number of eggs required for the sole production of male pupae (Figure 3). As a consequence, all of the release colony production is sterilised and goes to the field. It is not recycled except in exceptional circumstances. The release stream can in fact be placed under stress because there is no chance for the selection of recombinants and there is no chance for the adaptation of flies to high stress.

In a normal mass rearing configuration, two steps are required as an amplification bridge between the clean stream and the colony stream: the ***initiation stream*** could be called the first stage amplification and starts the filtering process. It is not cleaned because of the sheer numbers involved. The second stage amplification is the ***injection stream,*** to provide new replacement material to the colony stream, without interruption to operations.

The implementation of this filter will require a specific strategy in relation to configuring the rearing processes within a facility. However for the most part this will be easier in large facilities than in small facilities. The key component, the colony stream, needs to be maintained separately from the release stream. The fact that the filter has the potential to operate has been partially demonstrated by the way in which Seibersdorf has operated as the initiation and injection components to both Guatemala and to Argentina over the past year.

Figure 3: Diagrammatic Representation of the Recombinant Filter



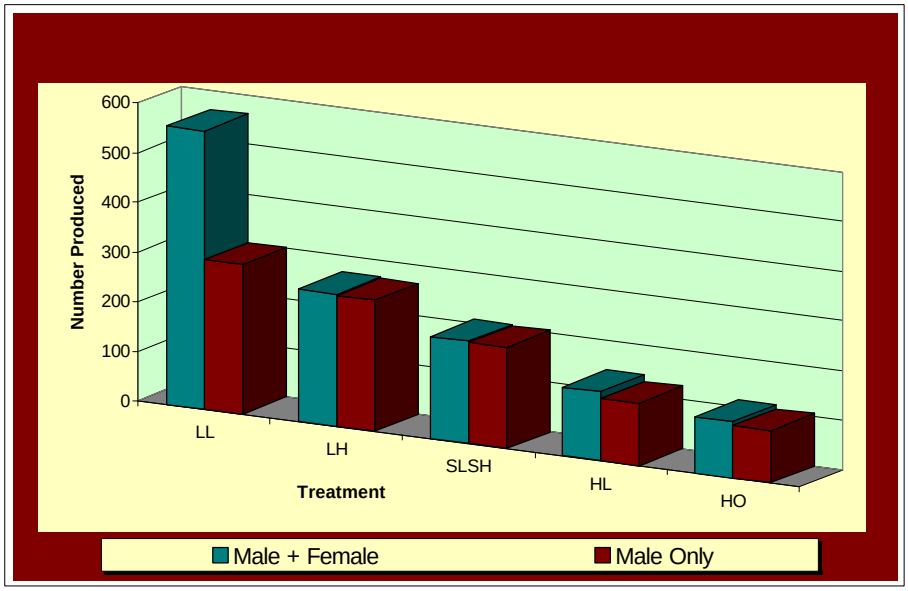
2.1.2.2. New Temperature Data for a Second tsl Strain

In 1995 a temperature treatment was found for VIENNA 6-94 which preserved the production of male individuals, thereby producing the maximum potential number of males. The treatment is known as the "Low-High" treatment, and is designed to kill female embryos after the lethal maternal effect in male embryos has passed.

However, the treatment may not be generic to all *tsl* strains, therefore a second *tsl* strain, the VIENNA 42/TOL 94, was tested in the same way. VIENNA 42/TOL 94 is reputedly more temperature sensitive than VIENNA 6-94, therefore additional treatment scenarios were tested. A series of treatments, including no temperature treatment (LL), 24 h at 23°C:24 h 34°C (LH), 20 h at 23°C:20 h 34°C (SLSH), 24 h 34°C:24 h 23°C (HL) and 24 h at 34°C with no further treatment (HØ), were tested.

The results of this research (Figure 4) showed that the "Low-High" (LH) treatment of eggs can be considered a generic treatment for *tsl***,** yielding nearly the same number of males as the un-treated eggs.

Figure 4. Temperature Treatments for VIENNA42/TOL-94



2.1.3. International Shipments: Strain Transfers

During 1996 the transfer of GSS to five countries represented everything from new strain adoptions and routine strain replacements to emergency colony supplements designed to maintain the production of action programmes. Much was learned about GSS technology transfer during the year, culminating in the realisation of the usefulness of the "recombinant filter".

Table 5 lists the GSS transfers achieved in 1996. Chile represents a country adopting a GSS for the first time. Three strains were transferred to Chile in order to test which is appropriate for both mass rearing in the Arica facility and for their field population. It is planned that Chile will mass rear 60 million pupae per week of one of the strains. Argentina and Greece both required routine replacements of the white pupae strain, SEIB 6-96. Argentina rears 250 million pupae per week of this strain and Greece rears 5 million per week.

During 1996, Guatemala moved its mass production from Petapa to El Pino. The El Pino facility (the largest in the world) is successfully rearing VIENNA 42/TOL 94 to a level of 85 million per week (equivalent to 150 million of a bi-sexual strain) and it is presently the largest mass rearing of a *tsl* GSS in the world. Guatemala represented a case for emergency action during 1996, requiring a special transfer to control recombination in a portion of their primary colony. This transfer, in combination with the adoption of the "recombinant filter" may enable Guatemala to provide consistent male-only shipments to Los Angeles (Ca.) and to Israel in the near future.

Madeira inaugurated its new medfly facility in 1995 and adopted a *tsl* GSS strain (VIENNA 6-96). It is successfully building both expertise and *tsl* GSS production after training assistance from Guatemala and Seibersdorf.

Table 5: Shipments of Eggs and Pupae during 1996.

	Strain	Month	Millions Shipped	*Millions Currently Produced*
Argentina	SEIB 6-96	June	11.3 (40.0†)	250
Chile	3 strains	November	0.2	1
Greece	SEIB 6-96	March	7.0 (8.0†)	5
Guatemala	SEIB 6-96	April	3.4 (4.0†)	
Guatemala	VIENNA 42/TOL 94	July	7.5	85
Madeira	VIENNA 6-96	April	0.2	5

†(potential million sent as eggs).

2.2. MATING BEHAVIOUR

The medfly mating behaviour studies were initiated in 1995 by the Entomology Unit in order to characterise the behavioural pattern of the GSS; to develop a quality control test for medfly mating behaviour; to assess the mating performance and compatibility of the GSS with wild populations and to provide training and expertise to the counterparts using GSS in SIT programmes. Depending on which information is required regarding the mating behaviour, different "tools" (testing procedure, equipment) may be used in the laboratory and/or under field or field-like conditions. The activities presented below review some of the techniques available to analyse the mating behaviour of medfly.

2.2.1. Laboratory Mating Activity of Genetic Sexing Strains

In order to characterise some aspects of the mating behaviour of the GSS mass-reared at the Seibersdorf, three strains (SEIB 6-95; VIENNA 6-94 VIENNA 42/TOL 94) were tested under laboratory conditions following the mating propensity protocol recommended by USDA.

The objective is to assess the mating activity (or mating speed) of mass-reared flies during one hour. In the present case, a combination of non-irradiated virgin males and females of four age groups (3, 5, 7 and 9 day-old, sex ratio 1:1) were tested and the duration of the experiment extended to seven hours (0700AM - 0200PM). Twenty-eight replicates were run for each strain. The time, duration of mating and age composition of pairs were recorded and compared between the strains. Data have been analysed with one-way ANOVA and Tukey's HSD tests.

Table 6: Mating Parameters of Three Genetic Sexing Strains

	VIENNA-42/TOL			*VIENNA 6-94*			*SEIB 6-95*		
Mating Propensity Index	42.84 a	[13.10]	N=28	6.38 c	[4.59]	N=28	19.73 b	[10.05]	N=28
Percentage of Mating after 9 hours of testing	74.11 a	[8.62]	N=28	29.46 c	[10.19]	N=28	44.20 b	[9.76]	N=28
Duration of Mating (min)	186.22 a	[38.35]	N=328	174.34 a	[40.80]	N=129	157.55 b	[43.37]	N=192

For each row, number with the same letter do not differ significantly according to Tukey's HSD test (P<0.01). Standard deviation is given in parentheses.

These data clearly indicate that VIENNA-42/TOL strain performs better than the two other strains (Mating Propensity Index = 42.84), and achieves a higher percentage of mating. The duration of mating is significantly longer for the two *tsl* strains than for the *wp* strain (SEIB 6-95). The analysis of the age composition of pairs indicates, that for the three strains, the females of the 4 age-groups were involved in matings in the same proportion, nevertheless the 3-day old males mated significantly less than the older ones.

The analysis of the hourly mating activity confirmed that VIENNA-42/TOL flies mate significantly faster and more frequently than the flies of the two other strains (Table 7). The mean percentage of possible matings per hour and the polynomial regression curves for the three GSS highlighted differences in the general mating activity pattern.

Table 7: Hourly Mating Activity of Three GSS

	HOURS AFTER FEMALES RELEASE								
	1h	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**F (df)**	**P**
VIENNA 6-94	15.70 a	10.46 a	14.83 a	12.06 a	15.83 a	10.48 a	8.63 a	4.83 (6)	0.00
	[5.81]	[2.44]	[6.32]	[4.50]	[4.40]	[3.56]	[7.30]		
SEIB 6-95	30.77 a	18.29 b	12.06 bc	13.69 bc	9.78 bc	8.34 bc	4.56 c	18.43 (6)	0.00
	[11.35]	[4.89]	[2.32]	[6.70]	[5.74]	[6.56]	[5.10]		
VIENNA-42/TOL	47.96 a	25.55 b	22.94 bc	18.08 bc	15.39 c	2.92 d	0.00 d	129.76 (6)	0.00
	[7.15]	[2.61]	[4.99]	[4.68]	[4.86]	[5.55]	[0.00]		

For each row, number with the same letter do not differ significantly according to Tukey's HSD test (P<0.01). Standard deviation is given in parentheses.

According to international standards used to quantify the mating efficiency of a strain (Mating Propensity Index), the VIENNA-42/TOL was the best strain. However, (a) these results were obtained with males and females of the same strain; and (b) the parameters measured under laboratory conditions must be interpreted with care. The Mating Propensity Index can easily characterise a mass-reared strain in the laboratory but is of little relevance to field performance with wild females. What is the advantage of rapid mating, when wild females penalise impatient males?. In most SIT programmes, bisexual strains (i.e. involving males and females) are released and a high value of the Mating Propensity Index can be a disadvantage with the sterile males mating with the sterile females. A longer mating duration (as was found for VIENNA-42/TOL) could also characterise a "good" strain by guaranteeing effective sperm and accessory gland fluid transfer. However, this characteristic could also become a

disadvantage in the field where less mobile flies may represent an easier prey for predators.

Figure 5. Hourly Mating Activity of the 3 GSS

Figure not available.

One of the major differences, in terms of mass-rearing, between these three strains is the number of generations and the "laboratory" age of the strains from which they were constructed. The VIENNA-42/TOL strain, with the most recently colonised wild background (1991), performed better than the other strain (originating from a wild strain colonised in 1983). It has already been shown that after an increasing number of generation (according to the species and the mass-rearing conditions) the mating competitiveness of the flies decreased significantly. In the present case the difference in performance between VIENNA-42/TOL and the other strains seems to confirm these findings.

However, because of the disadvantages of the test (see above), and as the goal of mass-rearing flies is to produce individuals with good field performance, it is recommended to assess the mating efficiency of mass-reared strains under field-like conditions (see 2.2.3)

2.2.2. Video Analysis of Mating Behaviour

A Co-ordinated Research Programme (CRP) on medfly mating behaviour was initiated in 1994 and is concerned with courtship and mating behaviour of medfly from different geographic areas. Within the framework of this CRP, wild medflies from Madeira Island (Portugal) have been video-recorded in the laboratory, according to the protocol agreed by members of the CRP in Tapachula (Mexico) in February 1996. A total of 39 single pairs (one male and one female) have been video-recorded for 45 minutes (or until mating), representing a total of 31.5 hours. Out of these 39 single pairs, only 6 resulted in successful matings.

In order to describe the courtship of the male, a series of 14 activities has been defined and they are listed in Table 8.

Table 8: Medfly Mating Activities

ACTIVITY	*DESCRIPTION*
Immobile	the male is resting or cleaning
Mobile	the male is moving, walking, flying
Immobile calling	the male is resting, cleaning and calling the female (the anal ampoula is visible)
Mobile calling	the male is moving, walking and calling the female (the anal ampoula is visible)
Vibrating	the male is vibrating the wings
Vibrating, head rocking	the male is vibrating the wings and rocking the head
Fanning	the male is fanning the wings in strong back/forward pulses
Fanning, head rocking	the male is fanning the wings in strong back/forward pulses and rocking the

	head
Jump	the male is jumping onto the female. The jump results in mating
Failed jump	the male jumps onto the female but it does not result in successful mating or the male jumps but misses the female
Quick jump	the male quickly jumps over the female and returns to its original position
Head rocking	the male is rocking the head but without any movement of the wings
Contact	the male and the female are in contact (with legs or head)
Mating	the mating pair is immobile

The time spent in each activity by successful and unsuccessful males is presented in Table 9 (for the successful males, the analysis lasted until the end of the activity preceding the successful jump; for the unsuccessful males, the analysis lasted for 45 min after the female release). The time spent in each activity is highly variable from male to male resulting in high standard deviation values making any interpretation difficult. To compare these data, the time spent in seconds has been transformed in percentage of the total duration of the courtship (total duration of the video-recording for the unsuccessful males). The data are presented in Figure 6 and Figure 7.

Table 9: Time(s) taken by Madeiran Successful and Unsuccessful Males for Each Activity

ACTIVITY	*MEAN DURATION IN SECONDS*	
	SUCCESSFUL MALES	**UNSUCCESSFUL MALES**
Immobile	16.75 [26.01]	392.15 [552.73]
Mobile	10.31 [16.61]	89.54 [163.87]
Immobile calling	689.05 [744.49]	1846.56 [725.89]
Mobile calling	59.31 [55.16]	181.17 [176.81]
Vibrating	88.03 [100.32]	152.23 [167.87]
Vibrating, head rocking	16.77 [11.32]	8.12 [13.56]
Fanning	0.18 [0.29]	2.62 [7.40]
Fanning, head rocking	56.43 [48.88]	10.13 [13.13]
Failed jump	6.04 [11.82]	0.84 [1.23]
Quick jump	0.09 [0.23]	0.97 [1.81]
Head rocking	0.79 [1.60]	13.55 [21.43]
Contact	0.75 [1.85]	2.14 [3.97]

Standard deviation is given in parentheses.

These two graphs show that the proportion of time spent by the male in calling the female is similar in both case (successful and unsuccessful). However, unsuccessful males spent more time in non-calling activities (immobile and mobile), indicating that they were less “motivated” in courting than the successful males. Wing vibrating and wing fanning occur when the female comes close to the male (usually closer than 2 or 3 cm), indicating that the male was able to attract the attention of the female (which does not necessarily mean that the male will be successful in mating). The graphs clearly show that the unsuccessful males spent little time in these two activities (vibrating and fanning) in comparison with the successful males.

Figure 6: Proportion of Time Spent by Madeiran Successful Males in Each Activity

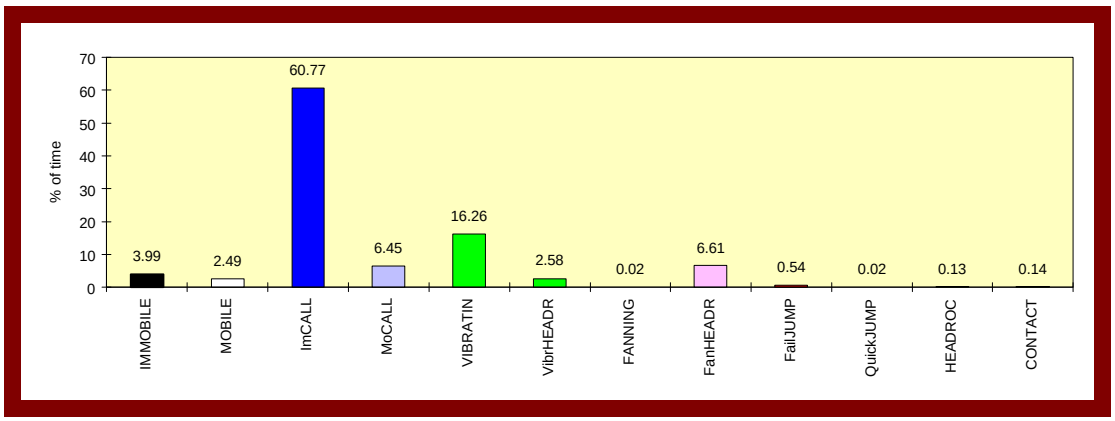
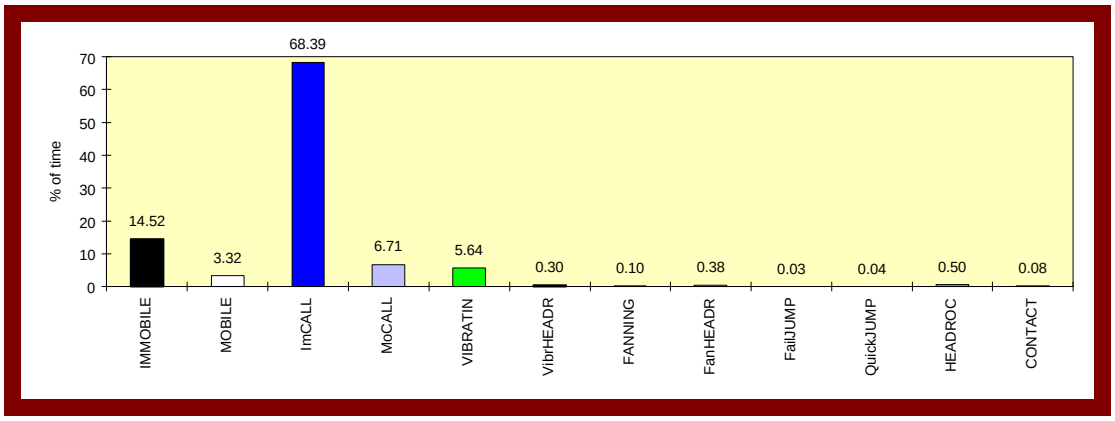


Figure 7: Proportion of Time Spent by Madeiran Unsuccessful Males in Each Activity



The reason why the female rejects or accepts the male for mating has not yet been identified and it is actually impossible to "predict" the future success of a male based on such analysis. According to the workplan of the CRP, the next phase of the analysis will consist in a detailed quantifying of the transitions between the different activities compared with the courtship of flies from different geographic areas. The aim of this comparative analysis is to try to identify the "key behaviour" determining the acceptance or the reject of the male by the female.

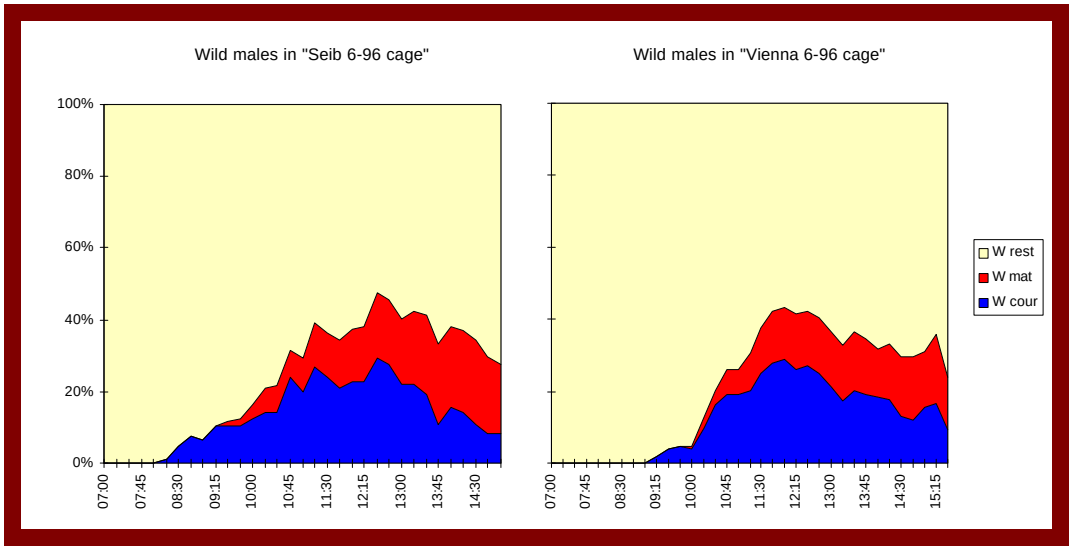
2.2.3. Field Mating Activity of Genetic Sexing Strains

The objective is to provide information on which GSS is more suitable for use in a particular programme, based on mating compatibility and competitiveness tests run in field cages. The test mainly consists of releasing wild and laboratory flies together in a field cage and to assess the type of mating which occurs (WW or WL). In 1996, these tests have been run for SIT programmes on Madeira Island and Chile-Peru.

2.2.3.1. Madeiran Flies

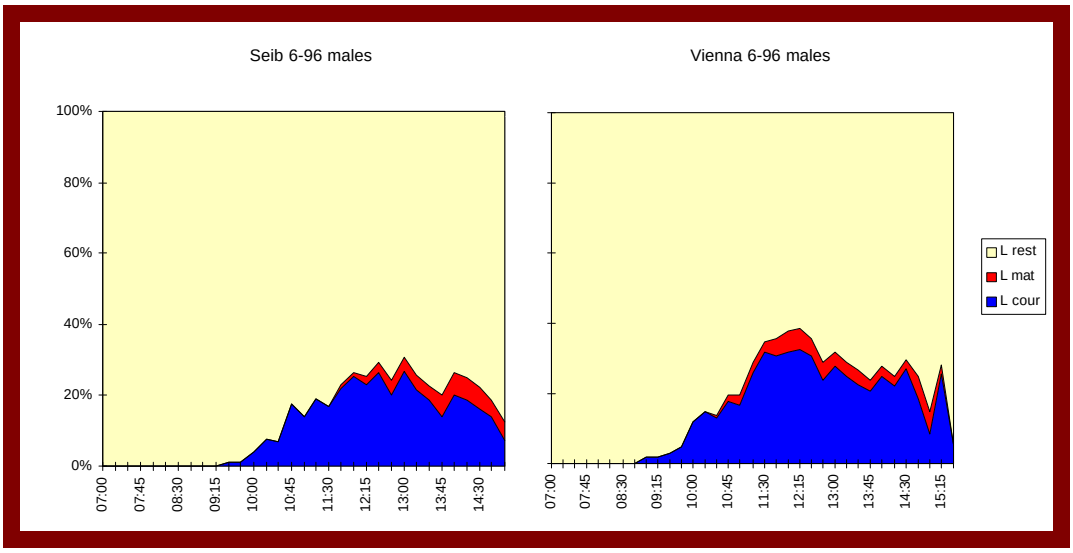
The first series of tests were run on Madeira Island in June 1996 with two GSS, VIENNA 6-96 and VIENNA 42/TOL. The two strains were reared in Seibersdorf and hand carried to Madeira. Probably because of the weather conditions (mostly cloudy and rainy), the sterile males did not perform very well, achieving only 6.67% (VIENNA 6-96) and 1.35% (VIENNA-42/TOL) of the total number of matings. Partly based on these results it was decided to construct a new GSS with a Madeiran genetic background. However additional tests were necessary to confirm the preliminary conclusions on low mating compatibility and a second series of field cage tests were run at Seibersdorf in August 1996 with wild flies shipped from Madeira Island and VIENNA 6-96 and SEIB 6-96 males. Data on the activity of the males during the tests are presented in Figure 8 and Figure 9.

Figure 8. Activity of Madeiran Medfly Males Inside the Field Cages



Cour: courtship/ Mat: mating / Rest: any other non-sexual activity / W: wild males. (Data expressed as percentage of individuals).

Figure 9. Activity of GSS Males Inside the Field Cages



Cour: courtship/ Mat: mating / Rest: any other non-sexual activity / L: laboratory reared males. (Data expressed as percentage of individuals).

During these tests, the SEIB 6-96 and VIENNA 6-96 males performed well, achieving up to 41.67 % and 40.00 % of the total matings respectively confirming that the low mating performance recorded in Madeira was not inherent to the strain itself. Tests run on Madeira Island by the local team trained during the first series of tests confirmed the performance of VIENNA 6-96 strain which is currently being mass-reared in the medfly facility in Camacha, Madeira.

2.2.3.2. Peruvian Flies

Field cage tests were run in July 1996 to assess the mating compatibility/activity of VIENNA-42/TOL and SEIB 6-96 strains with a wild-type population (first generation laboratory-reared) originating from Tacna (Peru). Two types of procedure were used a) males of the two GSS competed with wild males for wild females and b) males from one GSS release competed with wild males for wild females.

The proportion of mating achieved (Table 10) shows that, under these conditions, the SEIB 6-96 males were compatible with the Peruvian wild-type females, achieving about one third of the total matings. The VIENNA 42/TOL males achieved a negligible proportion of matings and seemed not to be suitable for large scale releases in Peru. However, considering the few replicates used, the same tests should be repeated in Peru before a decision is made on the choice of a strain.

Table 10: Mating Achieved by GSS and Wild Peruvian Males in Field Cages

	TYPE OF MATING		
TYPE OF TEST	**Wild x Wild**	**Seib 6-96 x Wild**	**Vienna-42/Tol x Wild**
Two strains	71.43%	24.49%	4.08%
One strain SEIB 6-96	71.43%	28.57%	n.r.
One strain VIENNA 6-96	100.00%	n.r.	0%

2.2.3.3. Standard Field Cage Protocol for Medfly GSS

Based on the experience of field cage tests in Madeira and at Seibersdorf and on protocols used to test normal bisexual strains, a standard protocol has been prepared to specifically assess the mating compatibility and competitiveness of GSS with wild population at the location of project counterpart. The protocol has already been discussed with counterparts in Argentina, where training will take place in 1997.

As the goal is to carry out these tests before taking any decision concerning which strain should be mass-reared and released and to train the local team, the workplan includes a one-week training and two weeks experiment. The training has already been completed in Madeira and the local team is now carrying out the tests according to the protocol prepared.

2.3. GENETIC SEXING

The possibility to release only sterile males in SIT programmes by utilising the appropriate GSS improves the economics of this pest control strategy in several areas., e.g. mass-rearing, efficiency in the field etc. Up until now, the genetic sexing technology has moved from a purely scientific level, through a semi-applied level to full implementation in certain programmes. The two primary programmes, using GSS are Argentina and Guatemala. In both cases, approximately 50 to 60 million males are produced per week.

At this level of production, weak points of the current GSS become apparent. Among them is the stability of the GSS. Rigorous strain management has to be introduced to keep degeneration of the GSS at an acceptable minimum. In addition, the problem can be reduced even further through the modifications of the structure of GSS, for example by introducing appropriate chromosome inversions.

Due to the genetic behaviour of such GSS, losses occur at certain steps in the production process, i.e. from egg to adult, current strains exhibit 50% sterility. As this is directly linked to the structure of such strains, improvements in this area are only possible by developing completely new strategies for the construction of GSS. The use of transgenic strains for this purpose appears to be the method of choice.

2.3.1. Medfly Transformation

Transgenic technology holds great promise for the improvement of the SIT. The aim of this project is to develop the relevant techniques required to transform the medfly. The primary target is the construction of novel GSS, i.e. strains where sex separation is accomplished by manipulation of suitable medfly genes and strains where molecular tags (silent or expressed) are incorporated.

Whatever gene will be used to transform pest insects, one basic tool is required in all cases, i.e. a method for the introduction and stable integration of genetic material into the target insect. Such a transformation system exists for some time in *Drosophila* (P element), but only recently the first breakthrough in that direction was reported for the medfly and the Queensland fruit fly (*Bactrocera tryoni*).

A transformation system, like the P-element system in Drosophila, consists of several components:

a) ***a mobile element***: Mobile elements are naturally occurring genetic elements that have the ability to excise and re-integrate into the host genome. All attempts to use the P-element from Drosophila in non-drosophilid species have failed. However, two different elements, *Minos* from *Drosophila hydei* and *Hermes* from the housefly (*Musca domestica*), have been identified. Both showed the potential that they might be mobile also in species other than their normal host. Recently, it was demonstrated that it is indeed possible to transform the medfly with *Minos* and the Queensland fruit fly with *Hermes*.

b) ***a suitable marker gene*** (plus the corresponding mutant recipient strain): In order to be useful as transformation vectors, the respective mobile elements have to be modified. One important modification is the addition of a selectable marker gene to be able to identify the transformed individuals. In the past, a bacterial gene that confers resistance to an antibiotic was used. However, this system is too leaky and too many false positive are generated. For the medfly this problem was solved by cloning the wild-type gene for the mutation white (*w*). Introduced into a medfly strain, homozygous for the *w* mutation, this gene rescues, at least partially, the mutant phenotype. Other types of markers are possible and are under development.

c) ***an inducible promoter***: The selectable markers, like the *w* gene described above, have to be engineered so that they are inducible. One possibility is to bring the gene under the control of a heatshock promotor, i.e. induction can be achieved by applying the appropriate temperature.

d) ***the "wings clipped" system***: The mobile elements mentioned so far contain two principle components required for their mobility; inverted repeats at the ends and a gene encoding a transposase enzyme located between these repeats. In order to obtain stable integration, these two components have to be separated. In the "wings clipped" system, two different types of molecules are injected into the target insect, one carries the transposase gene, but no inverted repeats and no insect origin of replication, the second molecule contains the inverted repeats and in between the marker gene plus the gene that has to be introduced into the insect. The transposase carrying molecule provides the enzyme activity required for the integration of the region between the inverted repeats. As no insect origin of

replication is present in either of the two molecules, they will be lost during the cell divisions following the injections; only the region between the inverted repeats can become stable and integrated into the target genome.

An attempt was made to transform medfly with the mobile element *Hermes* carrying the medfly white gene as selectable marker (under the control of a heat shock promoter). Two consultants, David O'Brochta (USA) and Peter Atkinson (Australia), performed these injections. They used medfly embryos from a white eye , white pupae strain (*we wp*). Table 11 summarises the statistics of these injection experiments and compares the result with figures published by the group from Crete for the injections using the *Minos* element.

Table 11. Effect of Injection of Hermes and Minos Constructs on Survival

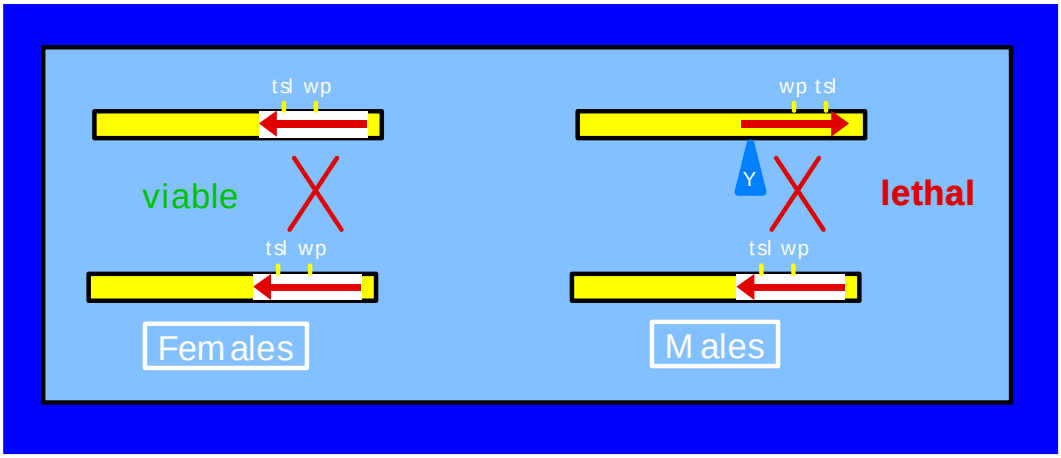
	Hermes		*Minos*	
Eggs injected	**2479**		**3998**	
Egg hatch	***813***	***32.8%***		
Number of pupae	***356***	***43.8%***		
Number of flies	***325***	***91.3%***	***390***	
Total recovery		***13.1%***		***9.7%***

The 325 surviving G_0 flies were mated, in single pairs, with flies from a *we wp* strain. In these crosses, only 205 produced offspring. From the egg stage until the late pupal stage the F1 was treated, 1 h per day, with a 37°C heatshock to activate the *white* gene in the *Hermes* construct. However, none of the families showed any rescue of the eye colour phenotype. This was unexpected as experiments using a plasmid-based transformation assay gave very high frequencies of transposition in medfly. In other words even though this element does show mobility in a medfly background it did not appear to functionally integrate into the genome. The causes of the failure to achieve integration are currently being investigated

2.3.2. Induction of chromosomal inversions

The aim of this project is to induce and isolate chromosomal inversions that cover the right arm of chromosome 5 which includes *white pupae* (*wp*) and the temperature sensitive lethal (*tsl*) gene. These genes are both used to construct genetic sexing strains when combined with particular Y-autosome translocations. Chromosome inversions are useful genetic tools as they suppress recombination in the affected chromosome region. This would allow the construction of GSS as shown in figure 10 where recombination is suppressed and stability of the strain increased. The sexing principle is the same as in current GSS. To increase stability an inversion (white box, orientation indicated by arrow) is added that covers the selectable markers (*wp, tsl*) and the Y-autosome translocation breakpoint (Y). Females would be homozygous for the inversion (recombinants are viable) while the males would be heterozygous (recombinants are lethal). Chromosomal inversions can also be used as balancer chromosomes to suppress recombination (most pronounced in females) in heterozygous individuals following a mutagenesis screen so that new mutations can be easily isolated.

Figure 10. Conceptual Structure of a GSS with Homozygous Viable Inversions.

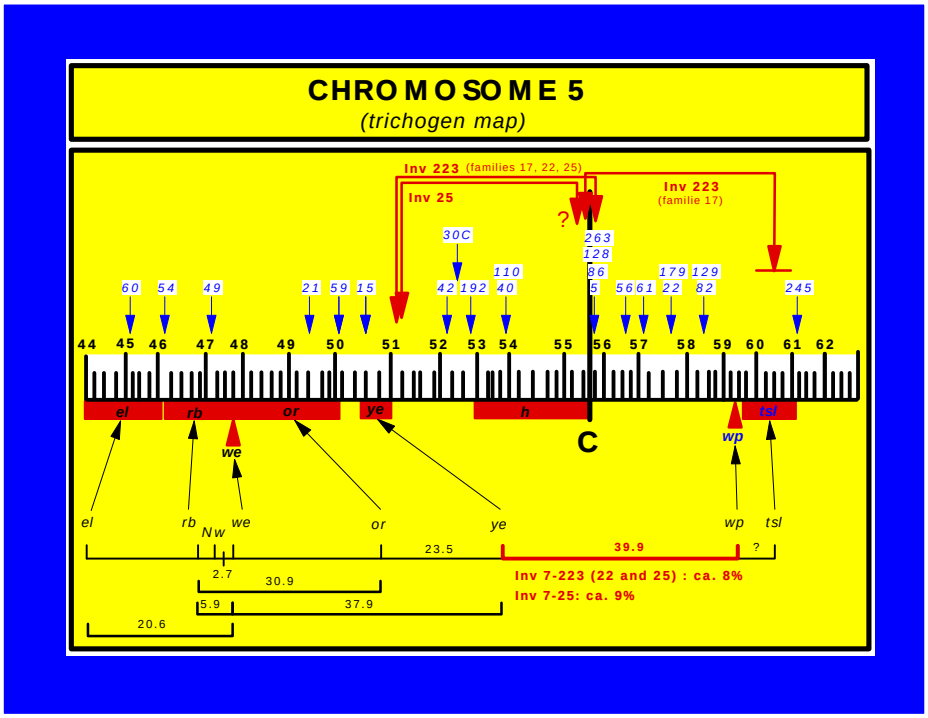


In 10 separate experiments, a total of 1855 irradiation treated 5th chromosomes (labelled with *wp* and *tsl)* was analysed. So far, three strains with translocations (in two of these the X chromosome was involved) and two strains with inversions were detected (families 7-25 and 7-223). About 10 to 20 families have still to be analysed. The analysis consists primarily of two genetic tests (recombination in females, and sterility tests in females and males) supplemented by cytological examination which was done by Dr. Antigone Zacharopoulou (University of Patras, Greece). With the recombination test we determine whether indeed a chromosome re-arrangement was induced that reduces female recombination between the two markers *yellow body* (*ye*) and *wp*, both located on the 5th chromosome (see figure 11). Location of Y-autosome breakpoints (blue arrows) and inversion breakpoints (red arrows) are shown. In addition, the cytological location and the genetic distance (in % recombination) of several mutations is given.

The normal recombination frequency between *ye* and *wp* is 39.9%. The recombination test consists of the following two crosses: i) *ye* wp^+ females x ye^+ *wp* (*tsl*) "inversion" males (mass-mating); ii) 5 single F1 females x *ye wp* males. Two parallel crosses were set up with F1 flies from the above test cross: i) 5 single F1 females x *ye wp* males; ii) 5 single F1 males x *ye wp* females. The following possibilities can be expected for families showing reduced female recombination.

If a translocation was induced then both sexes would show 50% or more sterility when heterozygous for the treated chromosome but if a pericentric inversion was induced then sterility would only be present in the female where recombination occurs and leads to lethality. If a paracentric inversion had been induced then no sterility would be observed. In addition to the tests mentioned so far, each family with reduced female recombination was tested for the presence of the *tsl*, i.e. approximately 30 single pair sublines were established for each family and tested separately for temperature sensitivity. This step was necessary because the *tsl* is located outside of the interval *ye-wp*. Only sublines that were homozygous for *tsl* were maintained for further analysis.

Figure 11. Schematic Representation of the Trichogen Polytene Chromosome 5.



2.3.2.1.Analysis of family 7-25:

Four sublines (7-25-1, -2, -13, -25), homozygous for *tsl*, were detected. Table 12 shows the results of the recombination test. The observed recombination frequencies range from 3.7% to 20% (the latter in a single female cross with only 5 offspring). On average all four sublines are very similar and recombination between *ye* and *wp* is reduced to 9.2%.

Table 12 Recombination and Egg Hatch Data from Family 7-25

Subline	Percent recombination (average of 5single females)	Percent egg hatch (from heterozygous females)	Percent egg hatch (from heterozygous males)
7-25-1	8.17	82.07	83.93
7-25-2	9.17	89.83	94.71
7-25-13	10.07	89.33	87.50
7-25-25	9.50	87.16	92.55
Total	9.17	86.96	87.76

Egg hatch is virtually normal irrespective of whether the potential inversion was heterozygous in a female or a male. This would indicate that this strain contains a paracentric inversion. The strain was analysed by Antigone Zacharopoulou (Patras, Greece) and all four sublines contained an inversion. The approximate position of the breakpoints on the polytene trichogen map are 51A and 55C/D (the positions were converted to the trichogen map, the initial analysis was done on salivary gland chromosomes). It appears that the right breakpoint is on the right arm of chromosome

5, i.e. this is a pericentric inversion. Further analysis is required to clarify the discrepancy between the genetic and the cytological data. In all sublines the inversion is heterozygous, i.e. it is apparently not viable as homozygote.

2.3.2.2.Analysis of family 7-223

In the temperature test for the presence of the *tsl*, six positive sublines were detected. One of them (7-223-17) behaves differently and will be discussed separately. From the remaining five, two (7-223-22, -25) were analysed so far. The results are shown in table 13.

Table 13. Recombination and Egg Hatch Data on Family 7-223

Subline	Percent recombination (average of 5single females)	Percent egg hatch (from heterozygous females)	Percent egg hatch (from heterozygous males)
7-223-22	8.92		
7-223-25	6.79		
7-223-17	10.07		
Total	8.32	64.55	94.99

In the single female crosses the recombination frequency varied from 5.38% to 14.29%. On average, recombination is reduced to 8.32% (Table 13). If the potential inversion is maintained through females, the fertility is reduced significantly while in males no sterility is observed. This indicates the presence of a inversion. In the sublines cytologically analysed a pericentric inversion was detected. The breakpoints are identical to the one in 7-25, i.e. 51A and 55C/D. Also here the inversion is not viable as a homozygote and additional test crosses confirmed this non-viability.

The family 7-223-17 showed a different behaviour as the parallel subline. The family is rather weak and is difficult to maintain: egg hatch is 43.7%, pupal recovery is 52.7%, emergence is 79.4%. This results in an overall recovery of only 17.5%. Polytene analysis revealed that this family contains two inversions, i.e. in addition to the one found in the parallel sublines a second inversion is present with breakpoints at 55B/C (left arm) and approximately 60A-D (right arm) (Fig 11).

Genetic tests showed that both inversions are on different chromosomes and that both are inviable in a homozygous condition. Our current description of the structure is that it represents a balanced lethal system. All flies are heterozygous for the two inversions. This structure is maintained in the inbreeding strain as any homozygote is inviable. In part this explains the severe lethality found in this family. Half of the offspring die because they are homozygous for either of the two inversions. In addition, both inversions together cover nearly the entire chromosome region between *ye* and *wp*. As recombination is 39.9% in this region, the equivalent number of offspring will die. This should amount in an overall recovery of roughly 30%, a little higher than was found.

2.3.3. Micro-dissection sex chromosomes

In medfly, male sex is determined by a *Maleness* factor located on the Y chromosome. Together with the genes for the ribosomal RNA, this is the only known functional gene on this chromosome. It is conceivable to utilise the *Maleness* factor to construct GSS that no longer require Y-autosome translocations as the current strains. The primary benefit of such transgenic GSS would be their full fertility. In addition, such a sexing system should be transferable to other fruit fly species. Therefore attempts are underway to clone the *Maleness* factor in the medfly.

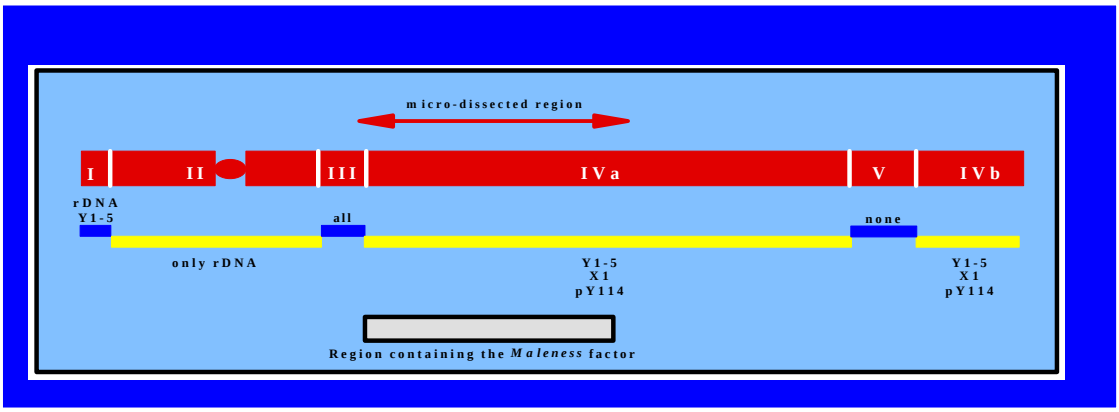
In the medfly, the sex chromosomes are heteromorphic, the X chromosome is twice as long as the Y chromosome. Most of the Y chromosome is heterochromatic, only a small constriction at the tip of the long arm, visible by the light staining in C-banded chromosomes, has euchromatic appearance. The short arm and the proximal third of the long arm of the X chromosome are heterochromatic while the rest of the long arm is intermediate grey in C-banded chromosomes. The sex chromosomes of medfly show the classical picture of differentiated sex chromosomes, with a degenerated, heterochromatic Y chromosome.

Through deletion mapping of the Y chromosome we have identified the sex-determining region on the long arm of the Y chromosome. A region, consisting of approximately 15% of the whole Y chromosome, is sufficient for the development of male adults with normal testes development and mobile sperm. In this region a *Maleness* factor and, potentially, Y-specific genes required for male fertility are located. Surprisingly, the male-determining region is not located in the constriction on the long arm, but in the heterochromatic part of the long arm of the Y chromosome.

Several different approaches are possible to clone the *Maleness* factor. One possibility is to micro-dissect a region of the Y chromosome that should contain this factor (see Annual Report 1995). This material was PCR amplified and used as a probe for fluorescence *in-situ* hybridisations (FISH). The intensity and distribution of the hybridisation signals enabled an estimate to be made of the degree of homology or divergence of repetitive sequences of the X and the Y. In the medfly not only the Y but also the X chromosome accumulated repetitive sequences in parts of their long arms. FISH of the micro-dissected probes to *C. rosa*, a closely related species, confirms, that some of these sequences were already present before the separation of these two species.

Combining these results with previous data enabled the medfly Y chromosome to be divided into five subregions depending on what probe hybridises to the respective region (Fig 12). The 5 sub-regions of the Y chromosome are shown. In addition, the approximate region that was micro-dissected is indicated by a red arrow.

Figure 12. Preliminary Cytological Map of the Medfly Y Chromosome



3. TSETSE GROUP

During the last two decades the Entomology Unit has advanced the development of effective rearing procedures for at least six species of tsetse. However, mass-rearing remains the most notable area in which improvements are needed before area-wide implementation of SIT against tsetse flies can be attempted. Tsetse fly rearing procedures are labour intensive with several manual steps which limits the industrial production of sterile flies. Based on accumulated knowledge of tsetse rearing, some areas of mass-rearing have been identified for automation namely; a system for holding, feeding and collection of larvae and pupae and sex separation of adult flies.

3.1. TSETSE PRODUCTION UNIT

In 1996 a prototype Tsetse Production Unit (TPU), which holds adult fly cages, moves cages to the feeding site and conduct automatic feeding while pupae and larvae are collected centrally was evaluated. The mechanics of the TPU were tested during the early part of 1996. From May 1996, *Glossina austeni* flies were used to evaluate the performance of the TPU. During this evaluation several related refinements have resulted in significant improvement in tsetse rearing so that production can be increased without a concomitant increase in labour or facilities.

The standard *in vitro* feeding of tsetse flies is through a silicone membrane which is placed on a tray of blood warmed to mammalian body temperature. For the TPU the feeding system initially involved moving blood through a closed system either by gravity or peristaltic pump. Blood was warmed as it passed over the heated surface which is covered by silicone membrane. Two problems were identified with this feeding system; the negative pressure on the blood did not allow flies to engorge, and it was difficult to attain the required level of sterility of the feeding equipment. This lead to the adaptation of a floating membrane.

3.1.1. Performance of *G. austeni* on the TPU

TPU cages were loaded with freshly emerged flies from the main colony, 300 females with 75 males per cage. The cages were held on the TPU and fed automatically. At intervals dead flies were removed from the cages and counted. Pupae produced from these flies were collected daily except Sundays and counted. A sample of pupae was sorted out by size and weighed out individually in each class size. The same batch of pupae was allowed to emerge into adults in order to estimate eclosion rate and sex ratio.

High initial mortality was observed among young flies put on the TPU. Investigations on fly density, cage movement, feeding efficiency, stress and toxicity of the material used in cage manufacture were carried out to identify the cause of this mortality.

The feeding efficiency test was carried out using teneral male flies starved for 48 hours in the standard cage (200 mm diameter x 50 mm deep with 100 female and 25male flies) and the TPU cage (200 mm diameter x 70 mm deep with 300 female and 75 male flies). Four cages of each type were fed on the TPU feeding system and a similar number was fed in the standard colony as control. After nine minutes of feeding, all flies were killed by chilling and examined to count the number fed. The results revealed that flies had access to blood, but better feeding was achieved in standard cages, 91% compared to 87% in the TPU. However, the cages were manually handled. The stress test in the normal colony showed that the rate at which flies died was not comparable to the massive death recorded among flies on the TPU where a daily mortality rate of up to 3% was recorded during the first week and experiments were carried out to see if toxicity of any cage component was responsible for the heavy mortality

For these tests, flies were put in cages with different treatments and observed for five weeks (Table 14)

Table 14. Survival of *G. austeni* in Different Types of Cages

Cage number	Treatment	Flies/cage	Survival	PPIF (%)
1	Old TPU, glued & washed, H	300f + 75m	91.8	0.29
2	Old TPU, glued & washed, V	300f + 75m	91.2	0.23
3	New TPU, glued & washed, H	300f + 75m	92.3	0.22
4	New TPU, glued & washed, V	300f + 75m	76.8	0.17
5	Old TPU, not glued & washed, H	300f + 75m	89.1	0.35
6	Old TPU, not glued & washed, V	300f + 75m	88.8	0.17
7	New TPU, no inserts & washed, H	100f + 25m	93.6	0.38
8	New TPU, no inserts & washed, V	100f + 25m	88.8	0.48
9	Old STD, no inserts & washed, H	100f + 25m	92.0	0.58
10	Old STD, no inserts & washed, V	100f + 25m	89.6	0.54

H=horizontal; V=vertical; TPU=tpu cage; STD=standard cage

One set of cages was placed in a horizontal (H) position while the second set simulated the vertical (V) plane as on the TPU. There was no obvious toxic effect from any of the cages indicating that neither the material used for cage housing, inserts in cages or

glue were toxic to flies. Flies in cages that were kept horizontal once more performed better than those held vertical.

3.1.2. Fly Density and Movement

After demonstrating that neither the material used in cage making nor lack of access to blood was causing mortality among young flies, it was decided to test the effect of density of flies per cage and movement of these cages on performance. On the TPU there are basically two types of movement; one "fast" to bring flies to feed and a second "slow" to enable all cages to experience the same environmental conditions throughout 24 hours.

Six cages with varying numbers of flies ranging from 100 females and 25 males per cage to 350 females and 87 males on the TPU were observed for survival and pupal production. Control cages with 100 females and 25 males and 300 females and 75 males were kept in a vertical plane but not on the TPU and they were manually fed (Table 15) Tests were replicated four times. In cages held on the TPU (continuous movement) the mean % survival decreased with the increase in fly density. The mean daily mortality rate ranged between 2.1% and 3.47% during the first week. The acceptable daily mortality rate is 1.2%.Among the flies in cages which were not on the TPU and which were manually fed, mean survival at the lowest density was 66.3% compared to 40.6% on the TPU. This difference increased with increasing density. Pupal production was also better among flies that were manually fed.

Table 15. Effect of Density and Movement on Performance of Flies

Cage Density	% Mean Survival	Pupae Per Initial Female (PPIF)	% Daily Mortality Week 1
With Movement			
100 f + 25 m	40.6	1.55	2.1
150 f + 38 m	39.77	1.46	2.32
200 f + 50 m	37.38	1.36	2.16
250 f + 63 m	29.02	1.35	3.03
300 f + 75 m	28.29	1.18	2.5
350 f + 88 m	25.89	0.95	3.47
Without Movement			
100 f + 25 m	66.3	2.69	1.2
300 f + 75 m	51.08	2.21	1.0

Pupal production was lowest for cages with the highest density, ppif of 0.95 in 10 weeks. Production (ppif) in cages which were manually fed (without movement), was 75% more than in TPU cages. The conclusion was that except for the cage with the highest density, the daily mortality rate did not vary with density on the TPU. The observations lead to the discontinuation of cage movement except during feeding.

3.1.3. Insert Designs and Fly Density

In a further search for likely causes of death among flies maintained on the TPU, insert designs were modified in the standard TPU cages and performance of flies was

monitored for 10 weeks. Two density of flies per cage were used; 100 females with 25 males and 300 females with 75 males.

The modifications of inserts were as follows:

Normal insert	cells 25 mm x 25 mm
Half insert	cells as for normal insert but only half the depth of the cage
Half division insert	cells 55 mm x 55 mm

Cages without inserts were also included. Mortality and pupal production was recorded weekly and tests replicated four times. Data was analysed using Tukey's HSD tests (Table 16)

Table 16. Effect of Insert Design in TPU Cages on Performance of *G. austeni*

	Density	No insert	Half insert	Half division	Normal
% survival	100 females	48.68 ± 17.60	65.00 ± 14.93	69.07 ± 13.48	51.60 ± 9.75
	300 females	20.71 ± 11.74	43.14 ± 12.01	48.75 ± 13.85	37.50 ± 14.18
Fecundity	100 females	0.24 ± 0.13	0.28 ± 0.10	0.28 ± 0.12	0.22 ± 0.10
	300 females	0.16 ± 0.27	0.15 ± 0.08	0.16 ± 0.06	0.13 ± 0.06

At 100 females per cage, there was no significant difference in survival between the groups although survival was best in cages with half division. In cages with 300 females the differences between the survival means were significant ($p < 0.05$) for all treatments. There was significantly better survival of flies in cages with 100 females compared to 300 females. Fecundity was generally poor for all treatment groups at the two densities but cages with the lower density still performed significantly better. Casual observations revealed a large number of expelled eggs and aborted larvae in the pupal/larval collector. Further tests are being conducted with half inserts and half division inserts.

3.1.4. Pupal Quality

Pupae produced by flies on the TPU were compared with those from the standard colony flies in terms of class sizes (Table 17), weight (Table 18) and emergence rate.

Table 17. Distribution (%) of Size Classes of Pupae

	COLONY	TPU G0	TPU F1
Class A	02.08 ± 1.42	10.56 ± 1.62	13.67 ± 1.7
Class B	24.01 ± 1.37	44.94 ± 1.64	46.28 ± 2.08
Class C	62.85 ± 1.5	40.94 ± 1.92	36.31 ± 2.18
Class D	10.99 ± 1.35	03.32 ± 2.04	03.65 ± 2.15
Class E	01.03 ± 1.6	00.24 ± 1.48	00.09 ± 2.41

The Class A pupae which are the smallest pupae made up 10.56 ± 1.62% and 13.67 ± 1.7% of TPU G0 and TPU F1, respectively, compared to 2.08 + 1.42% from colony pupae. The mean weight of pupae produced by flies on the TPU was generally lower than that from the colony.

Table 18. Mean Weight (mg) of *G. austeni* Pupae from Colony, G0 and F1 flies

	COLONY	TPU G0	TPU F1
Class A	12.93	12.28	12.31
Class B	16.94	16.18	15.86
Class C	20.45	19.46	19.27
Class D	23.49	22.62	22.34
Class E	26.41	24.95	22.90

The sex ratio at emergence was the same in all the three groups being 1:1 but the emergence rate was 90.39% for colony pupae, 78.54% ,TPU G0 and 78.96%, TPU F1 pupae. There were also more crippled flies at the time of emergence from TPU produced pupae.

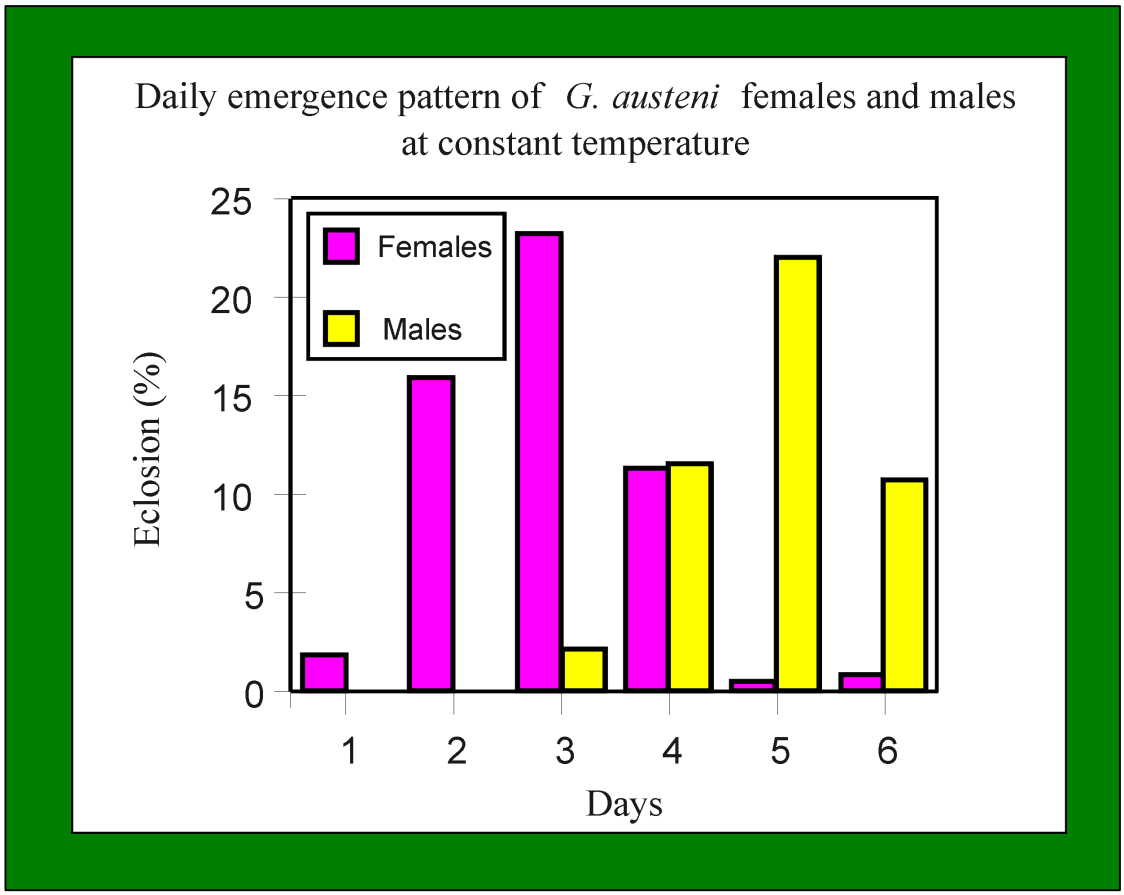
The indications are that the poor eclosion rate and high percentage of crippled flies could be due to high mortality of pupae in the early stage during development. Dissections of pupae from which flies failed to emerge confirmed that death occurred early during development. The quality of pupae produced on the TPU was therefore inferior, this could have been due to the physiological status of the mother (poor feeding or stress from movement) or the handling during the sensitive stage of melanization and hardening. Pupae and larvae remain in the cage until just before feeding when the cage collector on the cage tips them through an aluminium duct to the central collector.

3.2. SEX SEPARATION IN ADULTS.

Adult emergence pattern in tsetse flies has been shown to be driven by the daily cycle of temperature fluctuations and not by photoperiod. Three tsetse species investigated, *G. austeni*, *G. morsitans* and *G. pallidipes*, show similar differences in their intrapuparial development rate with females emerging earlier than males deposited at the same time (Fig 13).

In the search for the optimum conditions for sex separation useful in the mass-rearing of *G. austeni* it has been demonstrated that the temperature at the time of emergence is important. The emergence period of adults at 26.5°C, from pupae deposited on the same day spans a four day period. During the first two days virtually only females eclosed, and during the remaining two days mostly males eclosed. At 23-24°C the emergence period lasts 6-7 days (Fig 13), with the first three days being predominantly females, the fourth day mixed and the last two or three days being mostly males. At the higher emergence temperature the overall emergence period is shortened and the sex separation is better. In order to further shorten the emergence period for the females, low pre-emergence temperature of 18.5°C, followed by emergence temperature of 26.5°C was found to compress the overall emergence period to three days with 75% of the females emerging on the first day but with an increased mortality. Better sex segregation based on differential emergence time of males and females was further enhanced by keeping the puparia separately during the emergence period and not allowing them to touch each other.

Figure 13. Emergence of Male and Female *G. austeni*



3.2.1. Self Stocking of Production Cages

The above differences in eclosion time may be developed for self stocking of production cages. Flies would be made to emerge directly into production cages in the correct number and sex ratio eliminating the need for chilling and manual separation of sexes; a step which currently takes 23% of time for tsetse mass-rearing. Two systems have been tested; a system where flies are allowed to pass through a light breaker circuit and are counted and loaded according to cage requirements. Assumption is made that the male to female ratio is equal and using the established emergence rate, the number of pupae required to load the cage with the desired number and sex of flies is calculated. The second system uses weight loss from pupae or weight gain of production cages into which the flies emerge. This system requires a top loading balance to monitor the number of flies in a cage. However, a third system can be used which records the time elapsed from first emergence to the point when the cage has been loaded by the right number of flies at the required sex ratio.

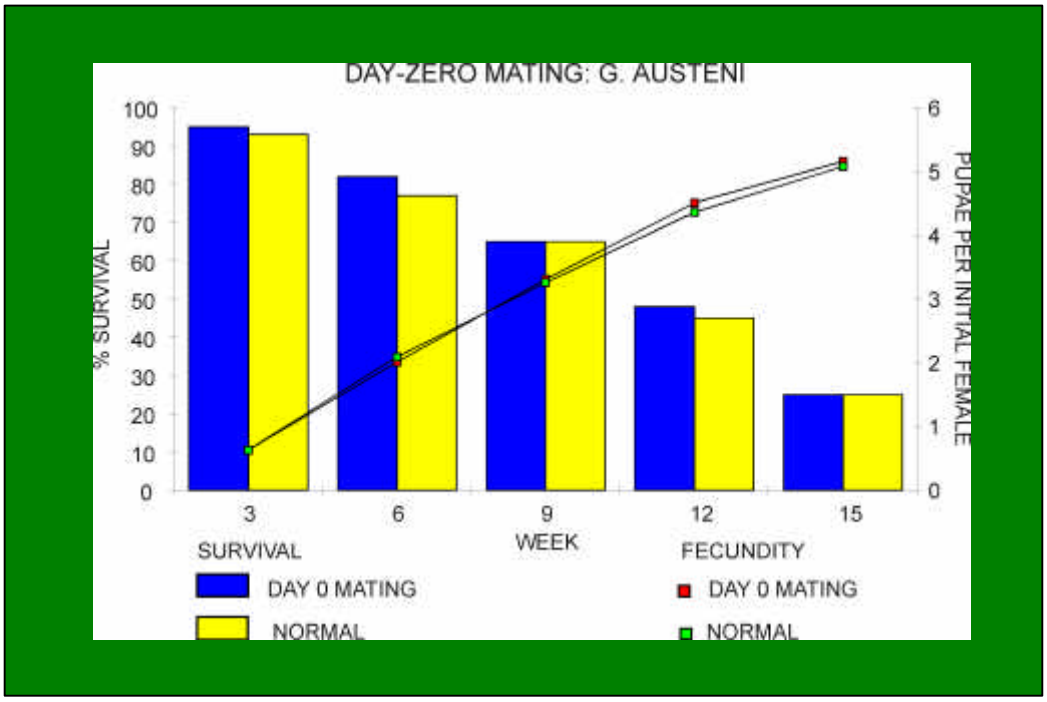
This system for direct stocking of production cages is being refined for application in colony maintenance initially using with *G. austeni*. By combining the system for self stocking of production cages with day 0 mating (see below) at the appropriate sex ratio, the time spent in tsetse mass-rearing would be reduced by 46%.

3.2.2. Day 0 mating

Previous laboratory investigations have shown that maximum insemination rates are achieved in a number of tsetse species when mating occurs between 3 day old females and adult males more than one week old. By this time females would have had the opportunity to feed twice and males several times. There are species differences and optimal fertility in *G. pallidipes* is achieved by mating females 7-9 days old with 10 days old or older males. However, in the field it is usually very rare to find virgin females in a sample of flies caught in traps or off a bait animal. In addition, laboratory reared *G. austeni* have been observed to mate in emergence cages before sexes are separated.

Having established that with *G. austeni* putting 1 male to 4 females in a production cage does not compromise survival and production, investigations were carried out to determine if it was necessary to allow flies to mature before placing them in cages for mating. Newly emerged flies at a ratio of 1 male to 4 females were put in production cages and their performance compared to that of 2 day old females mated with males which were 7 days old. The performance of flies in the two groups in terms of survival and pupal production were identical (Fig 14).

Figure 14. Effect of Day 0 Mating on the Productivity of *G. austeni*



The results showed that with *G. austeni*, successful mating takes place even when cages are stocked with freshly emerged flies at a 1:4 male to female ratio. Dissections of females from such groups confirmed that by day 10 after emergence, all the females are inseminated.

3.3. FREEZE DRIED BLOOD

Laboratory maintenance of tsetse flies originally depended on the availability of live hosts. Maintenance of a healthy and self-sustaining colony of live hosts for *in vivo* feeding of flies was more problematic than keeping the flies alive and it was also expensive. Other factors which affected the colony performance included adverse skin reaction of the host animals to frequent fly bites, the quality of feeds given to host animals and drugs used for treatment of host animals which may be detrimental to tsetse flies. These drawbacks lead to the development of the *in vitro* or membrane feeding system in which blood at mammalian body temperature is presented to flies through a suitable membrane.

With the membrane feeding system blood was collected on a weekly basis and kept at 4^{o}C. It was observed that when blood which was kept for more than six days at 4^{o}C was fed to tsetse flies it lead to decreased reproduction and loss of 20% of puparial weight. In an effort to overcome this, cells and serum from freshly collected bovine or porcine blood were separated and stored at -28^{o}C.The components were mixed before feeding in a 1:1 proportion and ATP added. Processed blood could be stored for up to four months without loss of quality. However, it was later found that there was no need to separate the cells from serum. Fresh frozen defibrinated bovine or porcine blood is the basic diet of tsetse flies to date. For most species a combination of the two in varying proportions is used and ATP is added before presentation for feeding.

Because of problems associated with obtaining a fresh supply of sterile blood especially during field projects, attempts were made to find ways of prolonging the shelf life of blood through freeze drying. Reconstituted freeze dried bovine and porcine blood was used for feeding a colony of *G. palpalis palpalis* during the Bicot project especially to provide the component of porcine blood to the diet. The procedure followed included reconstitution with sterile distilled water, irradiation at 1 kGy, mixing to the required proportion, dividing to the aliquot used in daily feeding and storage at -20°C. ATP at 10^{-3}M was added at the time of feeding. Oven drying of blood was found to be a cheaper alternative to freeze drying but was not pursued further. Tests with freeze dried and oven dried blood showed no difference in nutritional quality for *G. p. palpalis*.

The identification of the different components of blood which were essential for the maintenance of a productive colony of tsetse flies lead to the development of a semi defined synthetic diet. The synthetic diet was used to rear *G. p. palpalis* for five generations and the survival, fecundity and offspring size achieved were similar to those obtained when flies fed on whole blood *in vitro* but was expensive.

Decontaminated fresh frozen blood for the project in Zanzibar is shipped from Vienna, Austria in a refrigerated container to Tanga in Tanzania. Ninety percent of what is shipped is water and with freeze drying water is eliminated. A volume of 5000 litres in

5 litre containers would be reduced to 500 kilogrammes. It would therefore be more economical to reduce the volume to be shipped without loss of quality by freeze drying. Considering the volume of blood required to sustain a colony of more than one million female flies which is approximately 300 L a week, a fresh look was made at the use of freeze dried blood for tsetse flies colony feeding.

Sixty freshly emerged *G. pallidipes* (Uganda strain) per group were maintained on freeze dried and fresh frozen combinations of bovine and porcine blood and their survival and pupal production over a period of seven weeks recorded.

Table 19. Performance of *G. pallidipes* (Uganda strain) Maintained on Freeze Dried and Fresh Frozen Porcine and Bovine Blood

	Freeze Dried			Fresh Frozen		
	BB:PB 25:75	BB:PB 50:50	BB:PB 75:25	BB:PB 25:75	BB:PB 50:50	BB:PB* 75:25
% survival	86.50	77.00	85.00	76.00	80.00	79.00
PPIF	2.27	2.01	2.16	1.98	1.89	2.02

*standard combination used for *G. pallidipes* colony feeding
BB = bovine blood; PB = porcine blood

Two tests were run and the results showed that *G. pallidipes* performed equally well whether fed reconstituted freeze dried blood or fresh frozen blood. The next challenges is to find a source of dried blood which is suitable for rearing tsetse flies.

4. FELLOWS and VISITORS

Name	Reference Code	Field of training	Duration (months in 1996)	Fellowship period
BYAMUNGU, Ms. Mechtilda B.	URT/95003	Tsetse	0.07	95-07-03 to 96-01-02
MATEMBO, Mr. Sebastian I.R.	URT/95005	Tsetse	2	95-11-01 to 96-02-29
NGOTHO, Mr. Joseph Maina	KEN/95031	Tsetse	5	95-12-01 to 96-05-31
OLET, Ms. Pamela Akinyi	KEN/95032	Tsetse	6	96-02-01 to 96-07-31
TUN, Mr. Thein	MYA/95021	Fruit fly	6	96-03-02 to 96-09-01
MACHUCA LAGOS, Mr. Juan R.	CHI/95017	Medfly GS	5.9	96-04-01 to 96-09-28
ANDRADE, Mr. Luis Erwin	GUA/96001	Medfly GS	5	96-06-17 to 96-11-16
GONÇALVES SILVA, Ms. Maria	POR/96007	Medfly GS	2	96-07-01 to 96-08-31
MALELE, Ms. Imna Issa	URT/95029	Tsetse	6	96-07-01 to 96-12-31
MUTIKA, Mr. Gratian Nyambirai	ZIM/96001	Tsetse	4	96-07-01 to 96-10-31
NASSOZI, Ms. Annette	UGA/96001	Tsetse	1.9	96-09-04 to 96-10-30

5. SCIENTIFIC VISITORS

Name [Period]	Reference Code	Field of Expertise	Duration (months in 1996)
LOBOS AGUIRRE, Mr. Carlos R. 96-04-01 to 96-04-04	CHI/96003	Medfly	4
FRANCO RODRIGUES, Mr. A.P. 96-08-05 to 96-08-09	POR/96008	Medfly	5
OKELLO-ONEN, Mr. Joseph 96-11-11 to 96-11-15	UGA/96004	Tsetse	5
SHERENI, Mr. William 96-11-18 to 96-11-22	ZIM/96012	Tsetse	5
NDUNG'U, Mr. Joseph Mathu **96-12-09 to 96-12-13**	KEN/96013	Tsetse	5

6. TRAVEL

Staff member	Destination	Period of absence including travel days	No. of work days	Source of funds	Purpose of travel
Robinson, A.S.	Mendoza (Argentina); Guatemala City (Guatemala)	96-03-16 - 96-03-30	10	RIFA	Sci. Sec. RCM; Moscamed progr.
	Budweis (Czech Rep.)	96-04-24 - 96-04-26	3	NO COST	Evaluation of scientific programme
	Gainesville, FL (USA)	96-06-01 - 96-06-07	5	TC	Interregional training course
	Florence (Italy)	96-08-25 - 96-09-01	5	RIAL	Congress of Entomology
	Montpellier (France)	96-09-07 - 96-09-13	5	RIFA	IOBC Conference
Cayol, J.-P.	Tapachula (Mexico)	96-02-16 - 96-02-24	6	APO	RCM on medfly courtship behaviour
	Madeira (Portugal)	96-06-11 - 96-06-22	9	TC POR/5/005	TC mission
	Beirut (Lebanon)	96-08-18 - 96-08-29	9	TC LEB/5/013	TC mission
Caceres, C.	Madeira (Portugal)	96-02-18 - 96-02-24	5	TC POR/5/005	TC mission
	Madeira (Portugal)	96-04-28 - 96-05-10	10	TC POR/5/005	TC mission
	Madeira (Portugal)	96-06-09 - 96-06-22	10	TC POR/5/005	TC mission
Fisher, K.	Lincoln (New Zealand)	96-02-03 - 96-02-11	5	NO COST	Course on SIT
	Sydney (Australia)	96-02-12 - 96-02-21	6	RIFA - [PER DIEM ONLY]	Review SIT programme
	Mendoza (Argentina)	96-03-21 - 96-03-31	7	TC ARG/5/005	TC mission
	Guatemala City (Guatemala)	96-06-15 - 96-06-22	5	NO COST	Inauguration Moscamed Facility
	Palermo (Italy)	96-08-04 - 96-08-09	5	NO COST	Discuss new medfly mass rearing facility
	Mendoza (Argentina)	96-10-15 - 96-10-25	9	TC ARG/5/005	TC mission
	Arica (Chile)	96-10-26 - 96-10-31	4	TC CHI/5/015	TC mission
Franz, G.	Mendoza (Argentina)	96-03-17 - 96-03-28	9	TC ARG/5/005	TC mission
	Louisville, KY (USA)	96-12-07 - 96-12-13	5	RIAL	ESA meeting
Machuca Lagos, J.R.	Funchal (Madeira)	96-06-13 - 96-06-23	7	TC (CHI/5/015)	Medfly mating behaviour field tests

Staff member	Destination	Period of absence including travel days	No. of work	Source of funds	Purpose of travel
Mattiacci, L.	Gainesville, FL (USA); Guatemala City (Guatemala); Tapachula (Mexico)	96-01-22 - 96-02-07	13	JPO	Visit medfly parasitoid mass rearing and research centres
Nadel, D.J.	Nairobi (Kenya)	96-09-27 - 96-10-04	6	NO COST	Discuss tsetse mass-rearing at ICIPE
Opiyo, E.	Bamako (Mali)	96-05-25 - 96-05-31	5	TC MLI/5/012	TC mission
	Muguga, Nairobi (Kenya)	96-09-21 - 96-10-06	10	TC KEN/5/020	TC mission

7. PUBLICATIONS

BANKS, G.K., MALCOLM, C.A., ASCOLILLO, A., HALL, L.M.C., ROBINSON, A.S., "The evolution of an intron in the acetylcholinesterase genes of Diptera", 20th Int. Cong. Entomol. Firenze, 1996 (1996) 281 (poster abstract).

CAYOL, J.P., Box-thorn, key early season host of the Mediterranean fruit fly. Int. J. of Pest Management **42** (1996) 325-329

FISHER, K.T., "Queensland fruit fly (*Bactrocera tryoni*): Eradication from Western Australia", Economic Fruit Flies: A World Assessment of their Biology and Management (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 535-541.

FRANZ, G., KERREMANS, PH., RENDON, P., HENDRICHS, J., "Development and application of genetic sexing systems for the Mediterranean fruit fly based on a temperature sensitive lethal", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 185-191.

HENDRICHS, J., "Action programmes against fruit flies of economic importance", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 513-521.

HENDRICHS, J., KATSOYANNOS, B.I., GAGGL, K., WORNOAYPORN, V., "Competitive behavior of males of Mediterranean fruit fly, *C. capitata*, genetic sexing strain vienna-42", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 405-415.

MANSOUR, M., FRANZ, G., "A rapid test for distinguishing irradiated from unirradiated medfly, *Ceratitis capitata* (Diptera: Tephritidae), larvae", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp.

Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 505-510.

MANSOUR, M., FRANZ, G., Effect of gamma radiation on phenoloxidase activity in Mediterranean fruit fly (Diptera: Tephritidae) larvae, J. Econ. Entomol. **89** 3 (1996) 695-699.

MANSOUR, M., FRANZ, G., Gamma radiation as a quarantine treatment for the Mediterranean fruit fly (Diptera: Tephritidae), J. Econ. Entomol. **89** 5 (1996) 1175-1180.

RENDON, P., FRANZ, G., WOOD, R.J., "Assessment of irradiation doses for tsl strain Vienna-42", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 193-196.

ROBINSON, A.S., GOODING, R.H., "Initial studies to map the white gene in *Glossina morsitans submorsitans* using RAPD's", 20th Int. Cong. Entomol. Firenze, 1996 (1996) 779 (poster abstract).

ROBINSON, A.S., ZACHAROPOULOU, A., "Review of Session VI, Genetics/Biochemistry", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 199-203.

WILLHOEFT, U., Fluorescence *in situ* hybridization of ribosomal DNA to mitotic chromosomes of tsetse flies (Diptera: Glossinidae: Glossina), Chromosome Research (in press)

WILLHOEFT, U., FRANZ, G., Identification of the sex-determining region of the *Ceratitis capitata* Y chromosome by deletion mapping, Genetics **144** (1996) 737-745.

WILLHOEFT, U., FRANZ, G., Comparison of the mitotic karyotypes of *Ceratitis capitata, C. rosa* and *Trirhithrum coffeae* Bezzi (Diptera: Tephritidae) by C-Banding and FISH, Genome **33** (1996) 884-889

WILLHOEFT, U., FRANZ, G., MCINNIS, D.O., "Towards the application of genetic sexing in tephritid fruit fly SIT programs", Economic Fruit Flies: A World Assessment of their Biology and Managment (Proc. 4th Int. Symp. Clearwater, FL, 1994), (McPHERON, B.A., STECK, G.C., Eds), St. Lucie Press, Delray Beach, FL (1996) 179-184.