ANNUAL REPORT

1999

Entomology Unit

FAO/IAEA Agriculture & Biotechnology Laboratory

Seibersdorf

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1. Introduction

The activities of the Unit continued to be focused on the development of genetic sexing strains (GSS) in the medfly, Ceratitis capitata, *and improved production systems for tsetse. A new medfly genetic sexing strain was constructed, evaluated and transferred to several rearing facilities. This strain contains a mixed genetic background and has improved quality control characteristics. In tsetse, a system for the automated stocking of flies at the correct density and sex ratio was introduced into a large* G. pallidipes *colony. The results indicate that this system can be introduced into the tsetse rearing facilities in Africa. A slightly improved version of the tsetse holding and feeding unit, TPU2, was transferred to three facilities in Africa for evaluation.*

The Insect and Pest Control sub-programme underwent an external review in the beginning of 1999. One of the recommendations of direct relevance to the activities of the Unit concerned the development of an R and D programme for a second fruit fly. As most fruit flies are subject to quarantine regulation within Europe, modifications are required to the current rearing facility. During 1999 a consultant provided technical advice and initial funding was secured to complete Phase 1 of the conversion. Structural plans are also now available for the 2nd and last phase but funding has still to be identified.

Two professional staff left the Unit in 1999, Mr. J.P. Cayol took up a position in a fruit fly programme in French Guyana and Mr. K. Fisher was recruited by the California Department of Food and Agriculture to manage their medfly mass rearing facility in Hawaii. The retirement of three technical support staff at the end of the year led to a re-alignment of activities based on the recommendations of the external review and a new professional position will be created in the tsetse group. Six colleagues from abroad spent varying degrees of time in the Unit making contributions to the R and D programme in relation to support of Co-ordinated Research Projects (CRP's).

The supply of biological material to colleagues and institutions world-wide is an important component of the services provided by the Unit. Many tsetse research workers are totally dependent on the receipt of a regular supply of material from Seibersdorf. The vast majority of medfly rearing facilities are now using GSS supplied from Seibersdorf and this technical backstopping of operational SIT programmes will continue. The confidence built up by the customer in the use of the current strains provides a healthy environment for the new strains under development.

1.1 A New Medfly Genetic Sexing Strain and Chromosomal Inversions

In response to customer requirements a new genetic sexing strain was produced. This strain was constructed using a heterogeneous genetic background to improve biological fitness and utilizes a translocation which will produce a much improved quality profile. The strain was evaluated during mass rearing and tested under field cage conditions for mating competitiveness. It has now been transferred to three medfly programmes and is planned for transfer to several others this year. Major success was achieved in the long search for chromosomal inversions for inclusion in genetic sexing strains. A whole series has now been isolated in collaboration with the El Pino medfly rearing facility in Guatemala and the University of Patras. These inversions are being evaluated and selected ones will be introduced into a genetic sexing strain. The inclusion of an inversion will provide extra stability to the strain and ease the process of the introduction of new genetic material.

1.2 Field Cages for Tsetse

The use of field cage tests to monitor mating behaviour of sterile flies has been an essential component of quality control for most SIT programmes. In tsetse, these field cage tests have so far not been used. Experiments initiated in 1999 in the field cages previously used for medfly were surprisingly successful. Sexually mature, fed, male and female tsetse were introduced into the cage and many matings were observed. As well as providing an experimental system to monitor fly quality, it will now be possible, for the first time, to make detailed observations of mating behaviour in this species. The use of this new quality control procedure for sterile male tsetse will provide an increased level of confidence for field behaviour of mass reared and released flies.

1.3 Consultant's Contributions

Dr. M. J. Scott (Massey University, New Zealand) spent 4 months in the Unit working on the isolation of the male determining gene in medfly and initiating a molecular analysis of sex determination in tsetse. Dr. Scott was successful in making cDNA libraries from different medfly developmental stages which are still being screened for the Maleness factor. He also isolated several PCR fragments from tsetse using sequence data from sex determination genes in other insects. This work was continued on his return to Massey and will be reported on at the upcoming CRP on genetic transformation (D4.10.12).

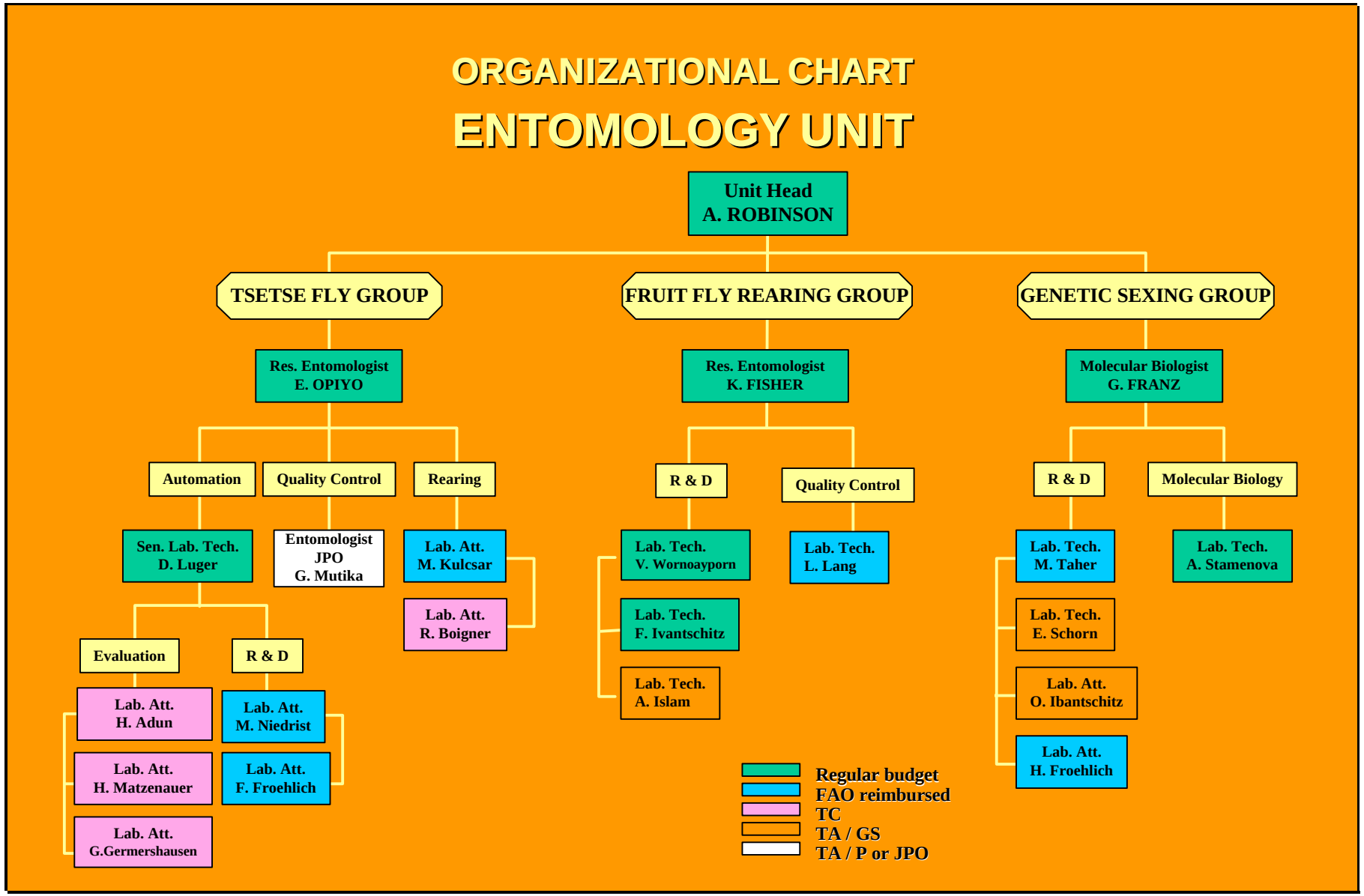
Dr. D. Briceno (University of Costa Rica) is a participant on a new CRP on quality control for fruit flies. He spent 3 weeks in the Unit examining the courtship behaviour of medfly mutant strains to understand the role played by vision and wing movement in successful courtship. Although both blind and wingless males could mate they did so much less effectively than normal males. The use of mutants of this type enables the significance of particular courtship behaviours to be assessed. This work forms part of the work programme for CRP D4.10.16.

Dr. Nestel (Volcani Insitute, Israel) returned to Seibersdorf to complete his evaluation of the use of an ELISA reader to measure lipid content in medflies. The ELISA protocol was successful and efficient, however lipid levels were inherently variable and no correlation with quality could be demonstrated. Evaluation of lipid content in relation to quality has not been easy and at the moment there is no adequate experimental procedure.

Drs McGraw and Akman (Univ. Yale, USA) carried out work on tsetse in relation to CRP D4.20.10. Two different aspects of tsetse biology were examined. Firstly an attempt was made to transfer *Wolbachia* symbionts between different tsetse species using injection of tissue extract. These bacteria have been shown to produce sterility in many insect species. Survivors from the injections were taken to Yale for analysis at subsequent generations. Secondly, cDNA libraries were made from different gut tissues to try to identify genes involved in trypanosome development. The libraries are currently being screened in Yale.

1.4 Other Activities

Research results of the Unit were presented at two important meetings. At the Working Group on Fruit Flies of the Western Hemisphere in Guatemala City, Guatemala, 3 posters were presented (see 4.1) and a session on fruit fly genetics was chaired by a Unit staff member. The International Council for Trypanosomiasis Research and Control held its 50th meeting in Mombasa. and three papers were presented during the meeting.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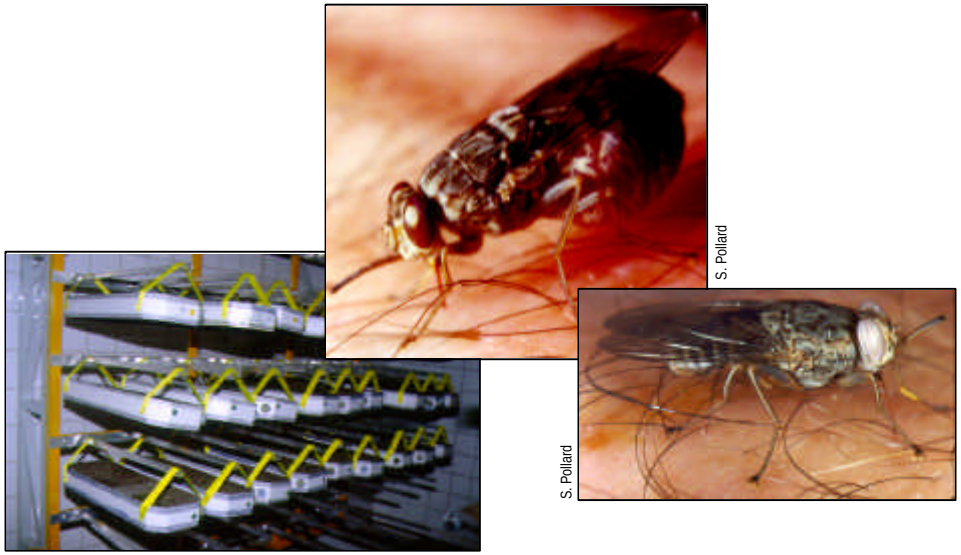
2. Quality Control and Rearing in Tsetse



The evaluation of a prototype tsetse holding and feeding system (TPU 2) was completed. The key factor in the evaluation was shown to be cage size and design. G. pallidipes *is very sensitive to adult density and fly movement in the cage is detrimental to survival. Good production can be obtained on the TPU 2 if fly density in the cage is low. The challenge is now to modify cage design so that more flies can be maintained in the same total space to ensure efficient mass production. A third fly holding and feeding system was designed and constructed and will be evaluated in 2000.*

During 1999 the self stocking procedure for the introduction of flies into cages was transferred to a large G. pallidipes *colony. With this system the colony is maintained without handling of the adult flies thus saving time and demonstrating its operational practicability. A very important by-product of the system are male pupae so that manual sexing of flies before irradiation and release is not required. For the system to work, pupae must be collected daily and incubated under carefully controlled environmental conditions. The successful evaluation of the system for the routine maintenance of a large colony suggests that it will be used for any future large scale tsetse SIT programme.*

Quality control protocols for mass produced tsetse need improvement and a field cage test system has now been developed. Sexually mature, tsetse males and females when introduced into a field cage show a high degree of mating activity and the mating competitiveness of male tsetse can now be evaluated.

The G. pallidipes *colony from Arba Minch, Ethiopia reached the level of 10,000 producing females during 1999. The quality of pupal shipments from Ethiopia has increased and the productivity of the colony at Seibersdorf has also risen. New colonies of* G. morsitans centralis, *field collected from Botswana, and* G. swynnertoni, *from an established colony in Canada, have been initiated to examine the use of hybrid sterility for tsetse control. The other colonies of tsetse maintained by the Unit provide essential material for many colleagues from many parts of the world. There are no cost recovery procedures available for the supply of these expensive insects.*

2.1 Fly Production

Efforts are still being directed towards improving mass rearing systems for tsetse and they include both new equipment and refined handling procedures.

2.1.1 The Final Evaluation of TPU 2

Structural details of the TPU 2 and the initial evaluation can be found in the **Annual Report 1998**. The large oblong cages (20cmWx60Lx7H) designed for this equipment were shown to cause high fly mortality due to an uneven distribution of flies probably due to a strong light gradient in the cages. There was significant crowding taking place as a result of the flies being attracted to the light and accumulating at the end of the cage nearest to the light. Investigations were conducted to determine the effect of light and cage position on survival and production of *G. pallidipes* using both the standard round cage (20cm∅x5H) and the oblong TPU 2 cages. Nine round cages were loaded with flies at the standard density of 48 females and 12 males and fixed on the TPU 2 in a 3x3 Latin square design. Three TPU 2 cages were loaded with flies at the standard density of 260 females and 65 males and placed on the same shelf as the nine round cages **(Figure 1).**The data on survival and pupae per initial female (PPIF) of the flies in these cages are given in **Table 1**.

Figure 1. Cage distribution on TPU2

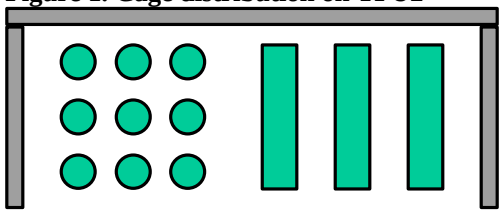


Table 1. Survival and PPIF of *G. pallidipes* in two types of cages on the TPU 2.

Round cages			TPU 2 cages		
Survival					
41.7	58.3	47.9			
47.9	47.9	35.4	20.4	14.6	48.8
23.1	39.6	37.5			
Mean	42.6		*Mean*	27.9	
PPIF	2.36			1.42	

There was a large degree of variation in survival, especially in the three TPU 2 cages, survival in the smaller round cages was superior. This might suggest that fly density per volume of cage is not the cause of increased mortality but that simply the number of flies in the cage is important. A statistical analysis of the survival data from the round cages revealed that there was no overall difference in survival but there was a trend that flies in cages towards the back of the TPU 2 had better survival. For the TPU 2 cages, the large variation in survival did not permit any meaningful conclusions to be drawn although mean survival was lower than for the round cages. Based on this preliminary experiment a second set of investigations was carried out on the TPU 2 using 27 standard round cages (20cm∅x5cmH) which were loaded with 48 females and 12 males and were fed 3 days a week. Mortality was checked weekly and pupae collected on a daily basis. By week 15, the PPIF was 3.12 and survival 47.1%. The PPIF for the same cages on the standard trolley is 3.6. This result shows that the TPU 2 rearing system is suitable for mass rearing of tsetse and can be used to produce males for an SIT programme. These tests using the standard round cages were carried out in relation to the evaluation of the TPU 2 by three facilities in Africa. TPU 2 units were shipped to the facilities in September. In these facilities only the standard round cages are available and will have to be used initially. The data from Seibersdorf will provide a comparison. None of the new cage designs tested on the TPU 2 has so far been satisfactory and the lack of a suitable cage is a serious concern and will be addressed again in 2000. The question of light distribution and availability will also need to be resolved.

2.2.2 Introduction of Self Stocking of Production Cages (SSPC) into G. pallidipes *Colony Maintenance.*

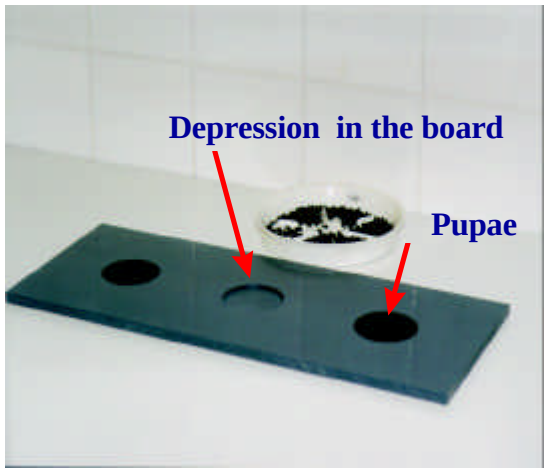
An experimental procedure (SSPC) for introducing tsetse into cages at the correct sex ratio and density was described in the **1998 Annual Report**. During 1999 the SSPC procedure was transferred to the large *G. pallidipes* Uganda colony for evaluation. Pupae from the colony were collected daily and incubated under normal conditions of 23-24°c and 75-80% RH. Thirty days after larviposition, 110 pupae together with 15 "male pupae" **(Annual Report 1998)** were placed inside a ring in a single layer under a production cage and the flies allowed to emerge directly into the cage at 26.5°C. The target number of flies required/ cage is 48 females and 12 males. Ten cages were loaded using this SSPC procedure and survival and production over 10 weeks was compared to cages manually loaded with 48 females and 12 males. The initial number of flies of each sex/cage in the SSPC cages was calculated *a posteriori* by adding the number of flies alive at week 10 to the number of flies that died during this period. There was a small error associated with the manually loaded cages.

Table 2. Survival and production of *G. pallidipes* in cages loaded in two ways.

	Mean No./cage		Mean % Survival		Pupae/cage
	Female	Male	Female	Male	
SSPC	42.6±2.9b	12.8±2.7a	41.4±12.1b	13.6±8.6b	203±28a
Manual	47.6±6.4a	11.9±1.9a	26.9±13.0a	4.0±7.3a	107±32a

The results are shown in **Table 2.** There was a significantly reduced number of females in the SSPC cages (F=5.10;df=1,18;P>0.05) but not males (F=0.97;df=1,18;P=0.05). Males and females introduced into cages using the SSPC had better survival than those introduced following a chilling procedure, i.e. manually. There was however, no significant difference in the mean number of pupae produced per cage (F=0.23, df=1,18, P>0.05). To be successful, the SSPC procedure involves counting the number of pupae to be placed under each cage. This time consuming activity could be avoided if a volumetric approach was used. For *G. pallidipes* the number of pupae/ml is *ca*. 16. Using this value, 7 ml mixed sex pupae and 1 ml male pupae will provide the correct number and sex of flies to load one 20cm∅ production cage. A second system was also tested in which a shallow depression, the equivalent size and depth of the ring used in retaining pupae below the production cage, was drilled in a plastic board. These depressions can then simply be filled with pupae, the excess removed and the cage placed on top (**Figure 2**).

Fig.2 Volumetric SSPC



Fourteen depressions were filled with pupae, without counting, and 15 "male pupae" added. After 48 hours the number of flies in the cage was counted. Fourteen samples of a known number of pupae, 110 mixed sex and 15 "male pupae", were emerged under the same conditions and acted as control. There was no significant difference between the number of females (F=2.44;df=1,26;P=0.09) or males (F=0.03;df=1,26;P=0.87) emerged in cages from the pupae which were counted or estimated volumetrically and the sex ratio was very close to the expected 4 females to 1 male. (**Table 3**). The time at which pupae

Table 3. Cage loading using volumetric method

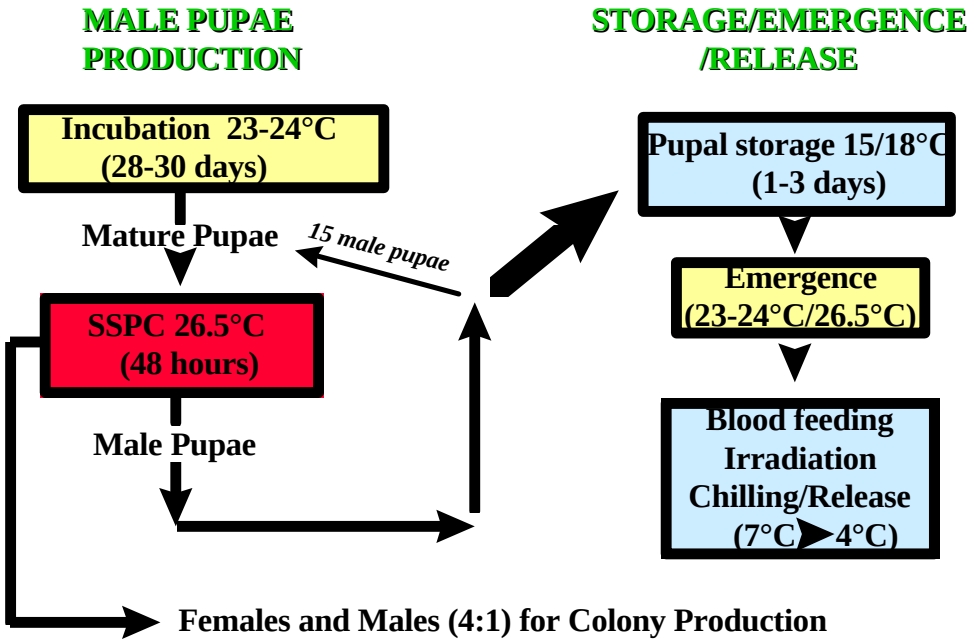
	Mean No./cage±s.e		Sex ratio
	Females	Males	
Volumetric	49.21±1.83	11.93±0.96	4.1:1
Counted	45.86±1.12	11.71±0.89	3.9:1

are placed under a production cage for emergence is crucial for the application of the SSPC technique. Tests were conducted to determine the best pupal age for transfer of pupae to SSPC conditions. The results **(Table 4)** show that pupae which are 30 days old when placed in the SSPC conditions can load cages with flies at the correct density and sex ratio. An essential component of this system is having predictable and reliable pupal incubation conditions. Based on these results we now use 30day old pupae for SSPC to maintain a large *G. pallidipes* colony of approximately 20,000 females. The successful introduction of the SSPC system into routine colony maintenance is important for two reasons, firstly it has demonstrated that, given good pupal incubation conditions, this system will be applicable for large scale tsetse production in Africa and secondly, it has eased considerably the work load for colony maintenance at Seibersdorf. The crucial by-product of this system namely male pupae, removes the need for hand-sexing prior to release of sterile males.

Table 4. Effect of pupal age on the sex ratio when using SSPC

	Mean No./cage		Sex Ratio	Range	
Pupal Age	Female	Male		Female	Male
28	45±8.3	13±4.1	3.5:1	23-63	4-32
29	45±6.6	20±8.4	2.3:1	26-55	5-41
30	42±6.5	9±2.3	4.7:1	21-50	5-14

Figure 3. Proposed procedure for production of sterile *G. pallidipes*



2.2.3 Handling of male G. pallidipes.

Male pupae produced from the SSPC system have been used to develop improved strategies for pupal storage and adult male handling. The first part in a series of investigations to determine the effect of low temperature treatment of *G. pallidipes* male pupae on important adult quality control parameters indicated no significant effects on emergence rate, survival without blood and insemination capacity **(Annual Report 1998).** Coupled with this, storage of pupae at 15°C delayed eclosion of *G. pallidipes* by 2-3 days, a feature which can be manipulated for synchronising emergence. These results demonstrated the potential advantages of the procedure to an operational SIT programme. Future sterile male production will utilise the SSPC procedure for sex separation at the time of emergence and, if required, male pupae could be stored at 15°C for 2-3 days before emergence. The adult males will be fed on blood containing Samorin, irradiated and released using the chilled adult release system. A schematic depicting these steps is shown in **Figure 3**.

It is necessary to determine the effect of these various pupal and adult treatments on sterile male quality. Tests were carried out to determine the effect of low temperature treatment of pupae, adult irradiation

and chilling on survival, flight ability, mating propensity, copulation duration and insemination ability. For each treatment a control group of untreated SSPC emerged males were used. Tests were conducted in both laboratory and field cages. Mature male pupae obtained after SSPC were incubated at 15°C for 24(group 2), 48(group 3) or 72(group 4) hours and thereafter allowed to emerge at 23-24°C. Group 1 were not chilled.

2.2.3.1 Effect of pupal and adult chilling on mating

Male flies that emerged from pupal treatment groups 2, 3 and 4 were irradiated in a ^{60}CO Gamma cell 220 source at 120Gy. Males from treatment group 1 were not irradiated. Irradiation was carried out on flies 10-12 days old. Following irradiation a sample of males was chilled at 7°C (±24% R.H) for 48 hours followed by 6-7 hours at 4°C. After this period of chilling the flies were returned to the control environment. Mating tests were conducted the following day. Males were mated with 8 day old control females by introducing them into 10 cm∅ cages into which the females were already resident. The flies were observed for two hours. Mating pairs were removed and placed in individually numbered single tubes to monitor duration of copulation. Males were discarded after separation. Females were dissected to determine if insemination had taken place and the amount of sperm in the spermatheca was quantified.

Table 5. Mating performance of *G. pallidipes* males that were irradiated then chilled

Pupal Group	% Mating	Duration (mins)	Insemination
1(not irrad)	89	31.6±2.3	75.1±7.7
2	43	36.6±2.3	66.0±6.0
3	74	35.2±2.2	77.0±5.8
4	45	42.9±3.6	86.1±7.4

The males that were irradiated and chilled as adults were able to engage in copulatory activity and successful sperm transfer although the proportion of irradiated males that engaged in mating was apparently lower than for un-irradiated males **(Table 5)**. The calculated lower proportion of irradiated males that successfully mated may be a result of a small sample size rather than a true reflection of effect of irradiation. The chilling of adults at 7°C for 48 hours followed by 6-7 hours at 4°C does not have a significant effect on duration of copulation ($F=0.18;df=3,73;P>0.05$) and on spermathecal fill ($F=0.28;df=3,73;P>0.05$). However in this experiment the chilled males were only presented with an opportunity to mate more than twelve hours after removal from 4°C. For further development of the envisaged chilled adult release system in a field programme it would be appropriate to conduct further tests soon after removal from 4°C to determine whether these parameters remain unaffected.

Table 6. Survival of male *G. pallidipes* after irradiation and chilling

Pupal Treatment	Adult Treatment Irrad.	Chilling	No. Adults Tested	No. (%) Surviving to Week 4
2	no	no	39	39(100)
2	yes	yes	38	28(74)
3	no	no	71	66(92)
3	yes	yes	100	61(61)
4	no	no	71	70(99)
4	no	yes	30	30(100)
4	yes	no	15	14(93)
4	yes	yes	91	78(86)

2.2.3.2 Effect of pupal and adult chilling on survival

A sample of the adult males emerging from pupal treatments 2 and 3 (this time using 18°C instead of 15°C) were irradiated on day 8 and then chilled at 7°C for 48 hours followed by 7 hours at 4°C. (This adult chilling regime was used because it might represent what is required in an operational SIT programme, 7°C would probably be the holding temperature on the ground and 4°C in the aircraft.) The males were then transferred to the insectary at 23-24°C and mortality was recorded daily for 13 weeks. The last surviving irradiated male lived for 70

days compared with more than 91 days for control flies. There was a significant reduction in the survivorship of irradiated males at week 4 ($F=11.45;df=1,24;P<0.05$). More than 50% of the irradiated and chilled males survived up to 35 days. Survival was generally above 90% for two weeks and above 60% for 4 weeks (**Table.6**). Survival of flies that were irradiated and chilled was significantly reduced compared to the flies that were not chilled ($F=18,25;df=1,24;P<0.05$). The trend in survival of the flies was similar irrespective of the temperature regime to which the pupae were exposed ($F=0.12;df=2,23;P>0.05$). In a field release it is not advantageous if released males live a long time as they could contribute to disease transmission.

2.2 Radiation Studies on *G. pallidipes*

Gamma radiation will be used to sterilize male *G. pallidipes* for the SIT component of a tsetse eradication programme in Ethiopia. For many tsetse species the appropriate dose of radiation for this purpose has been established, but not for laboratory reared *G. pallidipes.* It is also important to consider what stage should be irradiated, adults or pupae. In many SIT programmes, pupal irradiation is used and with the production of male pupae from the SSPC this would now also be possible for *G. pallidipes.* In the current study, adult male *G. pallidipes* from the Uganda colony were irradiated with different doses. The effect of radiation on male survival was monitored as well as the fecundity of the colony females mated with the males.

Flies were emerged using the SSPC and the sexes were kept separate under standard rearing conditions of 23-24°C and 75% RH until the females were 8 days and males 13 days old. The flies were irradiated in a ^{60}CO Gamma cell 220 at a dose rate between 32-36Gy/min. Six groups of 100-150 virgin males aged 13 days were irradiated with doses of 40, 60, 80, 100, 120, and 140Gy in air, one group was kept as untreated control. On the following day the males were mated with 8 day old virgin females in 20cm∅ cages. Twenty to 25 females were introduced into the cage followed by a similar number of males. Mating pairs were removed as they formed, placed in numbered tubes and copulation time recorded. The investigations were conducted between 11:00 to 13:30hr in full light (400 Lux), temperature 24°C and 40-50% RH. After the completion of mating each fly was placed in a separate tube. Tubes with females were placed in petri dishes and the dishes were examined daily under the stereo-microscope for the presence of eggs, larvae or pupae. Any pupae produced were kept for 40 days to monitor emergence rate and sex ratio of F1 flies. Tubes holding individual males were observed for 64 days and their survival was compared to that of untreated controls. Fecundity data included only females that survived 50 days. The total number of flies used in the observations varied with the number of pairs that formed for each treatment. A single classification of ANOVA for unequal samples showed that there was no significant difference in survival or duration of mating of males treated with different doses of radiation ranging from 40Gy to 140Gy compared to that of untreated controls ($F=0.17;df=6,566;P=0.05$ and $F=0.14;df=6,194;P=0.05$, respectively), **(Table 7).** Fecundity of females mated to irradiated

Table 7. Duration of copulation and survival of irradiated male *G. pallidipes*

	DOSE						
	Control	**40Gy**	**60Gy**	**80Gy**	**100Gy**	**120Gy**	**140Gy**
Copulation	30.3±0.8	30.4±1.3	29.1±1.2	31.5±0.8	32.6±0.9	33.5±0.9	31.3±0.4
Duration	(81)	(78)	(69)	(85)	(85)	(89)	(86)
Male	40.6±2.9	45.1±2.8	40.0±3.3	35.1±2.8	38.8±2.4	38.2±2.5	38.4±2.7
Survival	(24)	(29)	(25)	(28)	(35)	(30)	(30)

Table 8. Fecundity of *G. pallidipes* females mated to irradiated males

	No. of females	No. of Eggs	Larvae/ Female	Total Pupae	PPIF	Productivity
Control	42	10	0.26±0.50	77	1.83	100
40Gy	51	40	0.25±0.40	75	1.47	80.33
60Gy	42	46	0.20±0.46	36	0.86	46.99
80Gy	62	96	0.12±0.38	22	0.35	19.13
100Gy	52	87	0.19±0.53	7	0.11	06.01
120Gy	57	113	0.02±0.14	5	0.07	03.83
140Gy	59	109	0.06±0.25	0	0.00	00.00

males was assessed by the number of pupae, larvae or eggs produced (**Table 8**). Following a dose of 140Gy no pupae were produced and these data were excluded from the analysis. As expected, there was a significant difference in pupal production between females mated to untreated control males and females mated to males treated with varying doses of radiation ($F=15.24;df=5,300;P<0.001$). The number of pupae produced declined with increasing radiation dose whilst the number of expelled eggs increased. This increase in the number of expelled eggs was significant ($F=5.19;df=6,356;P<0.001$). There were also significantly less larvae produced by females mated to treated males than those mated to control males ($F=10.04;df=6,359;P<0.001$). The pupae produced by a sample of the females were allowed to continue development until eclosion. A very important observation was that irradiation had no effect on the emergence rate of surviving F1 pupae **(Table 9).** This would suggest that for an SIT programme a dose has to be used which guarantees that no pupae are produced. As reported by other workers there was a significant distortion of sex ratio in favour of males in the emerged F1 adults. Based on this study the recommended dose to ensure complete sterility of male *G. pallidipes* would be 120Gy as is the case with *G. m. morsitans* and *G. austeni*. The effects that this dose of irradiation will have on male mating competitiveness are under investigation.

Table 9. Emergence of F1 pupae from irradiated male *G. pallidipes*.

	No. of Females	No. of Pupae	F1 Females	F1 Males	Total (%) Emerged	% Males
Control		28	13	13	27(96.4)	51.8
40Gy	23	26	5	17	23(88.5)	73.9
60Gy	21	12	4	8	12(100)	66.0
80Gy	19	10	2	6	8(80)	75.0
100Gy	14	2	0	2	2(100)	100.0
120Gy	14	0	0	0	0	
140Gy	22	0	0	0	0	

2.3 Quality Control

The quality of sterile male tsetse is a crucial component for the success of operational SIT programmes. Improved quality control procedures are now being developed which address this problem.

2.3.1 Age and Mating

The relationship between the age and mating is important to know in order to maximise colony production and to ensure the correct release strategy for sterile males. In *G. pallidipes* previous work has shown that females reach peak sexual receptivity between 8-13 days and males between 7-9 days. In females sexual maturity is related to the development of the ovary and in males to the accessory gland. *G. pallidipes* virgin females of different age groups were mated with males of a fixed age in order to determine the age at which females are most sexually receptive and to try to correlate this with egg follicular development. In males the reverse procedure was followed and sexual maturity correlated to the size of the accessory gland. Virgin *G. pallidipes* males and females 1-15 days old were used. Three females were put in a cage, left for one hour and

Table 10. Reproductive parameters of *G. pallidipes* females

Female Age	Mating Index	Mating Duration (min±SD)	Follicular Size (mm± SD)
1	0.1±0.2d	n.d	0.69±0.04d
3	0.0	n.d	0.71±0.05d
5	0.5±0.5cd	36.1±8.0a	0.83±0.13c
7	1.2±1.0bc	26.5±11.8b	1.57±0.15b
9	1.6±o.8ab	26.4±5.6b	1.65±0.07ab
11	1.7±0.7ab	22.1±5.4b	1.66±0.06a
13	2.2±0.8a	24.9±4.9b	1.64±0.08ab
15	1.1±1.0bc	24.8±6.2b	1.70±0.16a

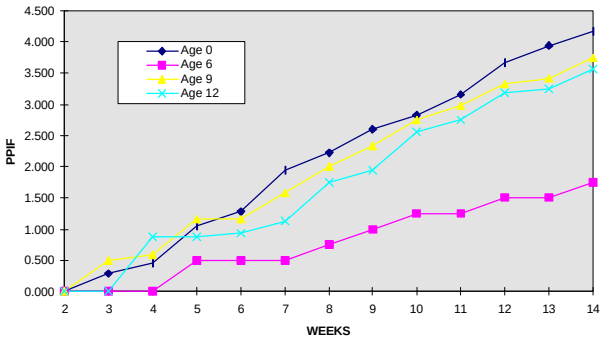
then three males were added. After 15 minutes, copulating pairs were removed. Each test was replicated three times.. The fixed age for both sexes was 7 days, Copulation time was recorded and females dissected 24 hours later for presence of sperm in the spermatheca. The males were dissected to measure the width of the accessory gland and the females to measure the length of the first egg follicle. Measurements were carried out with a calibrated eye piece micrometer to the nearest 0.01mm. In a second experiment, females and males of the same ages, (6, 9 and 12 days) were mated and pupal production monitored for 14 weeks. This was compared with males and females that were confined immediately after emergence.

The effect of female age on receptivity, copulation duration and follicular length is shown in **Table 10**. Female receptivity increased with age and peaked at day 13. Females at 5 days of age took significantly longer time in copulation whilst older females showed no effect of age (F=2.56;df=6,169;P<0.05,). The mean duration ranged from 22.1±5.4 to 26.5±11.8 at maturity (**Table 10**). Virgin females younger than 5 days had significantly shorter mean follicular lengths than other females (F=28;df=7,76;P>0.001) however older females showed no increase in size. No ovulation occurred in virgin females up to the age of 15 days. In the mated females however, ovulation had already occurred by day 9 indicating a significant effect of mating on the time of ovulation. In males a similar picture emerged (**Table 11**). Males started mating on day 3 and increased until day 13 followed by a decline (F=15.11;df=7,268;P> 0.001). Males younger than 5 days had long copulation times with little or no sperm transferred but there was no significant effect of male age on the duration of copulation (F=8.71;df=6,169;P>0.001) when males were older than 5 days. Mean spermathecal values as a measure of insemination potential of males ranged from 0.05±0.11 to 1.47±0.64 (**Table 11**). Males older than 7 days did not differ significantly in their insemination potential (F=10.33;df=6,100;P>0.001). The apical body width showed a significant increase with age (F=47.11;df=7,49;P>0.001). These studies on male reproduction would indicate that sterile males need to be retained in the rearing facility for up to 7 days before release.

Table 11. Reproductive parameters of *G. pallidipes* males

Male Age	Mating Index	Copulation Duration (min±SD)	Accessory Gland Size (mm±SD)	Spermathecal Value (MSV± SD)
1	0.0		0.12±0.01cd	
3	0.3±0.5cd	49.8±34.1a	0.11±0.01d	0.05±0.11b
5	0.6±0.6cd	26.9±9.2b	0.13±0.02cd	0.61±0.66b
7	1.0±1.0bc	26.9±8.1b	0.14±0.01c	1.26±0.45a
9	1.6±0.6ab	24.5±3.9b	0.17±0.01b	1.47±0.64a
11	1.7±1.0ab	25.1±3.1b	0.17±0.01ab	1.42±0.35a
13	2.3±1.0a	23.6±3.3b	0.17±0.01ab	0.73±0.48b
15	1.7±0.9ab	20.4±3.9b	0.19±0.01a	1.36±0.53a

Figure. 4. Productivity at different initial ages



The fecundity of the females that were confined on the day of emergence did not differ significantly from those that were mated at day 9 or 12. (**Figure 4**). but it is not clear why there was a significantly lower productivity in the flies mated when they were 6 days old ($F=7.97;df=3,47;P<0.001$). The females mated on the day of emergence produced the largest number of pupae/female. This data strongly supports the use of the SSPC for colony maintenance and have provided information of practical significance to future SIT programmes with this species. Issues related to colony maintenance protocols and the release strategies of sterile males can now be better addressed.

2.3.2 Field cage studies

In medfly, the use of field cages for assessing sterile fly quality and other mating characteristics has been invaluable in the development of meaningful quality control protocols. In tsetse this was missing, however recent experiments with a field cage have indicated that also in tsetse a similar system might be developed. Mating behaviour parameters have traditionally been observed in 20cm∅x5H production cages or in perspex tubes, 20cm∅x55H, with netting at either end. In these systems the flies are restricted to a limited space and lack freedom to make choices. To improve this situation we investigated the use of a large walk-in cage for mating behaviour studies as well as for other quality control procedures associated with tsetse mass rearing.

Table 12. Mating propensity of *G. pallidipes* in a field cage

Male Age	Male Treatment	Mating Propensity
14	irradiated	0.31
14	un-irradiated	0.39
12-14	irradiated, pupal chilling	0.47
15	irradiated, pupal chilling	0.40
15	un-irradiated	0.33
15	un-irradiated	0.73

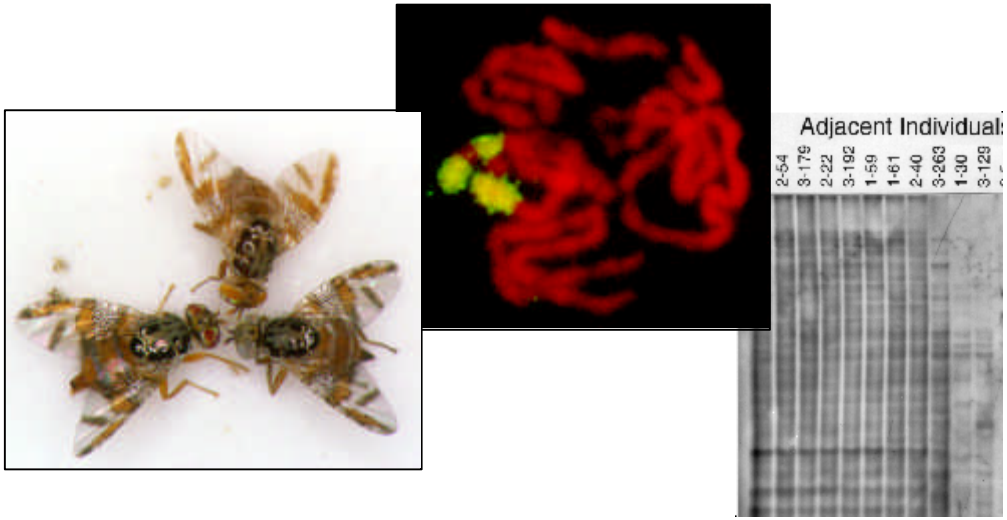
The initial investigations were conducted using the same cage arrangement as that currently in use for medfly mating studies. The cage is cylindrical, with flat floor and ceiling (2.9m∅x2.0mH) and contains potted citrus trees. The cage is made of netting and is erected inside a green-house **(Figure 23, 1998 Annual Report)**. Temperature was maintained between 23-27°C and the relative humidity and light intensity were monitored from 1100h to 1300h on the day of the experiments. Relative humidity ranged from 60 to 80%, atmospheric pressure was between 1019 and 1027hPa and all observations were made under natural light between 4000-6000Lux. Male and female flies used in the investigations emerged from pupae put through the SSPC procedure. Some male flies were used that had been cooled to 15°C for 24, 48 or 72 hours. Virgin females were first introduced into the cage immediately followed by an equal number of virgin males. The flies had been fed the previous day. The time when a mating pair was observed was recorded in minutes from the initial release. Observations were initially made from outside the cage by noting the mating pairs as they formed and if possible recording the duration of copulation. Subsequently all observations were made inside the cage. Some of the females that mated were dissected to estimate the amount of sperm and accessory gland fluid transferred. Following release most flies immediately flew upwards to the roof of the cage with a few landing on the citrus trees and walls of the cage. In some instances immediate mating strikes were recorded but frequently the flies would sit in one position preening themselves before attempting to mate. Males made more frequent short flights in the cage than females, which spend most of their time in one position. Some of the flights were directed at trying to find an escape route. The majority of successful mating pairs were formed at least thirty minutes after initial introduction of the flies into the cage. Mating pairs stayed in the same position until they uncoupled unless they were disturbed. A high pitched whistling sound could be heard prior to a male flying from its resting position and coupling with a female to initiate copulation. The significance of this sound is unknown. The mating propensity of the

females in the different experiments is shown in **Table 12**. At least a third of the females were observed to have mated within two hours. This level of mating propensity in the field cage will enable the effect of different treatments and rearing procedures on sterile male competitiveness to be readily assessed. It is remarkable that such a high level of mating occurred in the cage where the males sought and found females freely. There appears to be something that triggers the mating responses and with detailed observations from within the cage more information can be gained that will contribute to efficient application of SIT. The significant effect of pupal treatment on the time males successfully mate **(Table 13)** after introduction into the cage can be investigated further to determine the effects of such treatments on male competitiveness. There are no significant pairwise differences among means with same letters (P>0.05;LSD). These initial observations in the field cage open up prospects of validating laboratory results for application in SIT programmes in a situation that allows more space to the flies. For tsetse species that naturally do not favour humans as sources of food, observations can be done from within the cage.

Table 13. Some mating parameters of *G. pallidipes* in field cages

Pupal Treatment	Spermathecal Fill	Mean Mating Start Time	Mean Duration of Mating
Control	0.62±0.07	78.2±6.2a	33.4±??ab
15°C for 24h)	nd	63.1±8.5ac	36.8±4.0ab
15°C for 48h	nd	81.6±7.7abc	31.7±5.5ab
15°C for 72h	0.22±0.1	58.9±8.6c	29.2±1.5b
15°C for 72h	nd	99.7±8.0b	40.0±6.7a

3. Research On Medfly Genetic Sexing Strains



R and D, training and services provided by the medfly group continue to be focused on the use of genetic sexing strains for medfly SIT. All the major rearing facilities, with the exception of the plant in Mexico, now use these strains. There has been concern expressed by some of the customers that the genetic background of the current strains is too narrow and may compromise field fitness, although there is no field evidence for this. In order to address this problem a new strain was constructed which contains a mixed genome from 8 different field populations. This strain was evaluated in the mass rearing facility as well as in a field cage environment. Mass rearing characteristics were clearly better than previous strains although this was probably due to a different chromosomal translocation being used. No improvement in field cage mating competitiveness could be demonstrated.

The introduction of new genetic material into current genetic sexing strains is not always straightforward. The introduction of a chromosomal inversion into the strain will greatly simplify the procedure and add to the stability of the strain. During 1999 many chromosomal inversions were induced and isolated. Although the most appropriate inversion has not yet been isolated, it will now be possible to confirm the effects of the inclusion of an inversion on strain stability. Collaboration on the isolation of new inversions is continuing with colleagues in Patras and Guatemala City.

The isolation of the Maleness factor from medfly continues to present a challenge. This gene is being isolated in order to develop genetic sexing strains using transformation. The challenge is both technical and conceptual. The location of this factor is known to be on the Y chromosome where it is probably the only active gene and is surrounded by repetitive sequences. This gene has to be isolated from the other "junk DNA" using technically demanding protocols. Fortunately the position of the gene has enabled the majority of this DNA to be easily excluded from the further analysis. In 1997, transformed medfly lines were developed in Seibersdorf and are currently undergoing stability testing. In 1999 a grant application was submitted to the European Union together with other institutes to develop risk assessment protocols for transformed medflies.

3.1. Stability of gGenetic Sexing Strains

3.1.1. Genetic Recombination in Medfly Males.

For the practical application of genetic sexing strains (GSS), stability is very important, i.e. the sexing system should not deteriorate with time. All current GSS are based on Y-autosome translocations and two different genetic phenomena are known that lead to an uncoupling of the selectable marker from the male determining Y chromosome. Type 1 involves male recombination between the translocated and the free autosome and Type 2 involves male recombination between the two translocated Y-fragments **(Figure 5)**. This report focuses on type 1 recombination and genetic means to reduce its occurrence. The classical form of genetic recombination occurs during female meiosis. For example, recombination between two markers on chromosome 5, *white* (*w*) at position 47C/D on the left arm and *white pupae* (*wp*) at position 59B on the right arm **(Figure 6)** occurs with a frequency of approximately 50%, i.e. the two mutations behave as if they are on different chromosomes. Recombination in male medflies is significantly less than in females. Consequently, large numbers of flies have to be analysed in order to obtain meaningful results. **Table 14** shows the male recombination frequency in the same *w-wp* interval. Males from five different wild type strains (KEMMT is a mixture of 5 strains) were crossed with *w wp* females and the resulting F1 males were back-crossed with *w wp* females. The recombination frequency varies between 0.11 and 0.27% with an average of 0.18%. In GSS males carrying a Y-autosome translocation with a breakpoint on chromosome 5, *w* and *wp* recombine at a frequency of 0.084%, i.e. recombination is reduced by a factor of two (data not shown).

Figure 5. The two types of male recombination in medfly GSS

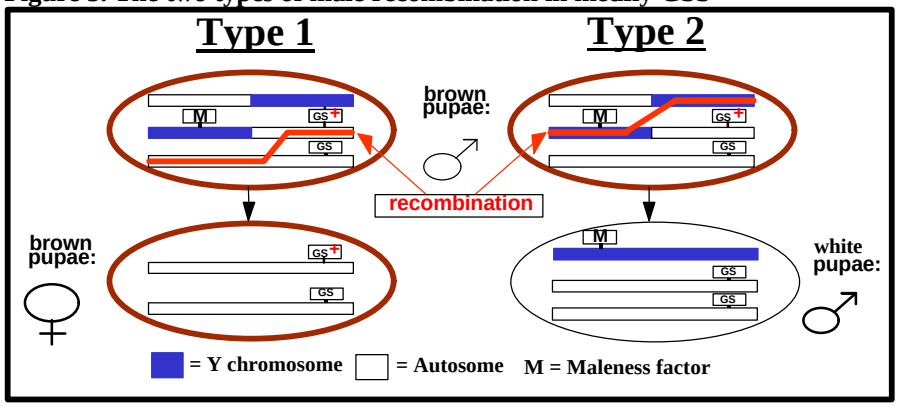


Table 14. Effect of genetic background on male recombination in medfly

Strain	Parental Types	Recombinant Types (%)
EgII	22693	49(0.22)
Mendoza	23751	45(0.27)
Kenya	25450	28(0.11)
Toliman	9781	14(0.14)
Madeira	20214	36(0.18)
KEMMT	31144	43(0.14)
TOTAL	133033	235(0.18)

Figure 6. A schematic of chromosome 5 in medfly

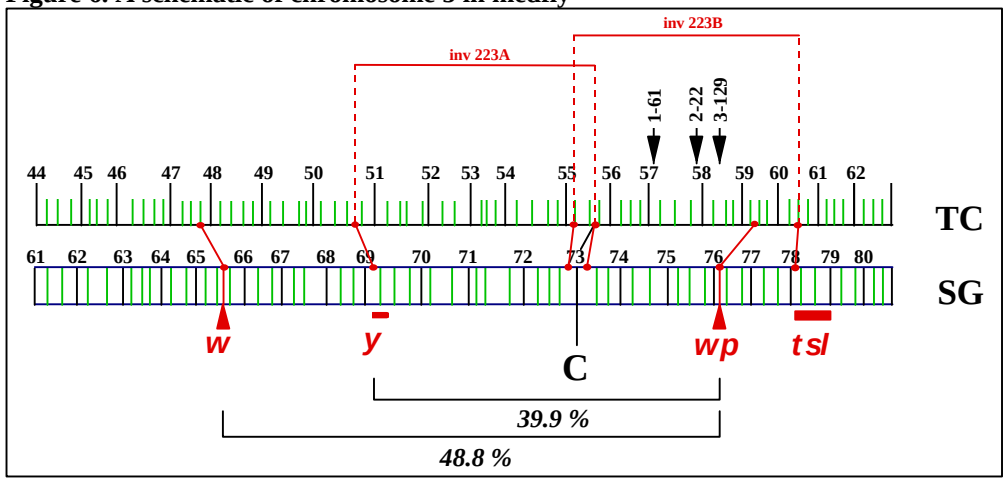
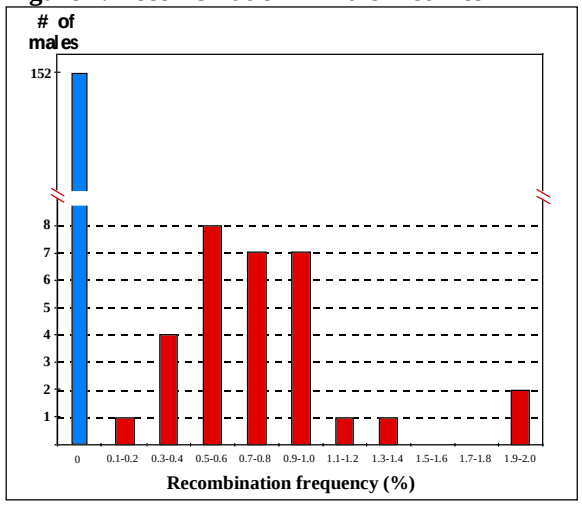


Figure 7. Recombination in male medflies



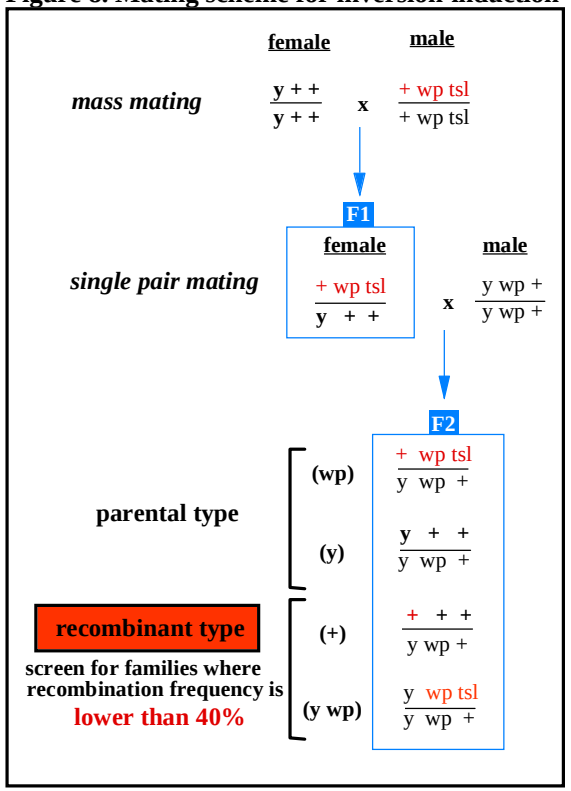
In *Drosophila*, male meiosis is achiasmate and recombination occurs pre-meiotically as recombinants were shown to occur in clusters. An experiment was designed to see if the same system is present in medfly. Among 282 single male crosses analysed, the vast majority did not show any recombinants although on average 547 F2 flies were screened per cross. Only in the offspring of 31 males (11%) were recombinants detected **(Figure 7)** and in these families the frequency was rather high reaching in two cases 2%. This strongly suggests that also in medfly male recombination is clustered and is, therefore, presumed to be pre-meiotic.

3.1.2. Induction of Chromosome Inversions

Individuals heterozygous for a chromosome inversion produce fewer recombinants due to one of two reasons. Firstly, recombination within the inversion can lead to embryo lethality and/or non-transmission of the recombinant chromosome to the offspring or secondly physical pairing between the inverted and the non-inverted chromosome is reduced leading to reduced genetic exchange. To be useful in the stabilisation of *tsl* based GSS an inversion must be viable as a homozygote and cover at least the chromosomal region containing *tsl* and the Y-autosome translocation breakpoint.

A first attempt to isolate inversions was reported in the Annual Report 1996. Inversions were induced by exposing pupae, one day before adult emergence, to 25Gy of gamma radiation followed by the appropriate test crosses (**Figure 8**). In this first screen 3 inversions were found but none of them fulfilled the requirements mentioned above. However, one of them, Inv223A:50D-55C, was used successfully as a starting point for the construction of a balancer chromosome (Dr. Zacharopoulou, Univ. Patras Greece) in which the whole of chromosome 5 is now protected against recombination. This is a very valuable tool for mutagenesis screens but it cannot be used to stabilise GSS as it is not viable as a homozygote.

Figure 8. Mating scheme for inversion induction



Here we report the results of a second screen conducted in collaboration with Dr. Zacharopoulou and Dr. Caceres of Moscamed, Guatemala. The screen is based on the mutations *yellow body* (*y*) and *wp*, i.e. the reduction of female recombination in this interval is used as an indicator for the presence of an inversion. The results of the genetic screen are summarized in **Table 15**. In total 1200 crosses with single F1 females carrying an irradiated chromosome were set up and out of these 876 (73%) produced offspring. In the F2 a total of 26742 flies was screened and among these 40.8±1.4% were recombinants. This is very close to the published recombination frequency of 39.9% in the *y*-*wp* interval. In only one family (D53) was a striking and reproducible reduction in recombination frequency detected.

Table 15. Inversion screen using *y* and *wp*

dosage ra				dosage ra				dosage ra				dosage ra				dosage ra				dosage ra			
males cro				males cro				males cro				males cro				males cro				males cro			
sterility: 5				sterility: 6				sterility: 6				sterility: 6				sterility:				sterility:			
single pai				single pai				single pai				single pai				single pa				single pa			
producing				producing				producing				producing				producin				producin			
F2 flies c				F2 flies c				F2 flies c				F2 flies c				F2 flies c				F2 flies c			
average r				average r				average r				average r				average r				average r			
families s				families s				families s				families s				families s				families s			
#	n	% rec	cytol.	#	n	% rec	cytol.	#	n	% rec	cytol.	#	n	% rec	cytol.	#	n	% rec	cytol.	#	n	% rec	cytol.
95	62	17.7	T(4;5)	10	35	28.6	normal	52	6	0	normal	9	7	0.00		98	7	14.29	NA	79	11	9.09	normal
109	123	25.2	normal	42	3	0	normal	61	7	0	normal	20	13	15.38						91	16	12.50	T(3;5;?)
				51	41	24.4	normal	131	5	20	normal	52	5	20.00						125	147	5.40	died
				116	5	0	normal	161	6	16.7	72C-73A	53	87	0.00	50D-59D								
				129	28	7.1	normal	162	18	11.1	normal	54	22	9.09									
				141	17	29.4	normal					133	22	13.64	NA								
				150	8	25	X-5 ?																
				166	30	23.3	normal																

Table 16. Effect of temperature on survival of 13 D53 single pair families

°C	1	2	4	5	6	7	11	12	13	15	16	17	18	tsl	EgII
% EGG HATCH (relative to 25°C)															
31	90.7	99.5	87.8	98.1	96.8	96.5	98.7	87.1	81.9	96.8	90.5	83.3	99.6	88.6	94.6
32	63.9	86.2	79.7	88.0	86.1	71.8	89.0	87.2	61.0	65.1	84.1	74.2	94.2	56.2	95.9
33	10.5	19.0	26.0	51.7	21.3	46.9	30.4	52.5	0.19	4.27	5.63	2.72	35.7	17.8	96.9
34	0.72	3.21	2.70	6.93	1.10	5.54	3.21	5.30	0.00	0.36	1.02	0.34	1.92	1.10	94.5
35	0.00	0.69	3.24	2.94	0.21	4.28	2.27	5.30	0.00	0.18	0.51	0.00	1.92	0.04	90.4
% PUPAL EMERGENCE (relative to 25°C)															
31	71.4	65.0	62.3	77.6	85.6	83.6	90.1	86.2		56.4	95.1	71.9	74.2	97.4	74.0	94.2
32	27.6	43.1	55.0	54.5	43.9	48.3	63.4	58.6	35.6	28.2	23.9	39.5	74.8	47.9	83.4
33	1.23	3.90	3.90	12.7	2.18	16.9	4.88	14.3	0.00	0.19	0.00	1.12	11.1	14.3	80.4
34	0.00	0.26	0.28	1.22	0.00	0.52	0.41	0.22	0.00	0.00	0.00	0.00	0.71	1.50	82.3
35	0.00	0.00	0.28	0.49	0.22	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	53.8

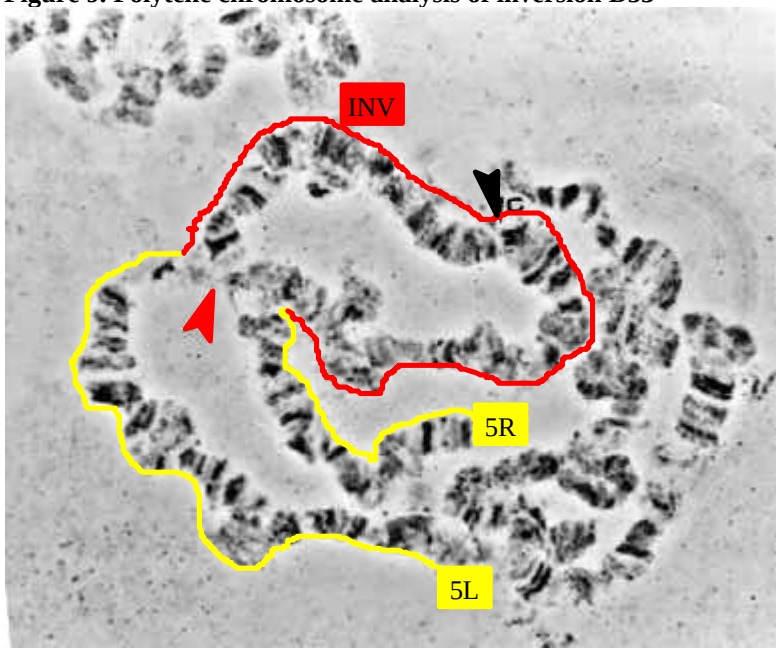
family still carries *tsl* is complex. All families clearly showed a temperature dependent lethality, i.e. they behaved very similar to the standard *tsl* strain but very differently from EgII. However, 10 of the families still showed some egg hatch at the critical temperature of 35°C. At the pupal and adult stage, 9 and 10 families respectively showed complete lethality at 35°C. For the construction of a strain homozygous for D53 it is sufficient to find a single family that is clearly homozygous for the *tsl* mutation. However, it is very difficult to estimate the recombination frequency between *wp* and *tsl* or, more importantly to judge whether *tsl* is included in the inversion. The cytological analysis of D53 showed that the breakpoints of the inversion are at 50D and 59D on the trichogen map, and 69A and 76B on the salivary gland map **(Figure 9)** therefore *y* and *wp* are included in the inversion but not *tsl*.

*3.1.3. Suppression of Female Recombination***.**

The ability of inversion D53 to suppress recombination was confirmed by crossing *y* wp^+/*y* wp^+ females with homozygous D53 males (y^+ *wp*/y^+ *wp*). The resulting F1 females were back-crossed with males homozygous for *y* and *wp*. Among 31,902 F2 flies scored, 20 recombinants were detected which is 0.063% compared with 40.8% with the normal chromosome. This confirms that the inversion really does reduce recombination. If the reduction in recombination

Following the isolation of D53 it was required to be made homozygous. During this process the irradiated *wp tsl* chromosome is maintained in heterozygous females where, if the inversion does not cover the *wp-tsl* region, recombination will lead to the loss of *tsl*. Consequently, each family had to be individually tested for the presence of *tsl* by heat treatment of eggs for 24 hours. The results for 13 families are shown in **Table 16** together with the standard *tsl* strain and a wild type strain (EgII). The decision as to which

Figure 9. Polytene chromosome analysis of inversion D53



frequency is solely caused by the lethality of the recombinant chromosomes, there should be an increase in lethality corresponding to the decrease in recombination frequency. **Table 17** shows the lethality at different developmental stages. At any developmental stage the % lethality is lower than the % reduction in recombination, i.e. at the adult stage, lethality is increased only by 27.3% while the reduction in recombination frequency is 40.7%. It is possible that the difference of 13.4% represents the second mechanism by which inversions reduce recombination, i.e. reduced pairing of chromosomes. The same phenomenon was observed during the construction of the balancer chromosome where the addition of more inversions did not significantly increase lethality.

Table 17. Lethality in females heterozygous for D53

Strain	% Egg Hatch	% Pupae	% Adults
D53	71.4	58.8	58.1
EgII	92.6	87.0	85.4
Difference	-21.2	-28.2	-27.3

*3.1.4. Suppression of Type 1 Recombination in Males***.**

The initial analyses of the inversion D53 were done in females as in this sex recombination is high and any reduction is easier to measure. However, for the stability of GSS only the recombination in males is relevant. To measure the effect of D53 on type 1 recombination in males, crosses as shown in **Table 18** were carried out. The results (**Table 18**) show that without the inversion the recombination frequency is 0.1076% whilst with the inversion the frequency drops to 0.0016, a reduction of about 97%.This is the first direct evidence that inversions will reduce recombination in male medflies and it suggests that they will make a significant contribution to stability of GSS.

Table 18. Effect of inversion D53 on male recombination

Mating Cross	Parental Types	Recombinant Types (%)
Without inversion:		
y females x *wp* males; F1 males x *y wp* females	32295	24(0.0743)
wp females x *y* males; F1 males x *y wp* females	29883	43(0.1437)
TOTAL:	62178	67(0.1076)
With inversion:		
y females x D53 (*wp*) males; F1 males x *y wp* females	30806	0(0.0)
D53 (*wp*) females x *y* males; F1 males x *y wp* females	31052	1(0.0032)
TOTAL:	61858	1(0.0016)

*3.1.5. Third Screen for Inversions***.**

A new screen was carried out using a new mutation named *Sergeant 2 Sr*^{2} This mutation is located *ca* 20 recombination units distal of *wp* and is in a much more suitable position for a screening for inversions in that region of chromosome 5 than the original mutation used, *y*. For this third screen it was decided to re-irradiate inversion D53. **Table 19** summarizes the results obtained from 200 single female crosses out of which 189 produced offspring. Four families (group 1) produced unrealistically high recombination frequencies (9-60%), 15 families (group 2) showed recombination frequencies between 0.55% and 2.27% whilst 170 families (group 3) produced no recombinants at all. The overall recombination frequency for the three groups is 0.277%, clearly far lower than the expected value of 20%. From group 3, 50 families were chosen at random and analysed cytologically. However none of them contained an inversion in addition to the original D53.

Table 19. Recombination in irradiated D53 families

No. families Tested	Ave. No. Counted per Family	Parental Types	Recombinant Types	% Recombination
#1 4 families	32	89	40	31.01
#2 15 families	129	1970	19	0.96
#3 170 families	113	19205	0	0
#2+3 185 families	115	21175	19	0.09
#1+2+3 189 families	113	21264	59	0.277

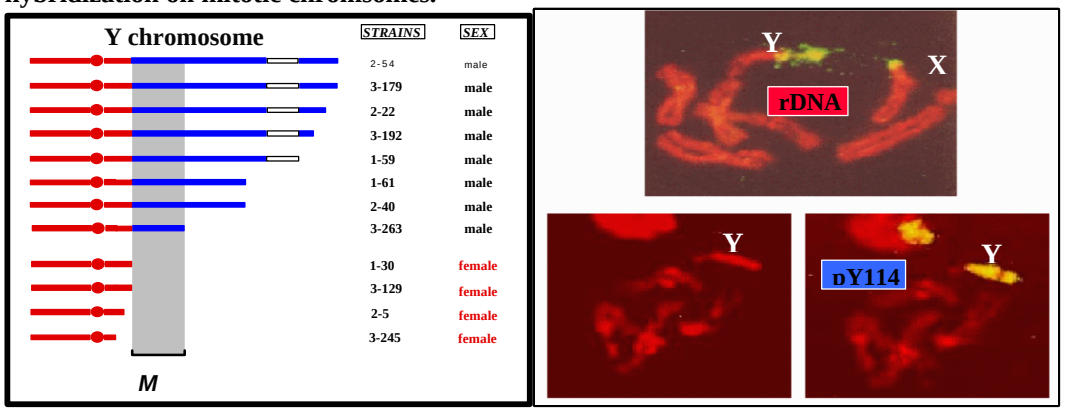
It is concluded that D53 has a very strong suppressive

effect on recombination in chromosome regions outside the inversion. It is possible that either the pairing of the inverted region causes structural constraints on the neighbouring regions or that the overall pairing of inverted and non-inverted chromosomes is reduced. It is clear that the reduced recombination in the chromosomal region adjacent to D53 makes it impossible to screen for new inversions in this region via recombination suppression.

3.2. Isolation of the Maleness factor from Medfly

In the medfly the male sex is determined by a dominant factor located on one of the sex chromosomes, the Y chromosome, as with other higher Diptera such as the house fly (*Musca domestica*) and the sheep blowfly (*Lucilia cuprina)*. In medfly the approximate location of this factor was determined by deletion mapping (**Figure 10**) by analysing the segregation behaviour of Y-autosome translocations. Using *in situ* hybridization with a short repetitive DNA probe (pY114) the Maleness factor was located in the proximal third of the long arm. The remaining part of the Y chromosome can be deleted without any obvious consequences. In general the long arm is believed to contain primarily repetitive sequences, i.e. very few essential genes. This would explain why the length of the long arm is very different between different fruit fly species.

Figure 10. Deletion mapping of the Maleness factor. Left: schematic drawing of different deleted Y chromosomes. Probes used are rDNA (red) and pY114 (blue). Right: *in situ* **hybridization on mitotic chromsomes.**

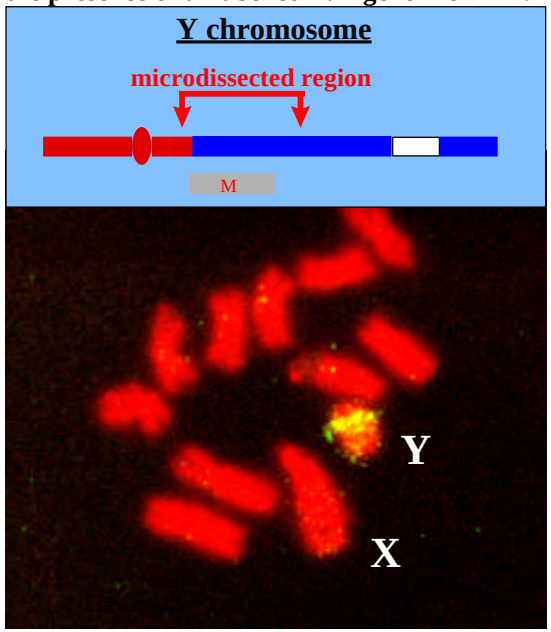


3.2.1. Micro-dissection of Y Chromosomal Material

The mapping information was used to micro-dissect the relevant region from mitotic Y chromosomes. It is estimated that these sequences represent approximately 50-100 Mbp of the Y chromosome. Using the micro-dissected material as a probe, it was shown that it hybridizes to the correct region of the medfly Y chromosome, i.e. to the proximal third of the long arm (**Figure 11**), to the medfly X chromosome and with less intensity and to one arm of the *C. rosa* Y chromosome. The cross hybridization between X and Y chromosomes was expected because the two sex chromosomes should show a certain degree of sequence homology due to their common evolutionary origin. The cross hybridization between the two species shows that at least some parts of the Y chromosome are conserved during evolution. In contrast, the repetitive probe pY114 hybridizes only to the medfly Y chromosome and there preferentially to the distal two thirds of the long arm. Material was also micro-dissected from the terminal region of the X chromosome (medX) in addition to that from the Y chromosome (medY) for amplification through DOP-PCR. This procedure amplifies sequences at random and does not selectively increase the frequency of certain sequences.

Four stable medfly strains are available where the Y chromosome is deleted to different degrees. These strains were generated as a consequence of type 2 recombination in GSS. In the most extreme case (Family 18) the long arm is the same length as the short arm, i.e. a large

Figure 11. *In situ* **hybridization with medY in the presence of unlabelled X/X genomic DNA.**



proportion of the long arm has been lost. This strain is fully fertile and can be reared without any obvious problems. The availability of such strains is of great practical importance as it allows the easy isolation of large amounts of RNA and DNA for analysis which contain different amounts of material from the Y chromosome.

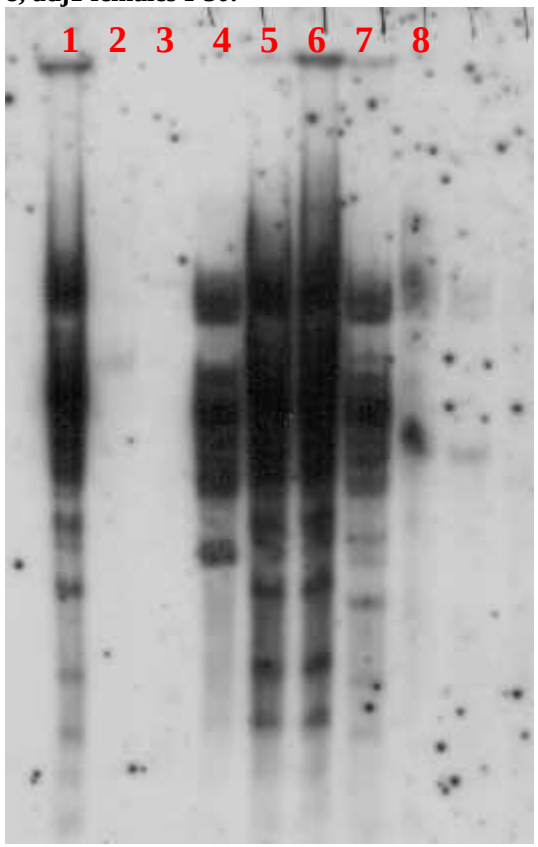
3.2.2. Current Status

3.2.2.1 Micro-dissected Y material:

As mentioned above, it is estimated that the micro-dissected material from the Y chromosome (medY) represents *ca* 50-100 Mbp of DNA. It follows that only a very small fraction therein represents the Maleness factor (less than 0.1%). The remaining material consists probably of more or less highly repetitive sequences. As there are currently no direct ways to isolate the Maleness factor from the micro-dissected material, indirect approaches have to be used, i.e. approaches where the repetitive sequences are removed and, thereby, an enrichment for the Maleness factor is achieved. The medY DOP-PCR products (average length *ca* 200-500 bp) were cloned into the plasmid vector pUC8 and 78 different clones were studied of which 45 contained an identifiable insert and these were blotted onto nitro-cellulose membranes following electrophoresis. The blots were probed with medX in an attempt to eliminate clones that contain stretches of DNA common to both the Y and the X chromosome. However, all clones hybridized with medX although to different degrees.

For further analysis the clones were divided into different pools, one pool with clones that gave a weak signal with medX and four pools with those clones that gave a strong signal. The pools were labelled and used separately to probe Southern blots containing EcoRI digested genomic DNAs from the following strains: a) EgII wild type males, b) EgII wild type females, c) Fam 18 (very short Y chromosome) males, and d) *C. rosa* males. The pool containing clones with weak homology to medX generated many bands, i.e. a result typical for highly repetitive sequences. The pattern generated by the other four pools was very different however similar between themselves. The number of bands was much lower but no sex specific differences were detected. In order to remove unwanted repetitive and non-Y-specific sequences from the medY library a subtractive approach was chosen utilizing the Clontech PCR Select kit. The following DNAs were used as drivers: a) rDNA, b) pY114, c) the combined 5 pools from step1, and d) either medX or total female genomic DNA. As a result, two subtracted medY pools are available, medY-XX (driver, medX) and medY-FF (driver, female genomic DNA). It is planned to clone these DNA sequences into plasmid vectors and also use them to screen a genomic library (see below). When medY-FF is labelled and used to probe genomic DNA from different origins, medfly females and *C. rosa* males show very little hybridization, i.e. medY-FF contains only Y-specific sequences from medfly (**Figure 12**). Furthermore, medY-FF contains sequences found primarily in the distal part of the long arm of the Y chromosome as with strain 1-30, which carries a deletion of almost the entire long arm, the pattern is only marginally changed **(Figure 12).**

Figure 12. Southern hybridization using labelled medY-FF (1, EgII males; 2, *C. rosa* males; 3, Eg11females, 4, Fam 18 males; 5-7, adj1 males from strains 3-263, 2-22 and 2-40; 8, adj1 females I-30.



3.3.2.1 Strain with deleted Y chromosome (Fam 18):

In parallel to the above approach, a second strategy was used that is based on the availability of a strain with a major deletion of the long arm of the Y chromosome (Fam18). Genomic DNA from this strain was isolated and a library was constructed. This library will be screened with the following probes: a) rDNA, b)pY114, c) medY-XX and d) medY-FF. The aim is to identify genomic clones that can be used as landmarks for the cloning of the Maleness factor. Firstly, clones will be identified from the relatively short region of the long arm that hybridize with both rDNA and pY114 that probably represent the proximal boundary of the Maleness factor region. Secondly, clones from the Maleness factor containing region that hybridize only with pY114 and which could serve as an entrance point for the further dissection of this region. Thirdly, clones isolated by screening with medY-FF and medY-XX would be pooled and used as probes for Northern blot analysis to identify clones containing transcribed genes.

4. Appendices

4.1 Publications

Cayol, J.P., World-wide sexual compatibility in Medfly, *Ceratitis capitata* Wied., and its implications for SIT, In "Area-Wide Management of Fruit Flies and Other Major Insect Pests" (TAN, K.H., Ed.), Universiti Sains Malaysia Press, Penang (in press).

Cayol, J.P., Wornoayporn, V. Trials assessing dry biowaste material to reduce costs of medfly (*Ceratitis capitata* Wied.) adult and larval diets. Working Group on Fruit Flies of the Western Hemisphere, Guatemala City, 4-9 July, 1999. Poster

Cayol, J.P., Zarai, M., Field releases of two genetic sexing strains of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) in two isolated oases of Tozeur Governorate, Tunisia, J. Appl. Ent. **123** (1999) 613-619.

Cayol, J.P., Vilardi, J., Rial, E., Vera, M.T., New indices and method to measure the sexual compatibility and mating performance of *Ceratitis capitata* (Diptera: Tephritidae) laboratory-reared strains under field cage conditions, J. Econ. Entomol. **92** 1 (1999) 140-145.

Coronado, P., Vijaysegaran, S., Robinson, A.S., Hendrichs, J., Wegensteiner, R. Mouthpart structure and feeding mechanisms of adult medfly (*Ceratitis capitata* Weid.) Working Group on Fruit Flies of the Western Hemisphere, Guatemala City, 4-9 July, 1999. Poster

Caceres, C., Fisher, K., Rendon, P., Mass rearing of the medfly temperature sensitive lethal genetic sexing strain, In "Area-Wide Management of Fruit Flies and Other Major Insect Pests" (TAN, K.H., Ed.), Universiti Sains Malaysia Press, Penang. (in press)

Fisher, K., Genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae): II. Quality of high temperature treated, mass reared temperature sensitive lethal strains J. Econ. Entomol. (1999), (in press).

Fisher, K., Caceres, C., A filter rearing system for mass reared medfly. In "Area-Wide Management of Fruit Flies and Other Major Insect Pests" (TAN, K.H., Ed.), Universiti Sains Malaysia Press, Penang (in press).

Franz, G., The "combi-fly concept" revisited: how much radiation is required to sterilize males of a genetic sexing strain? In "Area-Wide Management of Fruit Flies and Other Major Insect Pests" (TAN, K.H., Ed.), Universiti Sains Malaysia Press, Penang (in press).

Franz, G., Fisher, K. Comparison of two phenomenon leading to instability of genetic sexing strains in the medfly (*Ceratitis capitata*). Working Group on Fruit Flies of the Western Hemisphere, Guatemala City, 4-9 July, 1999. Poster

Gariou-Papalexiou, A., Yannopoulos, G., Robinson, A.S., Zacharopoulou, A., Polytene chromosome maps and RAPD polymorphisms in *Glossina austeni*. In "Area-Wide Management of Fruit Flies and Other Major Insect Pests" (TAN, K.H., Ed.), Universiti Sains Malaysia Press, Penang (in press).

Katsoyannos, B.I., Papadopoulos, N.T., Hendrichs, J., Wornoayporn, V., Comparative response to citrus foliage and citrus fruit odour by wild and mass-reared sterile Mediterranean fruit fly males of a genetic sexing strain, J. Appl. Ent. (in press).

Opiyo, E., Luger, D., Robinson, A.S., New systems for the large scale production of male tsetse flies (Diptera, Glossinidae). In "Area-Wide Management of Fruit Flies and Other Major Insect Pests" (TAN, K.H., Ed.), Universiti Sains Malaysia Press, Penang (in press).

Opiyo, E., Mutika, G., Robinson, A.S.., Effect of low temperature treatment on *Glossina pallidipes* pupae. In "Proc. 25th Mtg OAU/ISCTRC", Mombasa, 1999. (in press)

Robinson, A.S., Genetic control of insect pests. In Biological and Biotechnological Control of Insect Pests" (RECHCIGL, J.E., and RECHCIGL, N.A. Eds.), 1999, CRC Press LLC, Boca Raton, FL pp 141-170.

Robinson, A.S., Franz, G., The application of transgenic insect technology in the sterile insect technique, In "The Use of Transgenic Insects for Biological Control (HANDLER, A.M., JAMES, A.A., Eds), CRC Press, Boca Raton, FL (in press).

Robinson, A.S., Franz, G., Fisher, K., Genetic sexing strains in the medfly, *Ceratitis capitata*: Development, mass rearing and field application, Trends in Entomology. (in press)

4.2 Travel

Staff member	Destination	Period of absence incl. travel days	Purpose of travel
A.S. Robinson	Gainesville, USA	99-05-09 - 99-05-11 99-05-12 - 99-05-16	ITC INT-5.146 Seminar at CMAVE.
	Pavia, ITA	99-06-09 - 99-06-12	Seminar
	Guatemela City, Antigua, GUA Tapachula, MEX	99-06-28 - 99-07-18	WGFFWH meeting Sci. Sec at RCM D4.10.15
	Arava, ISR	99-07-25 - 99-08-03	Project review ISR/5/009
	Mombasa, KEN	99-09-26 - 99-10-07	ISCTRC meeting and Sci. Sec. at RCM D4.20.05
	Heraklion, GRE	99-11-21 - 99-11-27	Graduate lecture course
	Atlanta, USA	99-12-11 - 99-12-20	Two invited talks at the ESA meeting
K. Fisher	Stellenbosch, SAF	99-05-22 - 99-05-30	Project review SAF/5/002
	Stellenbosch, SAF	99-09-05 - 99-10-08	Project review SAF/5/002
G. Franz	Guatemala City, GUA	99-07-04 - 99-07-11	WGFFWH meeting
	Tapachula, MEX	99-07-12 - 99-07-17	Introduction of genetic sexing strain
	Funchal, POR	99-11-10 - 99-11-14	Present paper at SIT seminar:
E. Opiyo	New Haven, CT, USA	99-05-01 - 99-05-16	Advise on tsetse, discuss CRP D4.20.05
	Addis Ababa, Arba Minch, ETH	99-06-26 - 99-07-18	Project review ETH/5/012
	Mombasa, Nairobi, KEN Addis Ababa, ETH	99-09-25 - 99-10-17	ISCTRC meeting and Project review RAF/5/040:ETH/5/012
V. Wornoayporn	Bangkok, Pathumthani, THA	99-03-27 - 99-04-11	Lecture course at DOAE
	Bangkok, Pathumthani,	99-11-20 - 99-12-11	Project review THA/5/044

4.3 Fellows

NAME	FIELD OF TRAINING	DURATION (months)	FELLOWSHIP PERIOD
W. Kitwika (URT)	Tsetse	12	1998-02-06 to 1999-02-05
H.S. Nyingilili (URT)	Tsetse	6	1998-08-03 to 1999-02-02
A. Masaba Gidudu (UGA)	Tsetse	9	1998-10-01 to 1999-06-30
B.E. Chinsinjila (URT)	Tsetse	1	1999-01-01 to 1999-01-31
A. Beukers (SAF)	Medfly	2	1999-06-28 to 1999-08-27
P. Olet (KEN)	Tsetse	12	1999-10-05 to 2000-10-04
A. Mekonnen Tessema (ETH)	Tsetse	4	1999-11-01 to 2000-02-29
O.S. Chalo	Tsetse	6	1999-11-01 to 2000-04-30

4.4 Shipments of Tsetse Fly Pupae and Blood Diet

1. *Glossina austeni* (colony size 3,500 females)

Dr. Maudlin	16,750	Univ. of Glasgow, UK
Dr. Underwood	13,800	Univ. of Southhampton, UK
Dr. Aksoy	4,850	Yale Univ., New Haven, US
Dr. Zacharopoulou	600	Univ. Patras, GR
Dr. Gariou-papalexiou	600	Univ. Patras, GR
Dr Wetzel	200	Vet. Univ., Hannover, D
Dr. Disco	200	Univ, Munich, D
Dr. McCall	100	Univ. Liverpool, UK

2. *Glossina palpalis* (colony size 2,000 females)

Dr. Ahmed	7,300	NITR, Kaduna, NIG
Dr. Maudlin	6,600	Univ. Glasgow, UK
Dr. Molyneux	4,400	Univ. Salford, UK
Dr. Aksoy	500	Yale Univ., New Haven, US
Dr. Gomez	350	GAEC, Legon, Ghana
Dr. Bausenwein	200	Univ. Regensburg, D

Dr. Wetzel	150	Vet. Univ., Hannover, D.
3. *Glossina brevipalpis* (colony size 1,200 females)		
Dr. Guerrin	10,250	Univ. Neuchatel, CH
Dr. Aksoy	700	Yale Univ., New Haven, US
Dr. Disco	100	Univ. Munich, D
Dr. Wetzel	50	Vet. Univ., Hannover, D
4. *Glossina fuscipes* (colony size 1,400 females)		
Dr. Auerswald	7,750	Univ. Cape Town, RSA
Dr. Makumi	6,300	KETRI, Kikuyu, Ke.
Dr. Aksoy	100	Yale Univ., New Haven, US
5. *Glossina pallidipes* (colony size 14,000 females)		
Dr. Gariou-Papalexiou	2,400	Univ. Patras, GR
Dr. Aksoy	600	Yale Univ., New Haven, US
Dr. Wetzel	50	Vet. Univ., Hannover, D
6. *Glossina morsitans* (colony size 4,000 females)		
Dr. Brun	11,100	Trop. Inst. Basel, CH
Dr. Gibson	7,700	Sch. Biol. Sci., Bristol, UK
Dr. Tait	5,400	Univ. Glasgow, UK
Dr. Maudlin	4,650	Univ. Glasgow, UK
Dr. Gikonyo	3,950	ICIPE, Nairobi, Kenya
Dr. Turner	2,450	Univ. Glasgow, UK
Dr. Aksoy	2,150	Yale Univ., New Haven, US
Dr. Zollner	450	NRI, Chatham, UK
7. Blood Diet		
Dr. Mebrate	325L	ESC, Addis Ababa, ET
Dr. Omondi	500L	KETRI, Kikuyu, KE

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