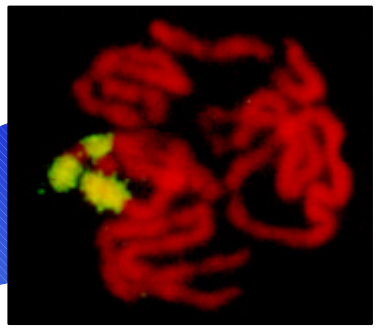
ANNUAL REPORT 1998



Entomology Unit

FAO/IAEA Agriculture & Biotechnology Laboratory Seibersdorf

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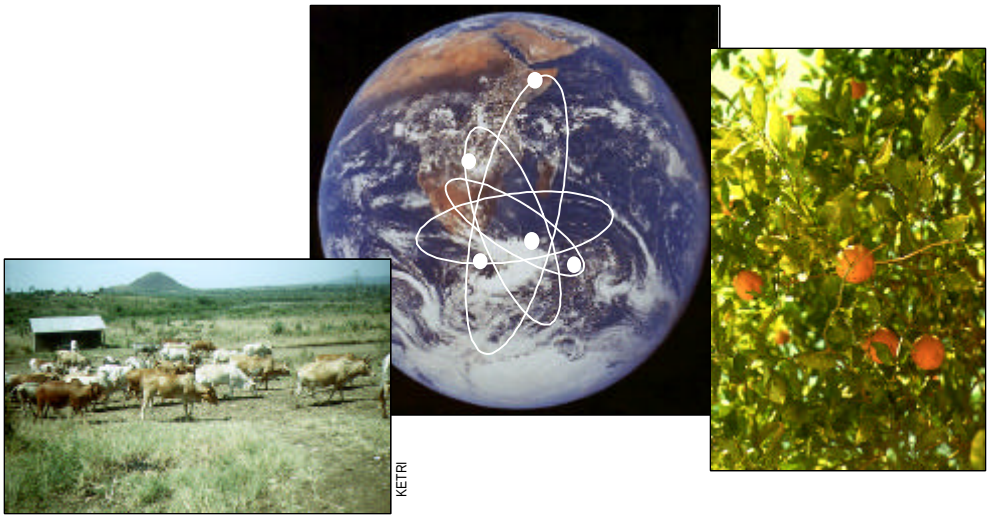
1. Introduction



IN TWO KEY AREAS, *genetic sexing in the medfly,* Ceratitis capitata, *and mass rearing of the tsetse,* Glossina pallidipes, *1998 has been a year of consolidation. For medfly, programme managers now view the use of genetic sexing strains (GSS) as an integral part of the Sterile Insect Technique (SIT) for control and/or eradication of this pest. Since its introduction only 4 years ago, this technology is having an increasing impact on the implementation of medfly SIT. For tsetse, a rearing system has been designed and tested which will form the basic rearing module for the mass rearing facility to be built in Addis Ababa, Ethiopia. The system is cheap, easy to maintain and can be locally constructed. The facility in Addis Ababa will produce sterile* G. pallidipes *males for the SIT component of a large tsetse eradication programme in the Southern Rift valley. The Unit will continue to play a unique role in the further development of these technology transfer programmes.*

In October, G. Mutika from Zimbabwe joined the staff as a Junior Professional Officer for an initial period of one year. He is working in the tsetse group to develop procedures for the handling of male tsetse pupae and adults. In addition, several colleagues from abroad have spent time in the Unit and have made important contributions to ongoing projects. These visits are extremely valuable as they allow both new techniques to be introduced into the laboratory and increased support be provided to selected tasks.

In May, the FAO/IAEA International Conference on "Area-Wide Control of Insect Pests Integrating the Sterile Insect and Related Nuclear and Other Techniques" was held in Penang, Malaysia in conjunction with the Fifth International Symposium on "Fruit Flies of Economic Importance". The Unit staff were well represented, presenting four papers and a poster. Several staff acted as chairpersons and rapporteurs for scientific sessions and conducted satellite Research Coordination meetings (RCM's). All the presentations will be included in the proceedings of the meetings to be published in 1999. All the professional staff of the Unit were able to attend these important meetings and make a significant contribution to their success.

The Unit continues to maintain many strains of tsetse and medfly which are used to supply numerous institutions and individuals with biological material for research work. In addition, medfly GSS are provided to operational SIT programmes as required. This support provided to Member States, especially in the area of tsetse research, appears to be of increasing importance as the number of requests rise each year. All of this material is provided free of charge.

1.1 GENETIC SEXING STRAINS AND THE FILTER REARING SYSTEM

There are now 4 medfly mass rearing facilities using GSS in operational SIT programmes and the use of these strains is now accepted as the norm for medfly SIT. New programmes in South Africa and Australia have also chosen to adopt these strains. The maintenance of quality of GSS during long term mass rearing has been addressed by the implementation of a Filter Rearing System (FRS), the concepts of which were developed at Seibersdorf and presented in the 1997 Annual Report. In 1998 all 4 facilities adopted this system as a way to maintain the genetic integrity of the GSS in the long run. It is likely that the FRS will have a range of application far broader than that required for GSS integrity. It has the potential to revolutionize the concepts upon which mass rearing strategies are developed.

Current thinking related to the maintenance of insect colonies in rearing facilities suggests that strains have to be replaced at regular intervals to maintain a certain level of quality, this also applies to GSS. To ensure that facilities are able to have access to new GSS on a predictable basis it will be necessary to implement a strain development and transfer protocol. In 1998 this was initiated by the construction of a new GSS which incorporates genetic material from many different geographical populations.

1.2 TSETSE MASS REARING

Based on experiences with the original Tsetse Production Unit (TPU) in 1997 a modified version was designed, constructed and evaluated in 1998. The TPU 2 is conceptually and technically simple and requires minimal maintenance, important characteristics to ensure efficient technology transfer. The TPU 2 holds 63 cages which can be simultaneously moved to the blood feeding station. Experiments have now established the appropriate fly density and sex ratio required in the cages and using *G. pallidipes* the TPU 2 has been evaluated. Some modifications in cage design and pupal collection will be necessary before several units can be ordered for further tests in rearing facilities in Africa.

It has also been shown in *G. pallidipes* that flies can be allowed to emerge directly into cages at the correct sex ratio and density without handling. A corollary of this procedure is that it will be possible to develop a very efficient sexing procedure to produce males only for sterilisation and release.

1.3 CONSULTANT'S CONTRIBUTIONS

Dr. Nestel (The Volcani Center, Israel), Dr. Gooding (University of Alberta, Canada) and Dr. Aksoy (Yale University, USA) spent some time in the Unit making important contributions to ongoing projects.

Dr. Nestel and colleagues have developed an assay to measure lipid content in medfly. Lipids are important components of metabolism and low levels can reflect poor larval nutrition and hence poor adult quality. Using the assay, total lipid content of individual medflies from different GSS was measured. The protocol is somewhat time consuming and will have to be developed into an ELISA type assay to be of use for routine quality control of mass reared medflies. If Dr. Nestel succeeds to develop an ELISA based assay then further work will be carried out in 1999.

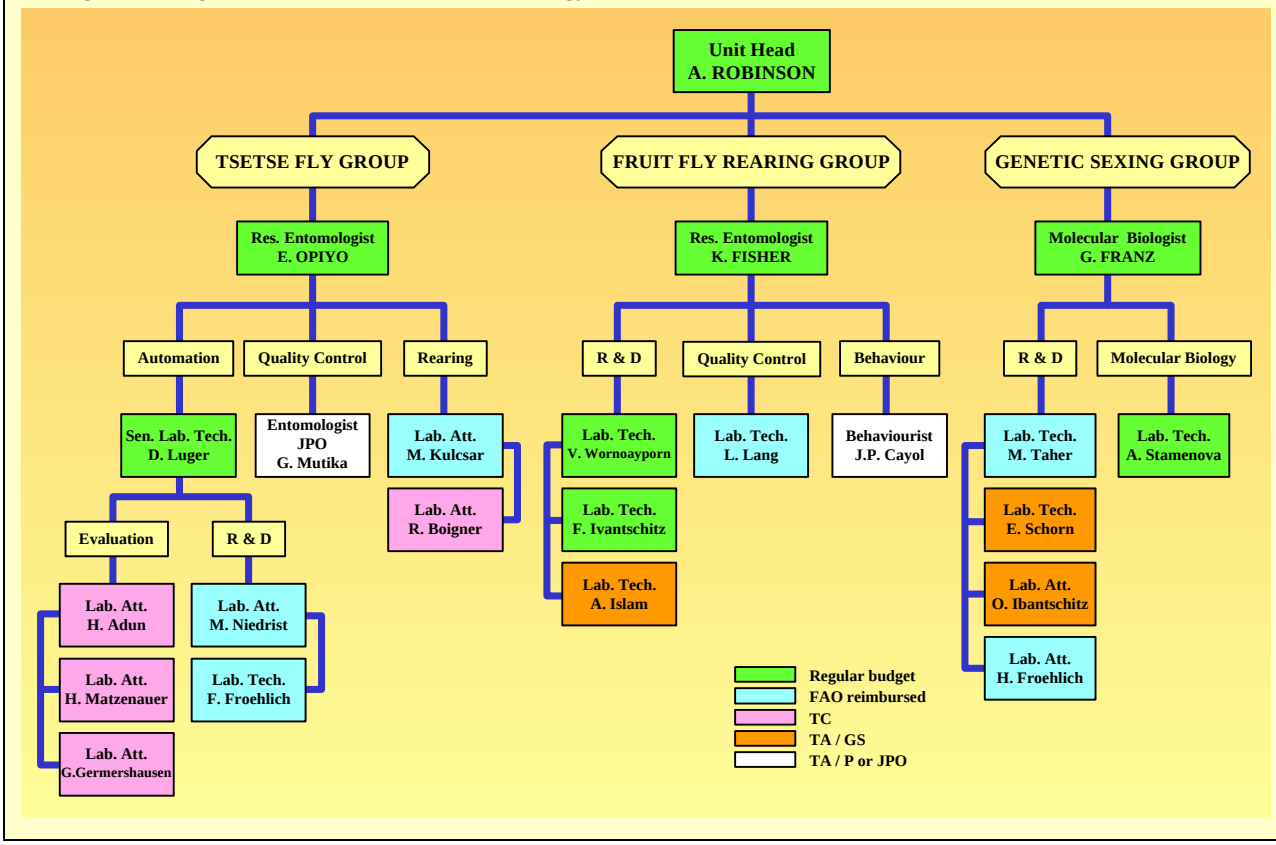
*The first cases of the production of transgenic medfly at Seibersdorf were reported in the 1997 Annual Report. This work was expanded in 1998 to include experiments with new gene-vector systems. Following an intense period of collaboration with colleagues from the USA, this new vector system was shown to be able to transform medfly (see **Section 3.2.2**). The increasing work on transgenic medfly strains necessitated an expansion of the transgenic working area. A plan is now being implemented which will provide an improved facility for this type of work.*

*Data discussed in the 1997 Annual Report indicated that medflies, sampled from populations anywhere in the world, showed no mating incompatibilities with each other. However, an exceptional case was found during a field cage evaluation with a particular GSS and a wild population from Madeira (see **Section 5.1**).*

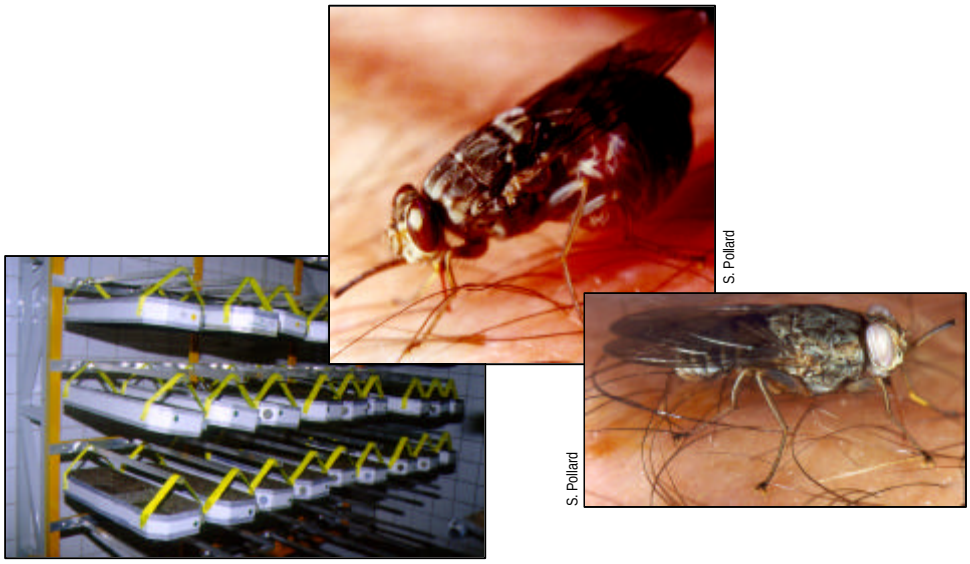
DNA markers are very useful characters to develop genetic maps and in tsetse, PCR (Polymerase Chain Reaction) based primers have been identified at Seibersdorf which can distinguish between populations of the same species. Dr. Gooding utilized some of these primers to try to map a visible marker gene in tsetse. During a sabbatical at Seibersdorf in 1996 he constructed several pedigrees using this gene and in the visit in 1998 he tried to identify primers which are linked to it. Two putative markers were identified.

Foreign genes can be expressed in tsetse by genetically manipulating the essential bacterial symbionts that are present in this species. This procedure has been called para-transformation. Dr. Aksoy is developing this technique with the aim to produce tsetse which cannot transmit trypanosomes. Non-transmitting tsetse would be of great value to any SIT programme. During her stay in the Unit, Dr. Aksoy succeeded in making a cDNA library from the gut of tsetse which she will use to screen for genes involved in trypanosome transmission.

Figure 1. Organizational chart of the Entomology Unit (as of 31 December 1998).



2. Tsetse Research and Development



IN 1998 EMPHASIS *has been placed on developing a system suitable for semi-industrial production of* Glossina pallidipes. *A tsetse production unit (TPU 2) was designed, constructed and is undergoing evaluation. It has already demonstrated that it has the basic requirements needed for holding a large number of cages with flies which can be easily transported for feeding and from which pupae can be collected. Based on this design, a basic module for the tsetse mass rearing facility in Addis Ababa, Ethiopia has been proposed.*

Because of the different mating conditions required by different species of tsetse it was necessary to determine if the rearing procedures which had been developed for G. austeni *were also suitable for* G. pallidipes. *These included: day 0 mating (introducing newly emerged flies directly into cages), sex ratio (number of males and females in the cage), resident males (leaving males permanently in the cage) and self stocking of production cages (allowing flies to emerge directly into the cage). It was also shown that alternate day feeding was not detrimental to performance of* G. morsitans *and* G. pallidipes *and this will go a long way to ease the logistics of mass rearing as only half a colony needs to be fed on any one day. Experiments with different diet components including freeze dried blood indicated the potential and limitations in the use of this type of blood for the maintenance of tsetse colonies.*

Studies have continued on the effect of low temperature treatment on performance of male flies and have been extended to cover mature male pupae with the aim of manipulating the last few days of the pupal period to have better control of male emergence. This study will be further extended to include the chilled adult release system for sterile tsetse.

2.1 EVALUATION OF TPU 2

The first tsetse production unit (TPU 1) was a system that automatically handled cages of flies and brought them for feeding while pupae/larvae were collected centrally, but exhaustive efforts failed to establish a self sustaining colony of tsetse on this unit (see 1997 Annual Report). However, the system did illustrate how large numbers of flies could be efficiently handled and fed. These successful concepts were used in the development of TPU 2.

Figure 2. Tsetse cage holding frame.

TPU 2 comprises a cage holding frame (**Fig. 2**), a pupal collector (**Fig. 3**) and a feeding station (**Fig. 4**). The cage holding frame (228cm x 69cm x 233cm) accommodates 63 cages in 7 shelves. At the time of feeding, the frame is moved to the feeding station into which it locks, the cages are then manually lowered onto the feeding trays and after 15 minutes the cages are raised and the frame is moved back to its original position with the pupal collector. Rectangular cages (20cm x 60cm x 7cm) with fine netting on top and a coarse netting at the bottom are held horizontally on the frame. Movement of cages and hence flies is limited to the time of feeding only. Shelves on the pupal collector provide a surface onto which larvae drop from the cages and then roll into a trough where they pupate. The angle of the shelf is adjustable to allow for its removal during the time of feeding. The final design of the pupal collector will allow pupae to be collected from each of the seven shelves to a central collector.

Figure 3. Tsetse pupal collector.

Figure 4. Tsetse adult feeding station.

2.1.1 Cage Density

Several fly densities were tested in the new cages to establish production criteria. Densities ranged from 260 females to 600 females at a sex ratio of 1 male to 4 females and some cages were tested which had more resting space. A summary of these preliminary experiments is

given in **Table 1** together with data from the standard round cage with 48 females and 12 males. Fly survival was very dependent on density, at 600 females/cage only 11% of flies were alive after 10 weeks compared with 65% in the control. However even at the lowest density survival was low (**Table 1**). These preliminary data were used to set up a more detailed test for the two lowest densities. A set of 9 cages were loaded with 260 females and 65 males per cage and 310 females and 78 males (ratio of 1 male to 4 females) and monitored for survival and productivity during a period of 13 weeks. A density of 260 females and 65 males is equivalent to the density/volume in the standard 20 cm diameter cage which holds 48 females and 12 males. Flies were fed 5 days a week and mortality was monitored using two cages from each density on a weekly basis starting at week 2. Pupae produced were collected each day, counted and a sample taken for size determination

Table 1. Performance of *G. pallidipes* in TPU 2 at different densities (week 10).

Density of /cage	% Survival	PPIF
600	11.00	0.39
450 *with inserts in cage*	4.22	0.67
450	10.67	0.53
360	16.39	1.10
260	29.81	1.96
48 *in standard cage*	64.58	2.19

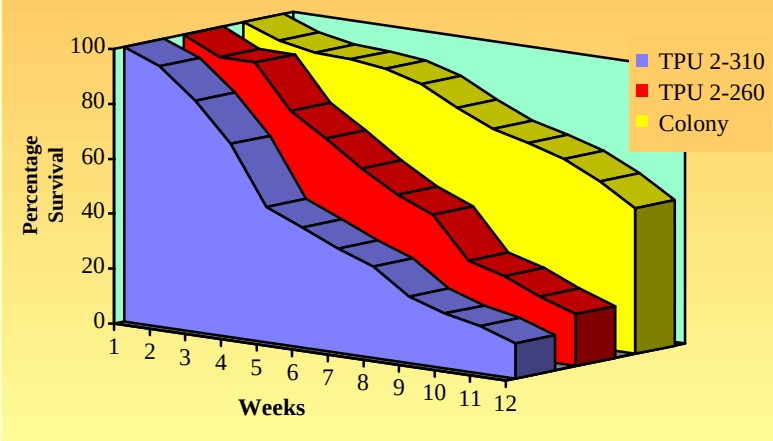
2.1.2 Survival

Cages with 260 females and 65 males demonstrated higher survival compared with those of 310 females and 78 males. However, the survival was still below that of the standard colony where the daily mortality was 0.54% compared with 0.89% for cages with 260 females and 0.95% for 310 females. A closer look at the mortality showed an equivalent survival till week 4 after which there is a faster decline in cages with higher densities (**Fig. 5**). This coincides with the period when females drop the first larva. It was also noticed that the distribution of flies in the cages was not uniform with significant crowding taking place which was dependent on the light direction. The provision of a uniform light distribution will be very important to ensure a better fly distribution and hence survival.

2.1.3 Productivity

Productivity was measured as pupae produced per initial female (PPIF). Cages with 260 females had a PPIF of 2.24 compared to 1.18 for cages with 310 females and 3.5 for the standard cage. Evaluation is continuing to determine the conditions under which a self sustaining colony can be maintained on TPU 2. So far the principle of automatically holding and feeding a large number of cages has been demonstrated. Emphasis is being placed on determining factors that ensure good survival and therefore production.

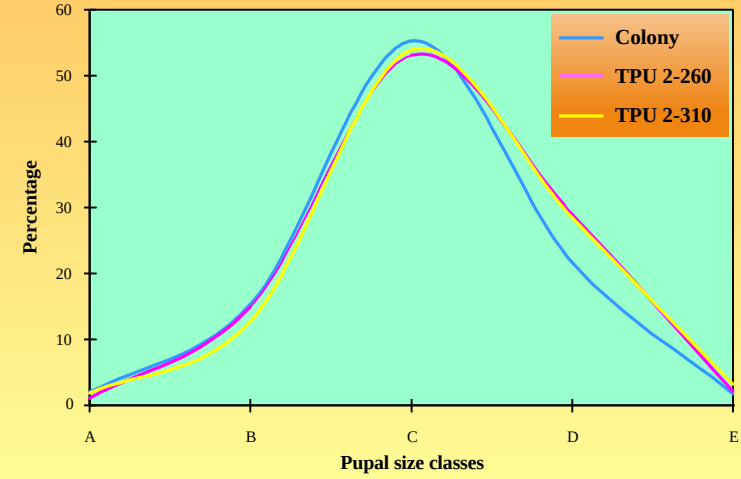
Figure 5. Survival of *G. pallidipes* at different densities.



2.1.4 Pupal Quality

Pupal quality is an indication of the nutritional status of the mother and is the key element in colony production. Poor quality pupae as measured by small size and low weight would indicate that the diet and the holding conditions are inadequate. Quality control tests were carried out on pupae produced by flies kept on TPU 2. Pupae collected on a daily basis from the different treatment groups were sorted according to size classes three

Figure 6. Pupal size classes (%) of TPU 2 and Colony *G. pallidipes*.



times a week. Pupal weights in mg were taken 24 hours after collection. Pupae from the main colony were used as control.

The results indicate that the pupae produced by flies in TPU 2 were of comparable quality with those of the colony flies. This was true of the proportion in each size class (**Fig. 6**) and also of the mean weights for each class (**Table 2**). Pupae produced from flies on the TPU 2 were set aside to monitor their emergence rate. A sample of pupae from the standard colony was used as a control. The emergence rate was 92.3% for cages with 260 females and 89.2% for cages with 310 females compared with 95.5% for colony pupae.

These initial experiments have confirmed that the concepts used to develop the TPU 2 were appropriate. However, adult survival will have to be improved by ensuring that the flies maintain an even distribution in the cages.

2.2 DAY 0 MATING AND SEX RATIO

Tests carried out with *G. austeni* showed that flies could be caged at the time of emergence (Day 0 mating), at the ratio of 1 male to 4 females and that males could be left in the production cages, without affecting either survival or production. These procedures were incorporated into colony maintenance at the Tsetse and Trypanosomiasis Research Institute (TTRI), Tanga to produce flies for the SIT project on Zanzibar. With the ongoing integration of an SIT component in a *G. pallidipes* eradication project in Ethiopia it was necessary to see if these procedures would be suitable for this species. *G. pallidipes* is known to have different requirements for successful mating and this appears to have been the main constraint to the establishment of laboratory colonies of this species. Published data suggest that female flies have to be more than 7 days old before they are receptive to mating and males more than 10 days. Females of the other tsetse species mate early in life from about day 3 post emergence.

Experiments were set up to determine the effect of Day 0 mating and sex ratio on production and survival of *G. pallidipes*. In all cases a total of 60 flies/cage was used. Four production cages per sex ratio were set up at

Table 2. Pupal weight (mg) of TPU 2 and Colony *G. pallidipes*.

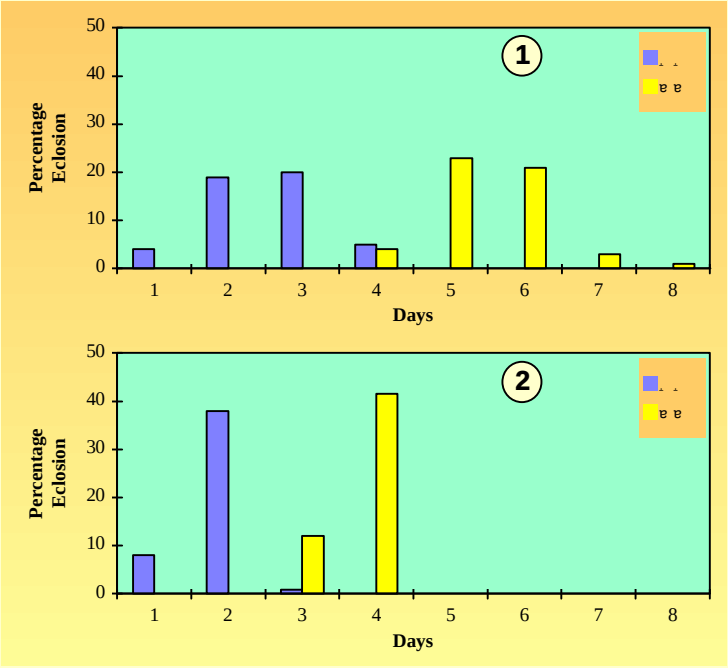
	Size Class				
	A	**B**	**C**	**D**	**E**
Colony	22.07 ± 2.66	29.62 ± 2.27	33.03 ± 2.37	38.59 ± 2.05	41.70 ± 2.72
TPU 2-260	21.56 ± 2.16	29.51 ± 1.99	33.91 ± 2.16	38.33 ± 2.03	41.41 ± 2.54
TPU 2-310	23.83 ± 0.31	29.69 ± 1.66	34.75 ± 2.30	38.40 ± 2.78	40.31 ± 2.81

Data presented as mean ± sd

the time of emergence together with a control group where 8 day old females were mated with 10 day old males for 3 days after which the flies were chilled and the males removed. Survival and production were monitored for 13 weeks. The goal of the experiment was to identify the lowest male to female ratio that would not compromise survival and production, and in so doing free more males for sterilisation and release. The male:female ratios used were 1:4, 1:5, 1:6 and 1:8 with a 1:1 ratio as a control. There was no significant difference in survival and pupal production of flies for all treatments (F=0.12, df=4, P=0.9719) (**Table 3**)

It was observed that the time to first larviposition is the same for all groups, and total pupal production during the first week (week 3) for cages with increasing numbers of females is not significantly different (F=5.19, df=4, P=0.0079), than for the females that were allowed to mature before mating (1:1 ratio). The results show that as with *G. austeni* it is possible to use Day 0 mating, resident males and a sex ratio in favour of females in production cages with *G. pallidipes*. This will impact positively on the efficiency of mass rearing the species.

Figure 7. Emergence pattern of *G. pallidipes* (1) at normal holding conditions and (2) at 26.5°C.



2.3 SELF STOCKING OF PRODUCTION CAGES (SSPC)

After demonstrating with *G. austeni* that production cages could be stocked with flies in the right number and sex ratio using differences in pupal development time (see 1997 Annual Report) and that Day 0 mating is acceptable in *G. pallidipes*, tests on the development of an SSPC were carried out with *G. pallidipes*.

Under normal holding conditions of 23-24°C and 80-85% RH, emergence of *G. pallidipes* pupae deposited on the same day spans a 7-8 day period. During the first three days only females emerge, with the males emerging over the last four days (**Fig. 7**). When pupae at the point of emergence were transferred to 26.5°C, the emergence period was reduced to 4 days (**Fig. 7**). Almost all the females emerged during the first two days and males emerged during the last two days. This allows for an excellent separation of sexes but in that 2 day period there

Table 3. Effect of Day 0 mating and sex ratio at mating on performance of *G. pallidipes*.

Age at Mating (days)		Ratio (♀♀ : ♂♂)	Survival (%)	Pupae per Initial ♂♂ (PPIF)	
♀♀	♂♂		Week 13	Week 13	Week 3
0	0	1:4	52.60 ± 13.32	3.31 ± 0.35	0.41 ± 0.14
0	0	1:5	50.50 ± 3.00	3.54 ± 0.18	0.35 ± 0.06
0	0	1:6	53.92 ± 9.80	3.08 ± 0.85	0.28 ± 0.11
0	0	1:8	51.89 ± 10.39	2.98 ± 0.83	0.24 ± 0.19
10	8	1:1 #	54.89 ± 6.14	3.47 ± 0.33	0.60 ± 0.08

Data presented as mean ± sd
 # pairs were held for 3 days before separation

would be insufficient males available for mating all the females. This problem can however be solved by adding "male pupae" to a batch of mixed pupae and then allowing flies to emerge into cages for 2 days.

Pupae, collected on a daily basis, were incubated at 23-24°C for 25 days after which time they were transferred to petri dishes and placed underneath special emergence cages. When the first flies emerged, usually 31 days after larviposition, pupae were counted into batches of 110 and divided into two groups. This number was used in order to stock the standard cage with approximately 48 females and 12 males. One group was kept at the same pupal incubation conditions and the second group was placed in a room at 26.5°C and RH 75-80% and 15 "male pupae" were added. In both rooms the pupae were placed in a single layer in a ring below a production cage. Flies were allowed to emerge directly into the production cages and counted on a daily basis in monitoring cages. The data from the first set of experiments is shown in **Table 4**. By adding the 15 "male pupae" to the mixed sample of pupae, sufficient males emerged together with the females, 48 hours from the time pupae are transferred to the higher temperature. The residual pupae will provide the additional 15 "male" pupae for the next batch of mixed pupae.

Table 4. Number of G. *pallidipes* that emerge per day when mature pupae together with male pupae are moved to 26.5°C.

Emergence day	♀♀	♂♂	Total ♀♀	Total ♂♂
1	18	6	/	/
2	30	7	48	13
3	3	18	/	/
4	/	26	/	/
5	/	3	/	/

110 mixed and 15 "male pupae" required for loading a cage with on average, 48 ♀♀ and 13 ♂♂ in 2 days giving a ratio close to 1 ♂♂ to 4 ♀♀. Data collected from 20 replicates.

2.4 POST PRODUCTION HANDLING OF *G. PALLIDIPES* MALE PUPAE

The success of tsetse SIT where males are released relies on the ability of the released males to successfully compete for mating with wild males for the wild females. Behavioural responses of mass-reared flies should therefore be similar to that of wild flies.

It is known that *G. pallidipes* males attain sexual maturity after about 10 days of adult life. It would therefore be advantageous in an SIT programme if males of that age could be released. The need to allow males to mature calls for a revised emergence protocol. In most SIT programmes, releases cannot be carried out daily thus increasing the need for an efficient emergence protocol. One possible way to achieve this is to stockpile male pupae and synchronise eclosion. Delay of eclosion can be achieved through lengthening of pupal period by lowering the pupal incubation temperature. However, it is possible that lowering the temperature at this critical time may have deleterious effects on the adults when they emerge.

Preliminary tests reported here were conducted to gain information on the effects of low-temperature treatments of mature *G. pallidipes* pupae on fly emergence and adult performance.

2.4.1 Pupal Treatments

All pupae used in this investigation, unless stated otherwise, were "male pupae" obtained from the procedure for the self stocking of production cages (SSPC) (see **Section 2.3**) whereby cages are automatically stocked by flies in the right number and sex ratio at the time of emergence without the need for chilling and separation of sexes. Using this approach females emerge during the first 48 hours and the remaining pupae are predominantly "male pupae". Because of lack of material it was not always possible to have the same number of pupae for each replicate. The "male pupae" were treated as follows.

Group 1. "Male pupae" were transferred to 15°C for either 24 (A) or 72 (B) hours and then returned to 26.5°C to monitor emergence.

Group 2. "Male pupae" were transferred to either 18°C (E) or 20°C (F) for 72 hours and

then returned to 26.5°C to monitor emergence.

Group 3. "Male pupae" were transferred to 23-24°C to monitor emergence.

Group 4. "Male pupae" were transferred to 15°C for 24 hours and then returned to 23-24°C (C) to monitor emergence.

Group 5. A control group of "male pupae" were left to complete emergence at 26.5°C (H) or at 23-24°C (I).

Table 5. The numbers of *G. pallidipes* mating pairs after mating colony females with males that received different treatments as pupae.

Treatment	Conditions	Type of Mating: 14 d. ♀♀ 7 d. ♂♂	Type of Mating: 15 d. ♀♀ 8 d. ♂♂	Time in Copula (min)
C	15°C for 24h	9	2	27.5 ± 5.0
H	26.5°C during EM	11	3	25.4 ± 5.0
I	Standard colony	6	1	28.8 ± 8.0

Data presented as mean ± sd

The majority of the pairs were recorded in the first hour of observation (81.8%, 85.7% and 69.2% for C, H and I respectively)

2.4.2 Mating Test

Males that emerged from treatment groups C, H and I were matured for 14 days before being mated with 7 day old females which had emerged under normal colony rearing conditions of 23-24°C and 85% RH. On the day of the experiment the flies were not fed. Fifteen females were placed in each of three 10 cm diameter round cages to which the same number of males from treatments C, H and I, were introduced and observed for two hours. Mating pairs were immediately removed and placed in single tubes to record duration of copulation and the males were discarded after separation. Males that did not mate (6 for treatment C, 7 for treatment H and 9 for treatment I) were used with fresh females in a similar set-up the next day. Mated females were dissected under a binocular microscope 24 hours after mating to determine the amount of sperm in the spermatheca, using x400 magnification in dark background.

Unsuccessful mating strikes were too frequent in the first ten minutes for treatments H and C to make any meaningful records so this parameter was ignored. However, it was noted that some of the females curved their abdomen such that any concerted and repeated attempt to establish copula was unsuccessful. The females were particularly refractory to mating attempts made by males emerged under the standard colony conditions (I) (**Table 5**). Successful mating pairs were formed by 73%, 60% and 40% of males from treatments H, C and I, respectively using males 14 days and females 7 days old and 43%, 33% and 11% of males from treatments H, C and I, respectively using males 15 days and females 8 days. Dissection of mated females 24 hours after mating showed that the spermathecae were 75-100% full for all females mated with males from the different temperature treatments. The majority of the pairs were recorded in the first hour of observation (81.8%, 85.7% and 69.2% for C, H and I respectively).

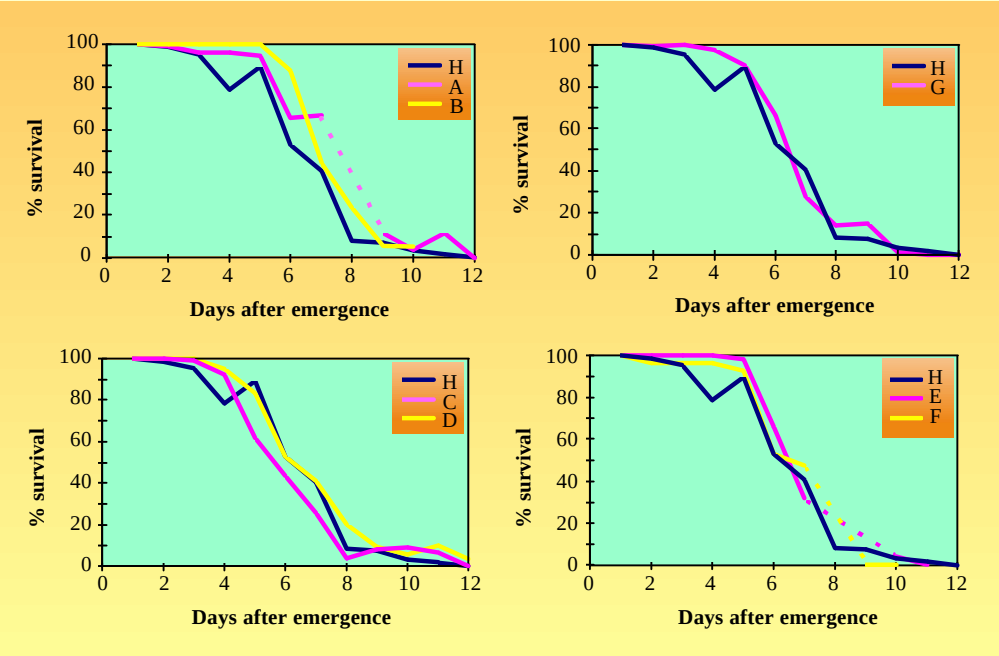
2.4.3 Stress Test

Male flies from the different treatment groups (average 30 per cage) were placed in standard colony conditions and not fed. The length of survival of tsetse flies without food after emergence is an indicator of the total fat reserves of teneral flies.

Mortality was noted for each cage and the dead flies were removed daily. Daily survival was then expressed as a percentage of the original number of males for each cage. The mean survival rate was then calculated for each treatment by averaging the rates in individual cages.

Survival of males that were not fed after emergence was 100% for three days, over 90% for four days with less than 10% surviving beyond eight days. 50% mortality was recorded on the 6th day for all treatments. There was 100% mortality by the 12th day after emergence (**Fig. 8**). There was no difference in the survival curves for all the treatments.

Figure 8. The survival of unfed *G. pallidipes* males after pupae were exposed to different temperatures prior to emergence.



"Male pupae" transferred to 15°C for 24h (A), 72h (B); transferred to 18°C (E), 20°C (F) for 72h before transfer back to 26.5°C. "Male pupae" transferred to 23-24°C (G). "Male pupae" transferred to 15°C for 24h before returning to 23-24°C, (C). Control group of pupae emerged at 26.5°C (H) or 23-24°C (I).

2.4.4 Emergence

The numbers and sex of flies that emerged from each batch of pupae were recorded for each treatment and expressed as a percentage of the original number of pupae in each of the treatments outlined in **Section 2.4.1**. A single classification ANOVA was carried out on arcsine transformed data. Numbers of flies with crippled wings were also recorded. Unemerged pupae were dissected and stage of development noted.

For all treatments except one, the emergence rate was 80-90%. The exception was for the flies that emerged at 23-24°C after incubation of pupae at 15°C for 24 hours where the emergence was 76.21±0.58%. There was no significant difference due to temperature treatment on emergence rates (F=0.007, df=8, P>0.05). For all treatments, more than 50% of the males emerged on the first day and emergence was completed on the second day except for a few exceptional third day emergences. Thus the pupal period for males was lengthened to 35-38 days with incubation at 15°C for three days without any apparent effect on emergence rate. Some flies were found to have emerged at the end of 72 hours incubation at 18 and 20°C. When pupae that did not emerge were dissected, over 60% were lysed with 10-40% having developed to the adult stage. Crippled males were less than 3% for all treatments.

From these preliminary observations it can be concluded that males which emerged from pupae subjected to low temperature treatment after SSPC and those allowed to emerge at 26.5°C apparently engage in mating activity more readily than the males that emerged from pupae kept in standard colony conditions. This may be an indication of early physiological sexual maturity for flies exposed to high temperature. The similarity in survival rate of the unfed males is an indicator that fat reserves were not affected by the different temperature treatments. It is therefore possible to delay eclosion of male *G. pallidipes* by lowering the incubation temperature of pupae after the female flies have emerged without apparent deleterious effects. This will enable release schedules of sterile flies to have a certain degree of flexibility.

This procedure will be tested in combination with the chilled adult release system to determine the effect of low temperature

treatment of mature pupae and adults on male *G. pallidipes*.

2.5 DIET

Previous tests showed that cohorts of both *G. austeni* and *G. pallidipes* could be maintained on reconstituted freeze dried blood (see 1997 Annual Report). However, these tests were conducted for only one generation of flies. In order to see if freeze dried blood could provide a long term substitute for fresh frozen blood a series of experiments was set up in which three successive generations of *G. pallidipes* were reared on several different diets.

Table 6. Survival (%) of *G. pallidipes* through several generations fed different diets.

		Generations			
Diet	**Composition**	**G0**	**F1**	**F2**	**F3**
1	FBB +ATP	37.78 ± 12.57	20.00 ± 6.28	46.67 ± 0.00	66.67 ± 18.86
2	FBB	30.00 ± 7.86	30.00 ± 4.71	50.00 ± 4.71	63.33 ± 14.14
3	FPB + FBB + ATP	24.45 ± 9.43	36.68 ± 26.72	40.00 ± 18.85	66.67 ± 0.00
4	FPB + FBB	31.11 ± 3.14	41.11 ± 1.57	73.33 ± 0.00	66.67 ± 9.43
5	FDBB + ATP	15.56 ± 3.15	50.00 ± 4.71	16.67 ± 4.72	30.00 ± 4.71
6	FDBB	14.45 ± 7.86	42.09 ± 6.48	23.33 ± 14.14	33.34 ± 18.86
7	FDBB + FDPB + ATP	30.00 ± 11.00	35.56 ± 12.57	36.67 ± 4.72	53.33 ± 0.00
8	FDBB + FDPB	27.78 ± 26.71	32.22 ± 01.57	43.34 ± 4.72	33.34 ± 9.43

Data presented as mean ± sd

The 8 diets used were:

1. FFBB + ATP
2. FFBB
3. 75%FFBB:25%PB + ATP
4. 75%FFBB:25%PB
5. FDBB + ATP
6. FDBB
7. 75%FDBB:25%PB + ATP
8. 75%FDBB:25%PB

FF = fresh frozen; FD = freeze dried; BB = bovine blood; PB = porcine blood

ATP = adenosine-5'triphosphate at a concentration of 10^{-3} M

Three cages (20 cm diameter) with 45 females and 15 males were used per treatment and were maintained on each of the eight diets for 13 weeks and their survival and productivity were monitored for three generations. Due to low productivity of some of the treatments smaller cages (10 cm diameter) with 15 females and 5 males were used for observations in the F2 and F3 generations. Pupal sizes were recorded for the different treatments.

Performance of flies maintained on the different diets was compared and data were analysed by ANOVA.

2.5.1 Survival

Percent survival for all the treatments improved through the generations although there were exceptions (**Table 6**). At the F2 generation the cage size and the number of flies per treatment had to be reduced and survival is generally better in smaller cages. There was a significant difference in survival through generations (F=14.296, df=3, P=0.000) with the highest being recorded for flies of the F3 generation. Flies maintained on fresh frozen blood (Diets 1-4) survived better than those maintained on reconstituted freeze dried blood (Diets 5-8) (F=21.477, df=1, P=0.000). There was also better survival recorded for flies that were maintained on a mixture of bovine and porcine blood rather than those fed bovine blood only (F=5.262, df=1, P=0.029). Addition of ATP to the diet had no effect for both fresh frozen or reconstituted freeze dried blood (F=0.415, df=1, P=0.524).

2.5.2 Pupal production

There were significant differences in pupal production between the different diets in the G0 generation (**Table 7**) (F=4.27, df=7, P=0.0294). In the F1 generation pupal production was higher for flies that were maintained on fresh frozen blood than on

Table 7. Pupal production as pupae per initial female (PPIF) of *G. pallidipes* maintained on different diets.

Diet	Composition	Generations			
		G0	F1	F2	F3
1	FBB +ATP	3.74 ± 0.83	3.29 ± 0.10	3.70 ± 0.33	5.70 ± 0.80
2	FBB	3.57 ± 0.46	2.63 ± 0.27	4.70 ± 0.61	5.07 ± 0.19
3	FPB + FBB + ATP	3.90 ± 0.14	4.20 ± 0.57	4.80 ± 0.57	6.04 ± 0.05
4	FPB + FBB	4.37 ± 0.52	4.17 ± 0.21	6.07 ± 0.00	5.50 ± 0.04
5	FDBB + ATP	3.60 ± 0.28	1.60 ± 0.62	0.67 ± 0.09	1.20 ± 0.66
6	FDBB	3.00 ± 0.85	1.55 ± 1.05	1.80 ± 0.57	1.24 ± 0.62
7	FDBB + FDPB + ATP	3.17 ± 0.05	2.59 ± 0.52	2.00 ± 0.38	2.03 ± 0.42
8	FDBB + FDPB	3.75 ± 0.78	2.57 ± 0.21	2.57 ± 0.90	1.97 ± 0.33

Data presented as mean ± sd

reconstituted freeze dried blood. Flies maintained on fresh frozen blood produced significantly more pupae than those fed reconstituted freeze dried blood (F=290.769, df=1, P=0.000). A mixture of bovine and porcine blood was better than bovine blood alone (F=39.730, df=1, P=0.000). Addition of ATP to the diet had no effect on pupal production.

From the above observations, it is clear that a colony of *G. pallidipes* cannot be continuously maintained on a diet of only reconstituted freeze dried blood but if necessary, freeze dried blood could be used in the event of a temporary shortage of frozen blood. The flies will survive well on freeze dried blood but will not produce the normal number of pupae. Addition of ATP to both fresh frozen blood and freeze dried blood did not significantly improve production and there is no justification for its continued use. For *G. pallidipes* a mixture of bovine and porcine blood whether freeze dried or fresh frozen was better than bovine alone. Freeze dried blood could also be used for feeding male flies destined for sterilisation and release since it has no effect on survival and the males once released will find a natural source of blood.

The distribution of size classes of pupae produced by females maintained on different diets indicated that for diets made up of a mixture of bovine and pig blood, there was a high proportion of larger pupae both for the fresh frozen and reconstituted freeze dried blood than in the equivalent bovine blood. The percent of total pupae in the largest class (Class E) being higher for those diet combinations with the mixtures (**Table 8**). The data in **Table 8** has been pooled for the three generations.

Conclusions drawn from these tests are:

- Tsetse colonies cannot be maintained through several generations using reconstituted freeze dried blood.
- Reconstituted freeze dried blood can be used in the event that there is a temporary disruption in the supply of fresh blood.
- Long term maintenance of a colony of *G. pallidipes* on freeze dried blood leads to deterioration in pupal production but does not affect survival.
- *G. pallidipes* can be maintained on a diet without ATP and pig blood.

2.6 OTHER TOPICS

2.6.1 Salivary Gland Infection

While comparing the mating competitiveness of male flies from two *G. pallidipes* colonies, it was observed that males from the Uganda colony readily mated with their own females as well as with females from the Zimbabwe colony while males from the Zimbabwe colony performed very poorly. The two colonies also displayed different levels of fecundity; the Zimbabwe colony being less productive than the Uganda colony.

It is known that male flies with enlarged salivary glands are sometimes aspermic and it was then decided to dissect male flies from the two colonies and also males that became available from the Ethiopian colony of *G. pallidipes* which is under colonization.

Table 8. Size class of pupae produced by *G. pallidipes* maintained on different diets.

Composition	Size Class				
	A	B	C	D	E
FBB +ATP	0	15.05 ± 03.99	56.06 ± 7.15	21.58 ± 15.17	2.23 ± 1.26
FBB	0	8.89 ± 5.63	46.65 ± 3.13	30.22 ± 23.47	4.22 ± 1.66
FPB + FBB + ATP	0.11 ± 0.18	3.35 ± 1.05	26.43 ± 11.16	33.28 ± 20.10	26.02 ± 11.71
FPB + FBB	0	3.36 ± 2.15	24.12 ± 10.67	38.44 ± 24.55	22.96 ± 9.11
FDBB + ATP	4.33 ± 5.07	26.51 ± 11.64	48.23 ± 19.06	24.19 ± 7.99	0.34 ± 0.59
FDBB	0	17.80 ± 1.51	55.68 ± 18.65	26.28 ± 9.09	3.38 ± 4.91
FDBB + FDPB + ATP	0.66 ± 1.14	9.45 ± 3.21	55.42 ± 8.67	20.58 ± 11.67	6.05 ± 1.69
FDBB + FDPB	0.19 ± 0.32	15.92 ± 4.49	44.44 ± 2.94	22.07 ± 17.40	7.57 ± 2.58

Data presented as mean ± sd

The Ethiopian strain did not have any enlarged glands on dissection. This does not rule out the possibility that other field populations of *G. pallidipes* in Ethiopia might be infected with the virus. All pupae that have so far been sent from Ethiopia to establish the colony have come from one location.

G. austeni males were also dissected. Sperm motility in the dissected flies was also monitored.

Enlarged salivary glands were present in two colonies of *G. pallidipes* with the Zimbabwe strain showing the highest incidence (**Table 9**). In some samples up to 75% infection was detected. Most of the males found with enlarged glands showed no sperm in the testes. The testes were smaller than those of normal males and females mated by such males were not inseminated. The Zimbabwe strain has since been terminated and the two remaining strains are kept in isolation of one another to reduce the possibility of cross contamination since fertile matings readily occur between these 2 strains.

Table 9. Occurrence of "enlarged glands" in various colonies of tsetse.

Species / Origin	Number Dissected	Percentage Enlarged Glands
G. pallidipes Zimbabwe	200	> 50
G. pallidipes Uganda	600	20
G. pallidipes Ethiopia	150	0
G. austeni Zanzibar	200	0

2.6.2 Alternate Day Feeding

The current feeding regime for *G. pallidipes* colony is 5 days a week, Monday to Friday with no feeding on Saturday and Sunday. In an operational programme it may be advantageous to feed half the colony on one day therefore feeding flies on alternate days. In nature, flies feed at intervals of 3 days. Investigations were conducted to determine the effect of alternate day feeding during the week, on fly performance.

Table 10. Effect of alternate day feeding on performance of *G. pallidipes* and *G. morsitans*.

Species	Parameters	Feeding Regime	
		3 day	5 day
G. pallidipes	% Survival	71.31	52.73
	PPIF	3.02	3.38
	Mean pupal weight (mg)	34.65 ± 3.81	34.31 ± 3.28
G. morsitans	% Survival	81.64	76.56
	PPIF	3.63	3.24
	Mean pupal weight (mg)	26.89 ± 2.67	26.81 ± 2.78

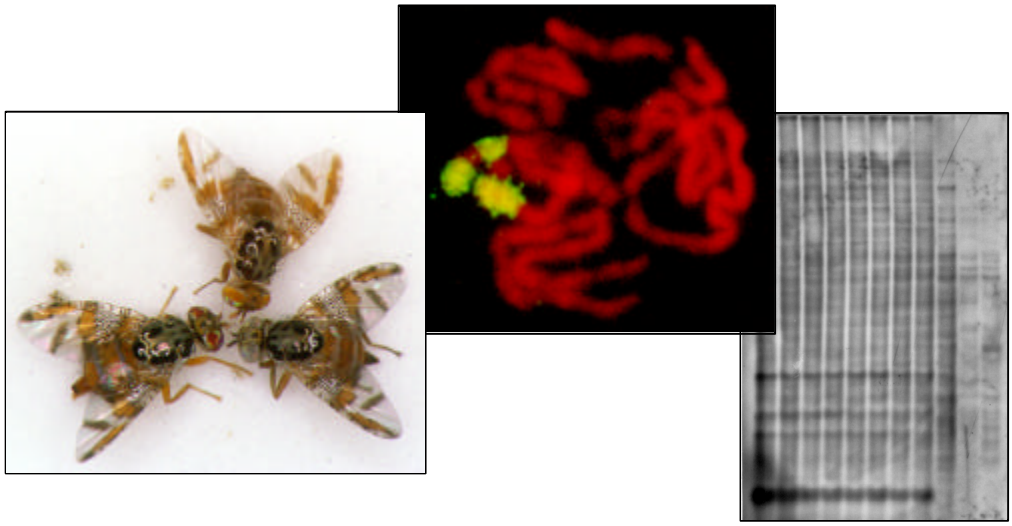
Data presented as mean ± sd

Standard cages were loaded

with 48 females and 12 males *G. pallidipes* and 64 females and 16 males *G. morsitans*. The flies were fed three days a week, Monday, Wednesday and Friday. A control group was fed 5 days a week. Mortality was checked every three weeks and pupae collected daily. The experiment was replicated 4 times. Observations was made over 13 weeks and survival and PPIF were measured. The data are shown in **Table 10**.

Pupal production from flies fed either 3 or 5 days a week was similar and so was the mean pupal weight indicating that it is probably not the frequency of feeding but the quantity of blood taken up that influences performance. When given a chance to feed daily, a fly takes on average 32.48 mg of blood. After two days without a meal up to 60 mg of blood can be taken during one feeding but the mean is 35.63 mg. Based on these results routine feeding of tsetse colonies at Seibersdorf has been reduced from 5 to 3 days per week.

3. Development of Medfly Genetic Sexing Strains (GSS)



THE USE OF GSS *in medfly operational programmes over the past 3-4 years has revealed some unexpected findings related to genetic recombination. In the absence of genetic recombination in GSS, male flies emerge from brown pupae and females from white pupae. Conventional recombination (Type-1 Recombination) reverses this relationship but due to fitness differences between the two new recombinant types there is always a tendency that the proportion of females emerging from brown pupae will increase far faster than the proportion of males emerging from white pupae. However, in several cases of GSS mass rearing a different picture emerged and there was a rapid increase in males emerging from white pupae. A hypothesis to account for this observation was proposed (Type-2 Recombination) and experimentally verified. Using this information, greatly increased stability of GSS has been demonstrated by incorporating a translocation with a breakpoint at a specific location on the Y chromosome.*

The use of genetic transformation to introduce foreign genes into the genome of pest insects has generated much interest. It is proposed that the use of this technology could help considerably in the development of new control techniques. For medfly genetic sexing, strategies have been proposed that incorporate this technology and that hold out the promise for strain improvement. Medfly, along with most other pest insects, has proven refractory to transformation attempts with the first successful experiment being carried out in 1995. In the 1997 Annual Report details were given of a successful series of transformation experiments carried out at Seibersdorf. These experiments were expanded in 1998 and once again successful transformation was demonstrated. Although the technique cannot yet be classified as routine, progress has been significant.

In relation to transformation, work has continued on the isolation of the gene that causes maleness in medfly. Experiments are being carried out try to isolate this gene from the large amount of non-functional DNA that is present in the part of the Y chromosome where the maleness gene is located.

3.1 GENETIC RECOMBINATION IN GSS

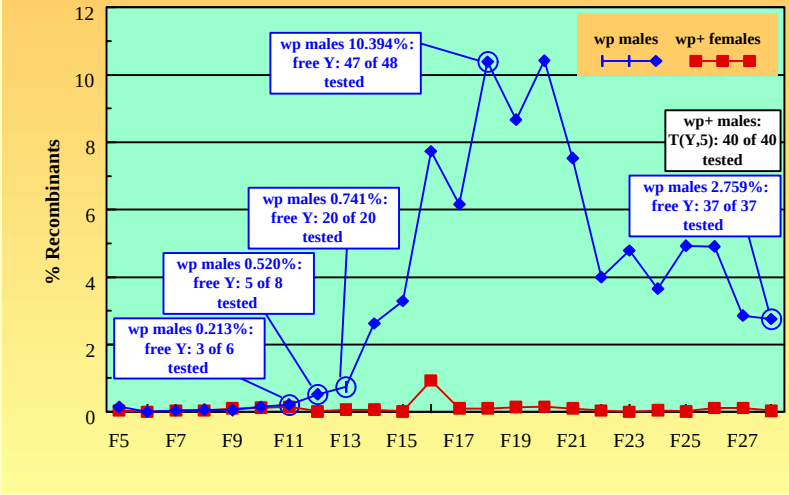
In GSS the males show a wild-type phenotype while the females are mutant with respect to the selectable marker used, ie either *white pupae* (*wp*) or *wp* in combination with a temperature sensitive lethal (*tsl*) mutant. This is achieved by linking the wild-type allele of the selectable marker via a Y-autosome translocation to the male sex. As a result of genetic recombination two aberrant types of individuals can be produced namely, mutant males and wild-type females. However, when GSS were reared at relatively low levels, predominantly wild-type females were observed as the aberrant type. Such wild-type females are the consequence of homologous recombination in the autosome region between the translocation breakpoint and the selectable marker. This type of recombination has been designated Type-1 Recombination. The wild-type females accumulate in a cycling colony because they have a selective advantage over the normal non-recombinant females carrying the mutant phenotype. The reciprocal aberrant type, mutant males, occurs initially with the same frequency as the aberrant females but do not accumulate because they have a selective disadvantage compared to the normal wild-type males.

3.1.1. Type-2 Recombination

When GSS were reared in much larger numbers, mutant males began to be detected at considerable frequencies (see 1995 Annual Report). It can be suggested that these mutant males initially occur at very low frequencies as this phenomenon was never observed in many generations of small scale rearing despite the fact that over 745,000 individuals were screened. The fact that such males accumulate in the mass rearing colony indicates that they represent a different type of mutant male than that resulting from the autosomal recombination mentioned above. This new type of event has been called Type-2 Recombination and was detected in all strains that have been mass reared irrespective of which translocation or selectable marker was used.

To better understand the occurrence and effect of Type-2 Recombination, the GSS VIENNA 6-94 was mass-reared and monitored closely with respect to the occurrence of mutant males. During the entire rearing period, almost no Type-1 recombinant females (wp^+) were observed (**Fig. 9**). From several generations, mutant (*wp*) males were sampled and tested for the presence of the Y-autosome translocation (**Fig. 9**). If these males were the product of Type-1 Recombination then the translocation should still be intact. In this test, single *wp* males were crossed with females from a *white* strain. This strain has white eyes due to a mutation, *w*, which has been mapped to the left arm of chromosome 5. F1 males were then backcrossed with *w* females. If the translocation is still present, F2 males should have a wild-type eye colour while the females should have white eyes. Conversely, if the tested *wp* males did not carry the translocation but instead carried a free Y chromosome then there would be no linkage with the sex and eye colour.

Figure 9. Mass rearing of VIENNA 6-94 at Seibersdorf. The frequency of recombinant individuals is shown. In several generations Type-2 recombinant males (*wp*) were tested for the presence of the translocation.

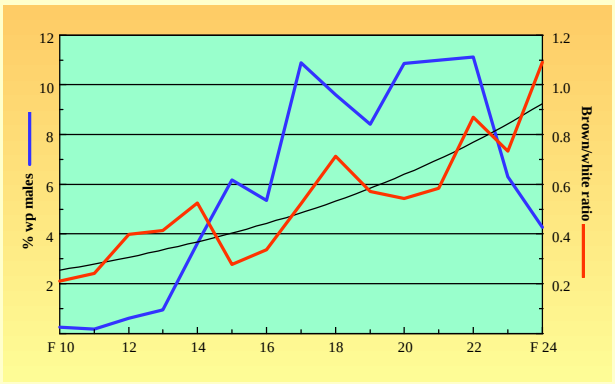


In the F11 generation the frequency of *wp*

males was 0.21%. Three of the six *wp* males analysed still contained the translocation and, therefore, it is assumed that their mutant phenotype was the consequence of Type-1 Recombination in males of the previous generation. The other three *wp* males showed no linkage between eye colour and sex and it was concluded that they contained a free Y chromosome. This was later confirmed by cytological analysis (data not shown).

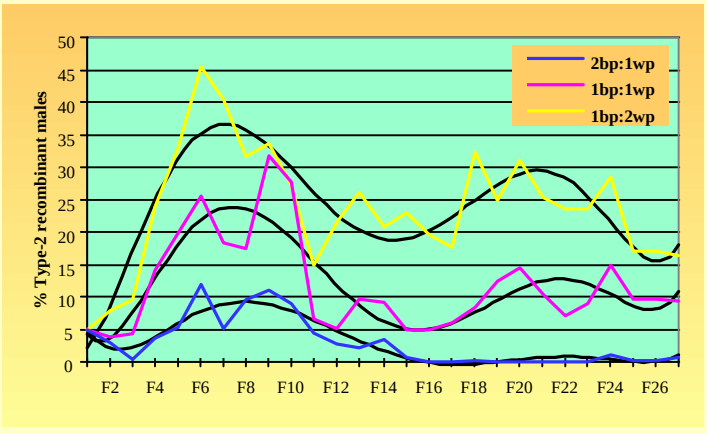
In subsequent generations the frequency of *wp* males increased significantly reaching 10.39% in F18 and all but one of the *wp* males tested from this generation carried a free Y chromosome. The reason why Type-2 males accumulate although they are mutant for the selectable marker(s) is the fact that a free Y chromosome restores full fertility while the other males in the population carry the translocation and are 50% sterile. From generation 21 onwards the *wp* male frequency declined. This is due to the way that the cages were stocked at each generation, ie what ratio of white and brown pupae were loaded into the cages to produce flies for the next generation (**Fig. 10**). In the F10 generation nearly five times as many white pupae (putative females) as brown pupae (putative males) were loaded into the cages in order to maximise egg production. Consequently, relatively more Type-2 recombinant males (white pupae) were added to the cage compared to when equal numbers of both types of pupae were loaded. At a later date the stocking of the cages was gradually shifted towards an equal number of white and brown pupae (**Fig. 10**) to avoid potential behavioural differences accumulating as a result of a distorted sex ratio. In this way the relative input of the two types of males was shifted in favour of translocation-carrying males and the frequency of the *wp* males decreased.

Figure 10. Accumulation of Type-2 recombinant males (*wp*) as a function of the ratio of white and brown pupae loaded into the production cages.



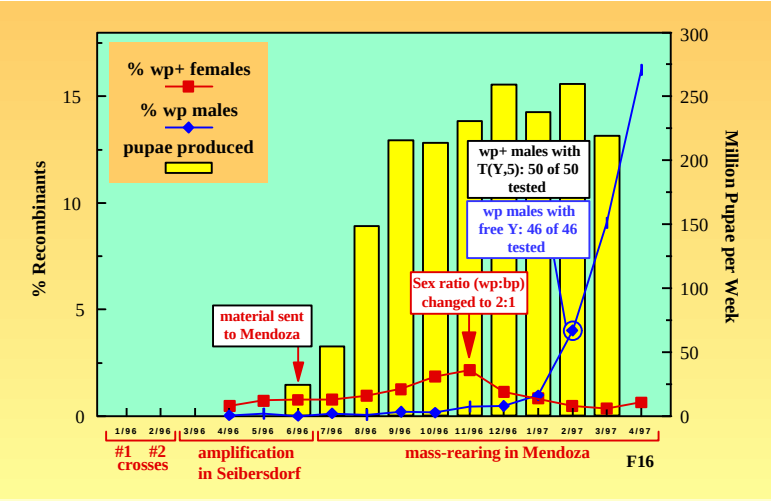
That indeed the sex ratio in the cages, determined by the relative numbers of white and brown pupae used to stock the cages, influences the frequency of Type-2 males was shown in a small scale simulation experiment. Starting with pupae from the VIENNA 6-94 colony at a level of 4.91% *wp* males, three parallel lines were established. Each line was set up and maintained with a different pupal sex ratio at each generation; 2:1, 1:1 and 1:2 for brown and white pupae, respectively. A simple theoretical model, based only on the difference in fertility between translocation carrying males (50%) and males with a free Y (100%), predicts that a 1:1 sex ratio would lead to an equilibrium of 12.5% Type-2 males. If the sex ratio was 2:1 in favour of white pupae then twice as many Type-2 males would be found and if the sex ratio was 2:1 in favour of brown pupae then the frequency of Type-2 males should approach zero. As shown in **Fig. 11**, the model provided a good simulation of the experimental data for the three lines and added credence to the explanation developed for the observations made during the mass rearing of VIENNA 6-94.

Figure 11. Accumulation of Type-2 recombinant males (*wp*) in 3 parallel colonies set up and maintained with different ratios of brown and white pupae.



3.1.2. An analysis of Type-2 recombinants in the colony from Mendoza

Figure 12. Mass rearing of SEIB 6-96 in Seibersdorf and Mendoza, Argentina.

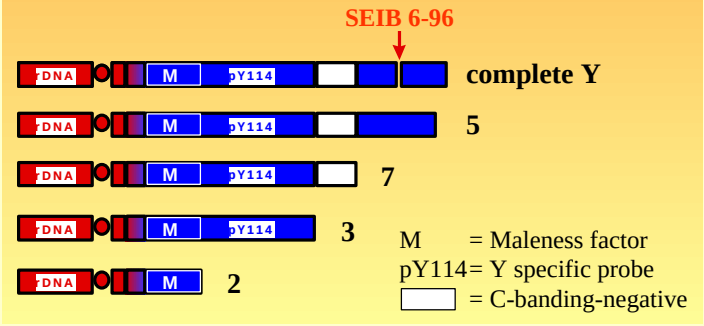


Data from Mendoza kindly provided by Gustavo Taret

The same scenario was observed from the mass rearing of SEIB 6-96 in the "KM8" factory in Mendoza, Argentina (Gustavo Taret, pers. com.) where this strain is being used in a large operational SIT programme. **Fig. 12** shows the history of that strain from its construction and initial mass rearing in Seibersdorf through the first 11 generations of mass rearing in Mendoza. Initially, Type-1 recombinant females, emerging from brown pupae, started to accumulate reaching approximately 2.5% in F11. However, at that time the sex ratio in the cages was changed to increase production, ie two times more white than brown pupae were loaded into the cages to obtain more producing females per cage. The consequences of this change for the integrity of the GSS were dramatic. The frequency of Type-1 females started to decline while Type-2 males began to accumulate very rapidly (**Fig. 12**).

Material from this colony at the F14 generation was analysed genetically and cytologically. In single male crosses, 50 males from brown and 46 males from white pupae were tested for the presence of the Y-autosome translocation. As expected, all males from brown pupae carried the translocation and all males from white pupae carried a free Y chromosome. From the latter group, 17 single male lines were analysed cytologically by Antigone Zacharopoulou (University of Patras). As expected, the cytological observations confirmed the genetical tests and all lines were shown to contain a free Y chromosome. However, the size of the Y chromosomes varied between the different lines. In the original Y-autosome translocation in SEIB 6-96, the breakpoint in the Y chromosome is in the region distal to the C-banding-negative segment of the long arm (**Fig. 13**). In the cytological analysis of the different lines no change could be detected in the short arm of the Y chromosome, ie size variations are found only in the length of the long arm. Based on overall size and on cytological features, the different free Y chromosomes from the Mendoza colony can be grouped into 4 distinct classes (**Fig. 13**). In 5 lines the free Y chromosome is virtually indistinguishable from a wild-type Y. In 7 lines the Y is clearly shorter, ie the distal C-banding-positive segment is absent. In 3 lines, the distal part including the C-banding-negative region is missing. In 2 lines the free Y is very short, ie both arms have approximately the same size. Despite this severe deletion, the males carrying such short Y chromosomes are fully fertile and show no obvious morphological defect.

Figure 13. Cytological analysis of Type-2 recombinant males detected in SEIB 6-96 from Mendoza.



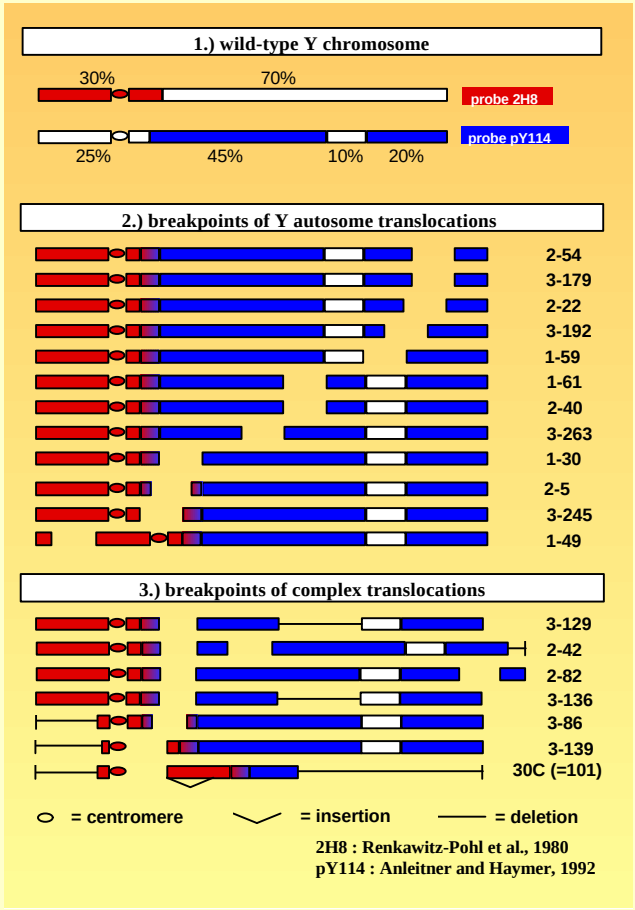
In total, 17 free Y chromosomes were analysed and could be separated into the 4 classes shown.

3.1.3. Origin of the different free Y chromosomes

The reversion of a Y-autosome translocation to a free Y chromosome can be explained by intra-Y recombination between the two translocated Y-fragments. The long arm of the Y chromosome consists of repetitive sequences some of which are found along the entire long arm. For example, the short Y-specific clone pY114 (1405 bp) hybridizes to virtually the entire long arm following *in-situ* hybridisation. It is assumed that this intra-chromosomal homology at the DNA level is responsible for the recombination between normally contiguous Y-fragments which are now involved in a Y-autosome translocation. It can be speculated that by breaking the Y chromosome into two fragments, as occurs in a Y-autosome translocation, the two fragments can align and recombine more easily than in an intact Y. During meiosis, chromosomal pairing takes place in the translocation complex and the degree of overlap between the two fragments will determine how long the resulting free Y chromosome will be. The analysis of the 17 free Y chromosomes from the colony in Mendoza reveals that the majority of the recombination events must have taken place in the distal half of the long arm while only in two cases did recombination take place between the distal part and a region in the proximal half. This seems to coincide with the finding that the distribution of sequences homologous to pY114 is not equal along the long arm. Southern analysis shows that in the proximal half the number of pY114-homologous sequences is reduced significantly (data not shown).

If indeed homology at the DNA level between the two translocated fragments is responsible for Type-2 Recombination, it can be postulated that for a stable GSS a translocation would be optimal where the breakpoint is situated such that the resulting fragments are very dissimilar. For this purpose several Y-autosome translocations were analysed using *in-situ* hybridization with two probes; pY114 and ribosomal DNA (rDNA). The results are summarised in **Fig. 14**. From the analysed translocations T(Y;5)3-129 was selected because one Y-fragment contains primarily rDNA while the other contains repetitive sequences. A second advantage of this translocation is that there is a low frequency of adjacent-1 individuals that survive to the pupal stage (see 1997 Annual Report). As Type-2 Recombination is extremely rare, it was necessary to analyse T(Y;5)3-129 at a relatively high level of rearing. The results of mass rearing a GSS, VIENNA 7-97, incorporating this translocation can be seen in **Section 4.1**.

Figure 14. Cytological analysis of Y-autosome translocations. The results of *in-situ* hybridisation experiments with two probes, pY114 and rDNA (2H8), are shown.



3.2 GENETIC TRANSFORMATION

The ability to introduce genes into the genome of the medfly will enable new approaches to be developed for improved GSS. Genetic transformation of pest insects is still in the experimental stage with a small but growing number of documented cases. Work in the Unit on this topic is restricted to the evaluation of

putative transformation constructs which are developed elsewhere. In this report results are given of several series of injections carried out with two different transformation vectors.

3.2.1. Transposable element *piggyBac*

In late 1997 a genetic transformation experiment was conducted in collaboration with Al Handler (USDA, Gainesville). A *piggyBac* vector was used where the transposase (the enzyme required for transposition) and the *white* (*w*) marker were under the control of a *Drosophila melanogaster* heat shock promoter (*hsp* 70). Embryos from *w/w* flies are injected and transgenic flies can be recognized as the eye phenotype is no longer mutant. From the 9 potential transgenic individuals that were detected in this screen a total of 16 sub-families are currently maintained. The different sub-families were the consequence of separating the two classes of eye colours found in the transgenics (red and yellow) and in some cases resulted from single pair crosses set up to make the transgene homozygous (**Table 11**). All families which were made homozygous via single pair crosses for either of the eye phenotypes are true breeding, ie only one type of eye colour is observed.

Table 11. Families from the *piggyBac* transformation experiment. True breeding refers to the uniformity of the eye colour.

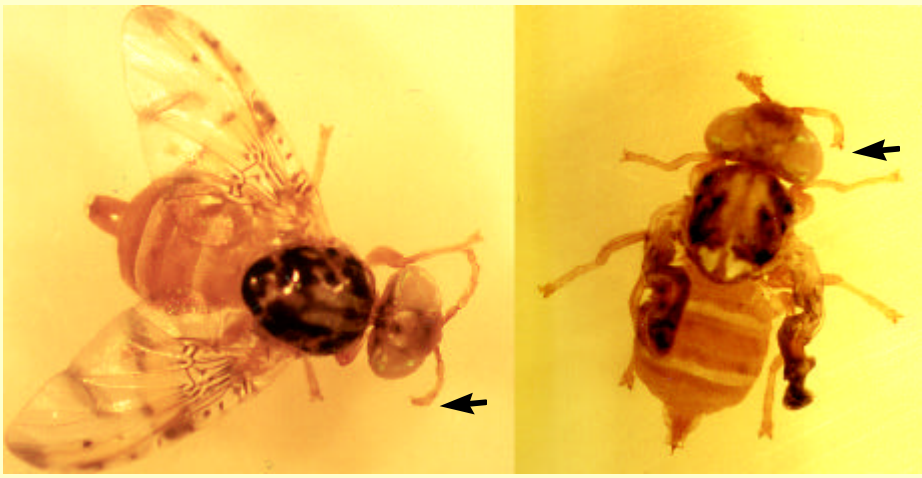
Original cross	G1 #	Sub-families	Eye Colour	Homozygous Through	True Breeding
Single G0 female #11	G1 #1	11-1d/7 RE	red	single pairs	yes
		11-1 RE	red	inbreeding	
		11-1 YE	yellow	inbreeding	yes
	G1 #2	11-2 RE	red	inbreeding	
	G1 #4	11-4/3 RE	red	single pairs	yes
Single G0 male #12	G1 #1	12-1 RE	red	inbreeding	
		12-1/2 RE	red	single pairs	yes
		12-1 YE	yellow	inbreeding	yes
	G1 #2	12-2RE	red	inbreeding	
		12-2 YE	yellow	inbreeding	yes
	G1 #3	12-3 RE	red	inbreeding	
	G1 #4	12-4/6 RE	red	single pairs	yes
		12-4 YE	yellow	inbreeding	yes
Mass G0 males	G1 #1	E-1 YE	yellow	inbreeding	yes
	G1 #2	E-2 RE	red	inbreeding	
		E-2 YE	yellow	inbreeding	yes

All families were maintained initially with heat shock treatment to induce the *white* gene. From late larval stages to late pupal stages a 1 hour heat shock (37°C) was given every day. Subsequently, the heat shock was removed and no difference was observed in the eye colour of any of the families, ie the heat shock treatment is apparently not required to activate the *white* gene via the *hsp* 70 promoter. From *Drosophila* it is known that this promoter has a low constitutive activity even without heat shock and it appears that this is sufficient to activate the *white* gene in medfly in order to rescue the eye colour mutation. It will be important to see whether the equivalent *hsp* 70 promoter from medfly will be subject to better temperature control.

In the screen for transgenic individuals certain mutant flies were detected (see 1997 Annual Report). One of these lines, initially started with a male carrying only one sub-orbital frontal (SOF) bristle, was maintained as an inbreeding family without heat shock treatment. On several occasions flies were detected in this line with a phenotype that resembles the *Drosophila* mutation *Antennapedia* (*Antp*). In this mutation the antennae are transformed to legs and in medfly a similar phenotype was observed (**Fig. 15**). In addition, nearly all bristles are missing in the medfly mutant, a condition which is not found in the putative *Drosophila* homologue. The mutation was detected in males and females. We attempted several times to mate such flies but were not successful. Most of the *Antp*-like mutants do not emerge fully and even those that do usually die very young. Currently it is not

known what the cause of this mutation is. As it was never observed in the original strain that was used for the transformation experiment it has to be assumed that it arose directly or indirectly from the injections with the *piggyBac* vector.

Figure 15. *Antennapedia* (*Antp*)-like mutant of the medfly. Arrows: antennae transformed into legs.



3.2.2. Transposable element *Hermes*

In collaboration with Peter Atkinson (UC Riverside) and David O'Brochta (University of Maryland) transformation experiments were conducted with the mobile element *Hermes* isolated from the house fly, *Musca domestica*.

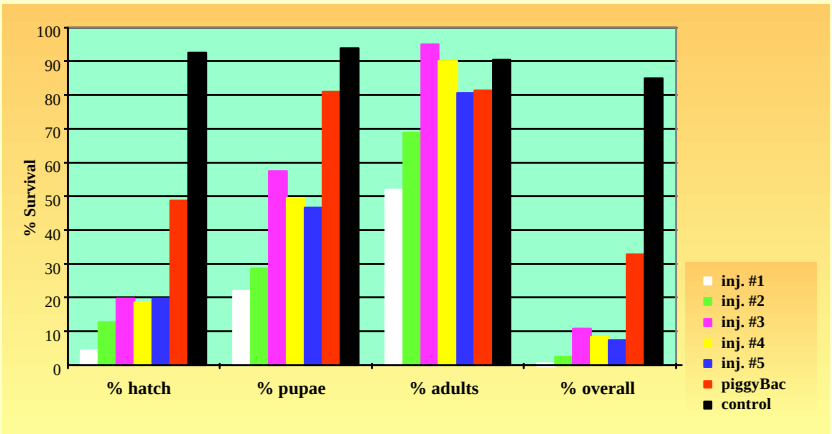
The following *Hermes* constructs were injected into medfly embryos:

- #170: medfly *white* gene with *Drosophila* heat shock (*hsp* 70) promoter
- #198: "Enhanced green fluorescent protein" (EGFP) gene with *Drosophila* Actin 5C promoter
- #II3: medfly *white* gene with *Drosophila hsp* 70 promoter and EGFP with *Drosophila* Actin 5C promoter (in cis).
- #205: medfly *white* gene with *Drosophila hsp* 70 promoter and EGFP with *Drosophila* Actin 5C promoter (in trans).

The helper plasmid carried the *Hermes* transposase either under the control of the *Drosophila hsp* 83 promoter (injection #1 and #2) or the *Drosophila hsp* 70 promoter (injections #3, #4 and #5). The EGFP marker when expressed in a transgenic fly enables the individual to be identified using fluorescence microscopy.

In total, 12,806 *w/w* embryos were injected. Combining the results for all the *Hermes* experiments, 645 G0 individuals survived to the adult stage of which 637 were crossed with *w/w* flies. **Fig. 16** shows the survival in the G0 generation at the different developmental stages for the 5 series of injections. The data from the *piggyBac* injections is included for comparison. The survival in the initial series of injections was low but improved with the later injections. However, overall survival was significantly below that obtained in the *piggyBac* experiments. It is surprising that not only the injected embryos themselves are affected (% egg hatch) but also the viability of the larvae (% pupae from hatched eggs). Adult emergence reached, at least in the later injections, more or less normal values. The

Figure 16. G0 recoveries in the different transformation experiments with *Hermes* vectors in comparison with the results of the *piggyBac* injections.



reduced survival from the embryonic to the pupal stages are most likely not due to any interaction between the genome of the medfly and the *Hermes* vectors. In one of the *Hermes* injection experiments reported here the *piggyBac* vector was used as control. The survival values obtained were equivalent for the two vectors suggesting that the reduced viability is caused by the experimental treatment/handling and not by any incompatibility of the medfly genome with the *Hermes* vector.

The test crosses to identify transgenic individuals were done with *w/w* individuals. Matings were carried out either with single G0 flies mated to 3-5 flies of the *white* strain, in mass matings or with groups of 3-5 G0 individuals. In **Table 12** the recovery of G1 flies per G0 individual from these three types of crosses is summarised. Crossing single G0 males with 3-5 *white* females is, on average, the most effective way to produce G1 offspring. The reciprocal cross yields only 67% of this number. Even fewer G1 flies are produced when small groups or mass mating is used, ie per G0 individual only 30 to 38% of the numbers obtained with single G0 males are reached, irrespective of the sex of the G0.

In one of the group matings with G0s from the fifth injection, 12 flies (5 males, 7 females) with red eyes were detected among a total of 400 G1 adults. These potential transgenics were mated individually with flies from the *w/w* strain. Attempts are being made to generate homozygous strains by inbreeding. Unlike the *piggyBac* transgenics, the overwhelming majority of flies show red eyes ie full rescue of the mutant phenotype. Only after several generations were a few flies with yellow eye colour found in 3 of the 12 families. These were set up as separate lines and have so far maintained their yellow eye colour.

In the laboratory of Peter Atkinson (UC, Riverside) the different families are being analysed by PCR to determine whether they are carrying the *Hermes* vector. Preliminary results show that this is indeed the case. More detailed analysis will be required to show that the transformation event was a precise integration of the entire vector mediated by the *Hermes* transposase.

3.3 OTHER AREAS OF ONGOING RESEARCH

3.3.1. Isolation of the *Maleness* factor

Work continues towards the isolation of the Y-linked factor that is responsible for the induction of maleness. The cloning of this gene(s) would be the first step to develop genetic sexing strains that no longer require the use of Y-autosome translocations. Starting from microdissected material from the appropriate region of the Y chromosome two rounds of subtraction with different types of driver DNAs were performed. From each round of subtraction, libraries of the remaining material were constructed. Random clones were

Table 12. Number of G1 offspring produced per G0 individual.

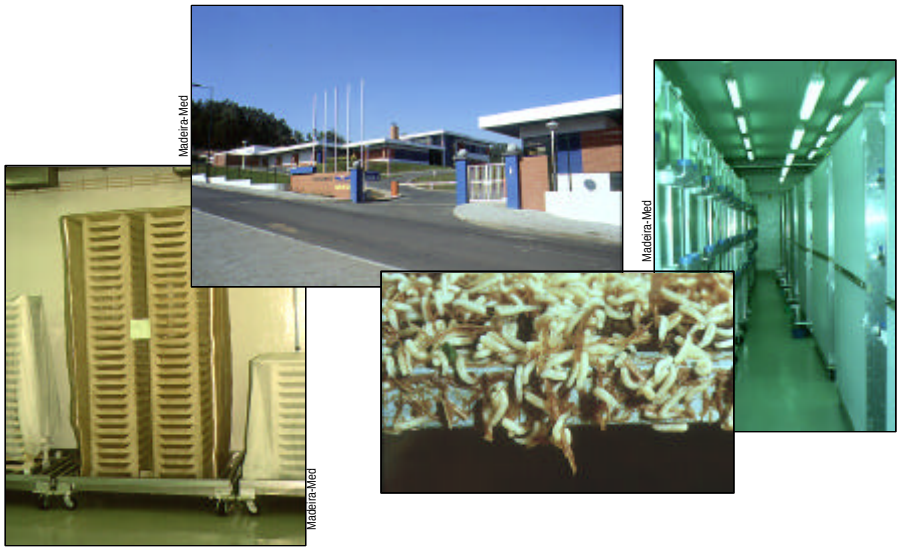
Vector	Injection	Single G0 crosses		3-5 G0 crosses		G0 mass mating	
		G0 ♂	G0 ♀	G0 ♂	G0 ♀	G0 ♂	G0 ♀
#170	#3	249	150				
	#4	223	121				
	#5			77	75		
#205	#3	264					70
	#4	68				64	53
Average		**201**	**136**	**77**	**75**	**64**	**62**

picked and used to probe genomic Southerns. The analysis of these clones is currently ongoing and, based on the results, alternative subtraction strategies will be designed.

3.3.2. Screen for pericentric inversions

Pericentric inversions can be used to increase the stability of GSS with respect to Type-1 Recombination. In collaboration with Antigone Zacharopoulou (University of Patras, Greece) and Carlos Caceres (Programma Moscamed-Guatemala) large numbers of irradiated chromosomes were screened for inversions. In total, 8 pericentric inversions were isolated and analysed genetically and cytologically. Out of these 2 are viable as homozygotes. So far no inversion was detected that covers the region on chromosome 5 where *wp* and *tsl* are located. To improve the chances that such inversions can be found, a new screening strategy was devised that is based on a new mutation isolated by A. Zacharopoulou.

4. Mass Rearing Characterization of Medfly GSS



MEDFLY GSS ARE TESTED *and characterised under mass rearing conditions in Seibersdorf so that, following transfer to operational facilities, managers know what to expect of them and can make informed decisions relating to SIT implementation. The Filter Rearing System (FRS), developed in Seibersdorf during 1996-97, for mass production of insects, has the capacity to maintain the essential characteristics of these strains so that they continue to perform according to their established character. However, with the development of new, more stable strains, it is equally important to document the long-term character of GSS in mass rearing, particularly under different colony maintenance strategies and varying levels of stress.*

A detailed study of the mass rearing of VIENNA 7-97, a temperature sensitive lethal GSS, was completed during 1997. This strain demonstrated excellent characteristics for the production of high quality males for field releases (1997 Annual Report). After 12 months in mass rearing, VIENNA 7-97 remained genetically stable as compared with previous strains, but there still remained the question concerning long-term genetic stability, especially under the different colony maintenance strategies. To examine this question, the VIENNA 7-97 colony was divided into 2 separate colonies and reared under high and low density mass rearing regimes. The stability and quality of production in the two colonies were studied.

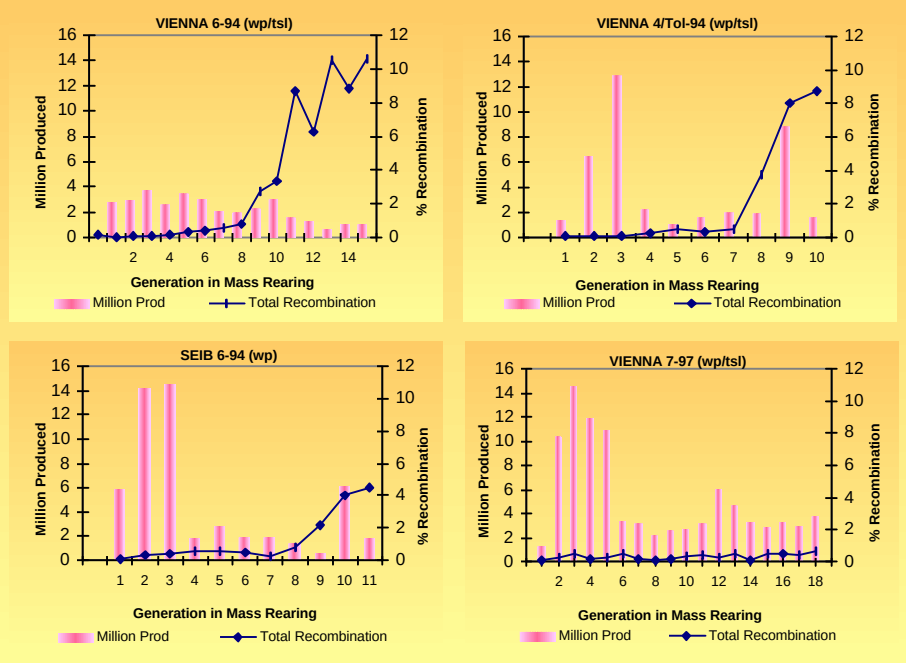
An extension of the FRS concept, called an "open filter", was explored in 1998 to test the idea of maintaining flies under more natural conditions within a factory. To be successful, an FRS needs to maintain a suite of traits in the insect that would enhance (or maintain) field related attributes such as mating behaviour, whilst retaining those attributes required in mass rearing, such as oviposition through nets. Using VIENNA 7-97 an open filter rearing system was established and some production and behavioural traits documented.

In many GSS, individuals which carry a triplication of some genetic material are able to survive to the pupal and even adult stage. These individuals have a very low fitness and can decrease the quality of pupal and adult production. The role of these triplication males in modifying apparent pupal quality in colony production was confirmed, using a GSS (AUSTRIA 6-97) (see 1997 Annual Report) which carried an extra genetic marker, y, yellow. *Research in 1998 continued to audit the impact of triplication males in male-only production, by studying their occurrence after the application of generic heat treatments of eggs of AUSTRIA 6-97.*

4.1 VIENNA 7-97 UNDER STRESS

As described in **Section 3.1**, this GSS was mass-reared in order to monitor the occurrence of Type-2 recombinants and it has shown remarkable stability in comparison with others that have been mass-reared at Seibersdorf (**Fig. 17**).

Figure 17. A comparison of genetic stability of various genetic sexing strains that have been mass reared in Seibersdorf.

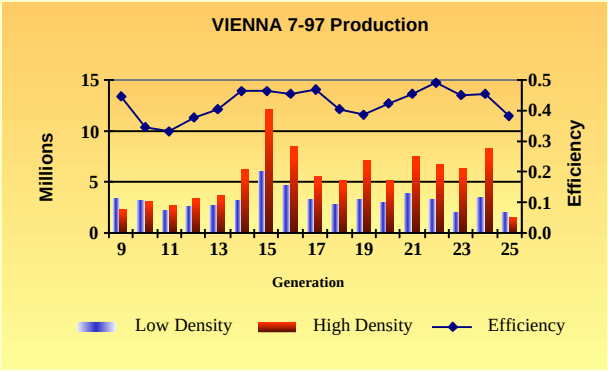


During March-August 1997, 6 generations of VIENNA 7-97 were maintained using ca. 213,000 flies per cage (1.2 m³) with a sex ratio of 0.8 females per male (the preferred ratio for colony cages). In September 1997, this colony was split into two experimental colonies: a "low-density" colony and a "high-density" colony, with attributes shown in **Table 13**. The "high-density" colony was used to simulate the rearing system that had been used in the Mendoza facility and which had led to the rapid increase in Type-2 recombinants. Both of these colonies have been reared for a further 16 generations with separate adult colony maintenance, larval rearing and pupation processes. The level of pupal production for the high-density colony was 75% higher than for the low-density colony, but in each colony only 10% of females produced were used in cages. Low-density cages retained the same basic number of flies per cage as used traditionally in Seibersdorf (**Table 13**) and with equal numbers of males and females per cage. The number of flies per high-density cage was increased by 12.5%, and females outnumbered males (**Table 13**). The high density scenario had 3 implications:

Table 13. Values of parameters in VIENNA 7-97 low and high density colonies.

	Low density cages	High density cages
Female/Male ratio	1.0	2.5
Flies/Cage	213,500	240,250
Cm²/Fly	1.29	1.15

Figure 18. Production of VIENNA 7-97 low and high density colonies during 1998.



1. a large proportion of white pupae (putative females) in relation to brown pupae (putative males) were used, in order to bias the sex ratio in favour of females. However, in most studies in Seibersdorf, instability was usually expressed by males emerging from white pupae so it was

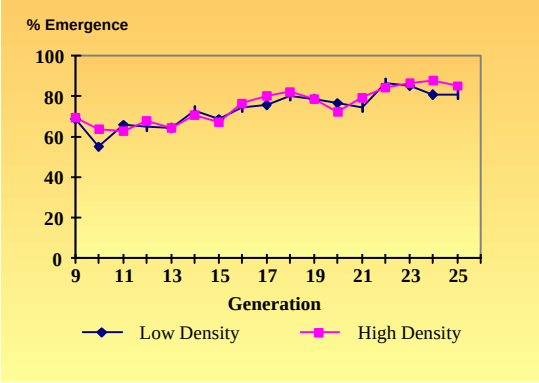
expected that a distorted sex ratio in favour of white pupae would exacerbate this pattern of breakdown.

2. space for individuals to lek, mate, oviposit and rest was more limiting. This type of additional stress would generally favour recombinants and lead to an increase in their number.

3. since females were abundant, males would no longer be compelled to compete as vigorously for mates, as in the low density scenario.

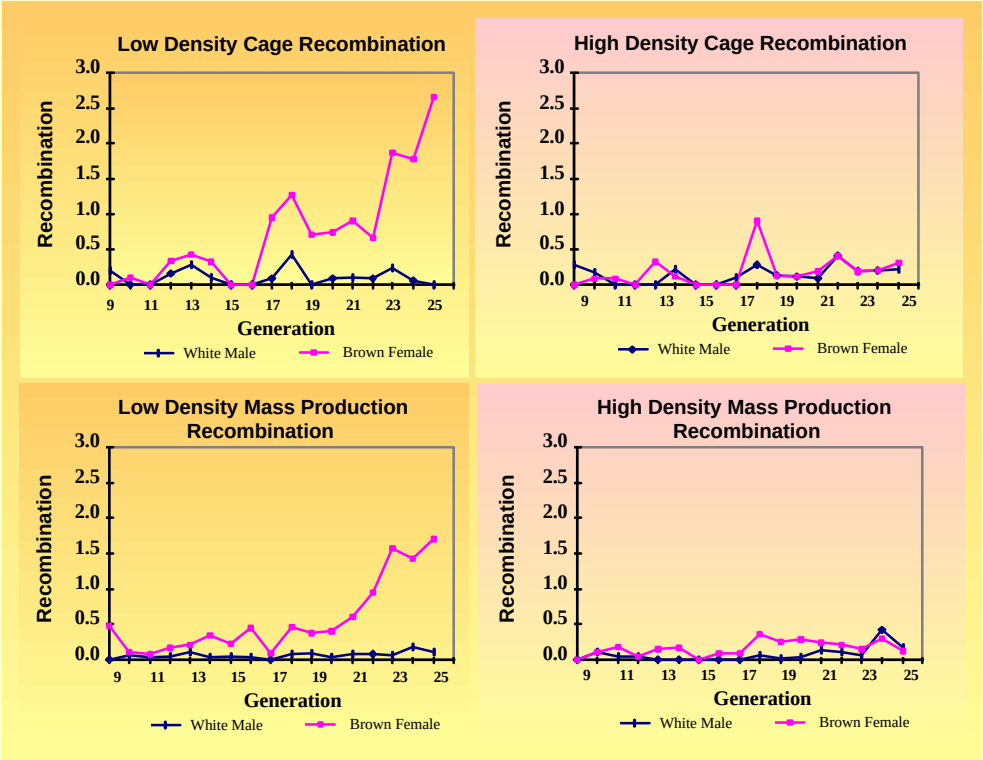
Mass production for low and high density colonies averaged 3.2 and 5.6 million pupae/generation respectively (**Fig. 18**). Cages were maintained in egg production for 8-10 days, new cages were made weekly and seeding was done weekly. Production increases in generation 15 were due to a related experiment, but production in both colonies was maintained in proportion to each other. Both colonies demonstrated equal efficiency in production (the proportion of eggs that become pupae) with an average of 42% (**Fig. 18**), which is higher than the average efficiency reported in 1997.

Figure 19. Emergence rates from pupae produced in the low density and high density production colonies.



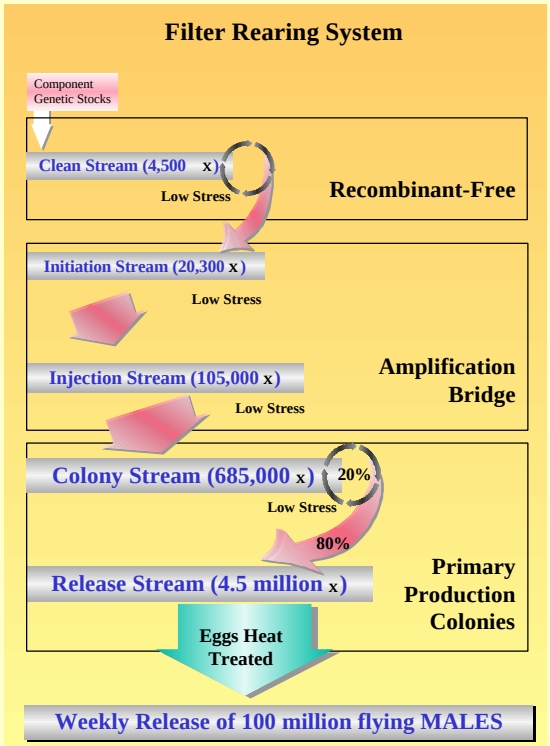
Rates of emergence of flies from pupae, and the proportion of recombinants in the total production and in the progeny that were loaded into cages, were monitored in each colony. Emergence rates were the same for both sexes, in both colonies, and showed a significant increase over time (**Fig. 19**). This and the increased efficiency of rearing may indicate some degree of domestication in these colonies.

Figure 20. Rates of recombination found in low and high density colony production, and the level of recombination in parent cages each generation.



The primary interest of this experiment was to investigate the occurrence of Type-2 recombinants during mass rearing. Contrary to expectation, Type-1 Recombination occurred in the low density colony (**Fig. 20**), where females emerged from brown pupae, while no increased recombination developed in the high density colony. Type-1 Recombination takes place between the part of the autosome which carries the wild-type gene and is translocated to the Y chromosome and the free autosome which carries the mutant gene. Recombination occurs in the region between the translocation breakpoint and the gene. This results in the production of wild-type females (from brown pupae) which have a selective advantage over the homozygous mutant females and depending on the rearing conditions, they can accumulate in the colony. These females cannot be separated from the males and therefore they reduce the accuracy of the sex separation and contaminate male production (see 1997 Annual Report). The frequency of males emerging from white pupae remained low and probably reflected the base line frequency of triplication individuals in the strain (**Fig. 20**).

Figure 21. A representation of the Filter Rearing System (FRS) and the relationship between its various components.



The level of recombination, as measured by pupal colour, in the low density colony increased to ca. 2%. However, temperature tests performed on these colonies in generation 25 indicated that the *tsl* mutation in females was decreasing, but they retained the *white pupae* trait. This implies a second Type-1 Recombination event in this colony in which recombination occurred between the *wp* and *tsl* genes. The conditions or stimuli that promote or accentuate these events are not known, but clearly some conditions of mass rearing favour these recombinants. In this case, it is presumed that loading cages with more brown pupae than white pupae, increased the probability of selection. The increase in *wp*/*tsl*$^{+}$ females is probably due to the increased fitness of non-*tsl* individuals.

It must be concluded that under certain mass rearing conditions, VIENNA 7-97 is also prone to recombination. Clearly, the filter rearing system in mass production of GSS cannot be avoided.

4.2 THE OPEN FILTER CONCEPT

The Filter Rearing System (**Fig. 21**) was developed in Seibersdorf in 1996. It has been instrumental in resolving the problems associated with the accumulation of recombinants in large scale mass rearing of GSS in Argentina, Chile, Guatemala and Madeira. Fundamentally, the Filter Rearing System (FRS) is a protocol designed to maintain a small colony of insects (called a clean stream), in which desired traits are selected (e.g. zero recombination) and undesirable traits are removed. The clean stream is linked via an amplification bridge to the large mass rearing colonies required in operational programmes.

Apart from the value of controlling recombination in mass reared GSS, the FRS has the potential to control other quality aspects of mass reared insects which might enhance field performance, such as a high level of mating competitiveness. Adaptation, selection, acclimatization, colonization and domestication are a balance of integrated forces that must be considered in rearing colonies of insects. However, care must be taken to maintain those traits which have been selected and are needed

in mass rearing, such as oviposition through nets for example. Traditionally, colonies of medflies have been housed in very large cages and females lay their eggs through the netted side walls of the cages. Eggs are collected in troughs of water placed under the cages. The cages generally contain a high density of individuals in optimized environments but with a certain level of stress. It is essential to retain the egg-collection system, but the high cage densities undoubtedly change mating effectiveness, especially in colonies that are many years old.

This antagonism between desirable traits for the field and special traits required in mass rearing might be resolved using the FRS. Research into an "Open Filter" concept was initiated which aimed to demonstrate that:

a) a small colony (a clean stream) of medflies could be maintained outside of a cage,

b) females in such a system would retain the ability to oviposit through nets and

c) male mating performance may improve in such a system.

A colony of VIENNA 7-97 was housed in a 3m x 4m room, and excluded from air conditioning and humidifying vents. Flies emerged from containers of pupae placed on the floor of the room. They had to fly more than 2 meters to obtain food and water suspended from the ceiling. A standard oviposition cage was placed in the middle of the room and was modified to allow oviposition through the oviposition nets into the cage, rather than out of the cage; eggs were collected in a tray of water within the base of the cage.

Table 14. The space allocation for males and females (cm² per individual), Total number of individuals and the ratio of females to males in the "open filter".

Generation	Cm²/♂	Cm²/♀	Total flies	Sex Ratio ♂/♀
1	486	325	475,172	0.7
2	514	466	378,857	0.9
3	474	311	494,710	0.7
4	503	618	334,837	1.2
5	358	330	196,581	1.1
6	1,944	1,812	98,680	0.9
7	8,637	7,223	23,647	0.8
8	8,518	10,391	19,872	1.2
9	7,107	7,172	26,164	1.0

The room was cleaned of adults between generations and nine discrete generations were reared during 10 months of 1998. The first 5 generations were set up using ca. 420,000 flies (**Table 14**) in order to select females which would retain their orientation to oviposition nets in the "open" environment. Generations 7-9 were maintained with a reduced density (23,000 flies) in order to promote a greater search for mates. The sex ratio in the open filter was not held constant (**Table 14**), and varied by 30%. Egg production improved from 2.5 to 4 eggs/female/day over 9 generations, but was effectively only 25% of normal production.

This preliminary research into the open filter concept did not seek to measure a wide range of parameters, but rather the qualitative potential of such an idea. Egg production was not measured specifically, but indications were that reduced egg production was likely and would necessitate much larger clean streams, or perhaps another stage in the FRS amplification bridge. Egg production may even be improved by using a smaller oviposition site. However, the apparent increase in mating performance of males over 7-8 generations has obvious potential (see **Section 5.2**). The results of the open filter concept are encouraging and need to be explored further. It needs to be established that egg productivity does not decrease when clean-stream flies are put inside the standard cages. It also needs to be established that a functional clean stream can be maintained and that other selective traits such as longevity could be included in the open filter operations. Finally it needs to be established that the gains in such traits as mating performance are retained during the other stages of the FRS and into the field.

4.3 HIGH TEMPERATURE TREATMENT OF AUSTRIA 6-97

Temperature sensitive lethal (*tsl*) GSS of medfly allow female zygotes to be killed following high temperature treatment (34°C) during the egg stage. The high temperature treatment should produce the highest potential number of males, with few if any females (ie high accuracy). The results of studies of VIENNA 6-94, VIENNA 4/Tol-94 (1995/96) and VIENNA 7-97 showed that the application of the high temperature treatment during late embryogenesis (24-48 h after egg collection; the Low-High (LH) treatment) produces significantly more male flies compared with heat treatments during early embryogenesis (0-24h after egg collection). The LH treatment conserves nearly all potential male flies, with less than 0.5% "contamination" with females.

VIENNA 7-97 is much more efficient for rearing only males than VIENNA 6-94. This was assumed to be an effect of the very low survival of triplication males (unbalanced males) to the pupal stage. VIENNA 7-97 is the first GSS to demonstrate a level of recovery which is close to the theoretical maximum (25% of eggs becoming males), and which is not contaminated by triplication individuals. However with VIENNA 6-94 and VIENNA 4/Tol-94, the proportion of triplication individuals after the LH temperature treatment of eggs increased with consecutive daily pupal collections. This reduces the emergence quality from these pupal collections. In addition to reducing overall quality, it requires a rearing strategy to control both the rate of rearing (therefore the inherent quality of consecutive collections) and the tracking of separate larval collections so that management for releases could be enhanced.

The effect of triplication males on *tsl* colony production was established during 1997, using the yellow body GSS AUSTRIA 6-97 [T(Y;A) 2-22 $y^{+}wp^{+}tsl^{+}/y$ *wp tsl*] (see 1997 Annual Report)). AUSTRIA 6-97 is based upon a VIENNA 6-94 *tsl* strain, but contains a third selectable marker; a body colour mutation (*y*), located on chromosome 5. The inclusion of this mutation enables the fate of triplication individuals to be followed and as with all *tsl* strains, the inclusion of the *wp* marker enables the genetic integrity of the strain to be monitored. White and brown pupae are produced, with males emerging from brown pupae and females emerging from white pupae. The additional *ye* marker means that females have a yellow body as well as being *wp* and *tsl*. However, male triplication individuals will also be *yellow*, whereas normal males will have the wild-type body colour, and in this way the appearance of triplication individuals can be audited in pupal collections. Research in 1998 continued to measure the impact of triplication males in GSS, by studying their occurrence following heat treatment of eggs for male-only production of AUSTRIA 6-97.

Eggs of AUSTRIA 6-97 were collected over 5 hours and placed into 6 liter plastic flasks containing 5 liters of tap water, with the eggs being kept in suspension by using aeration through aquarium air stones. Flasks were placed into water baths which were maintained at a constant temperature using thermostatically controlled circulating pumps. Only 2 temperatures were used: 34 ± 0.5°C was used to kill temperature sensitive individuals and 23 ± 0.5°C was used as the non-lethal (control) temperature. Treatments of eggs were conducted over the 48 h immediately after egg collection. The treatment protocol was divided into two, 24-h periods. A control treatment of 2 concurrent periods of 24 h (48 h) at the low temperature of 23°C (low-low, LL) was used to compare the production and quality of flies from 3 high temperature treatment regimes: 24 h at 23°C followed by 24 h at 34°C (low-high, LH); a reciprocal treatment of 24 h at 34°C followed by 24 h at 23°C (high-low, HL); and 2 concurrent periods of 24 h at 34°C (high-high, HH).

Water baths were maintained at set temperatures with flasks being physically moved between baths according to their respective treatment protocols, to facilitate faster equilibrium to temperature changes. Egg samples were taken at 24 h and 48 h to measure the impact of treatments on egg hatch.

After 48 h of treatment, 3.6 ml of eggs was seeded onto trays of standard diet. Each tray was maintained individually in open-ended

Table 15. Total hatch (from larvae at sampling plus from remaining good eggs) for Austria 6-97 and VIENNA 6-94, after 24 h and 48 h of the different heat treatments, after 5 d incubation at 23°C post sampling.

	After 24 h		After 48 h	
Treatment	**AUSTRIA 6-97**	**VIENNA 6-94**	**AUSTRIA 6-97**	**VIENNA 6-94**
High-High (48 h 34°C)	23.2 a	39.0 a	18.4 a	30.9 a
High-Low 24 h 34°C - 24 h 23°C)	23.3 a	35.8 a	25.0 b	38.2 a
Low-High (24 h 23°C - 24 h 34°C)	81.5 b	80.8 b	47.8 c	55.5 b
Low-Low (48 h 23°C)	82.8 b	82.1 b	81.0 d	81.6 c

Means followed by the same letter in each column are not significantly different (P > 0.05, Tukey HSD test)

plastic bags for 3 days at 23 ± 1°C and 100% RH. Trays were removed from the plastic bags after 3 days and were placed separately over large metal collection trays containing dry bran. Collecting trays containing larvae were replaced daily for 5 days from the first larval popping. Larvae were allowed to pupate in the bran for 8 days at 23 ± 1°C before pupae were separated from the bran by using a mechanical winnowing machine.

Daily pupal production from each tray was measured volumetrically. A 5-ml sample of pupae from each daily collection of each treatment was separated into white and brown pupae. The ratio of brown to white pupae, sex ratio of flies, emergence, partial emergence, and deformed males and females, and non-emerged pupae from white and brown pupae were counted from the sample.

Untreated eggs had an egg hatch (EH) of ca. 80% (**Table 15**) and early treatment markedly reduced EH as expected. After 48 h of treatments, EH for all treatments was significantly different, with 48 h of high temperature treatment (HH) resulting in the lowest EH, followed by HL, LH and LL respectively. The results are similar to those for VIENNA 6-94.

The number of pupae produced from equal aliquots (3.6ml) of eggs, for each heat-treatment, is shown in **Table 16**. Treatment during the second 24 h (LH) produced more than double the number of brown pupae (potential males), compared with heat treatment during the first 24 h (HL). In all heat treatments brown pupae represented more than 99.7% of pupal production (ie predominantly male).

Triplication individuals are identified as yellow bodied males emerging from brown pupae (balanced males emerging from brown pupae will have the wild-type body colour). **Table 17** shows that emergence of males from brown pupae from the LH treatment was significantly greater than all other treatments in this experiment. These results are similar to VIENNA 6-94 for the various parameters and in both cases it should be noted that the number of brown pupae from the LH treatment is significantly less than the number of brown pupae produced from untreated eggs (LL). It is

Table 16. The mean number of brown and white pupae produced from the different heat treatments of eggs of AUSTRIA 6-97 and VIENNA 6-94 and the accuracy of production as the proportion of brown pupae in total pupae.

	AUSTRIA 6-97			VIENNA 6-94		
Treatment	**No. Brown Pupae**	**No. White Pupae**	**% Brown in Total Pupae**	**No. Brown Pupae**	**No. White Pupae**	**% Brown in Total Pupae**
High-High	2,156 a	2 a	99.9	5,543.6 a	13.6 a	99.8
High-Low	8,752 b	27 a	99.7	6,990.0 a	403.0 b	94.5
Low-High	17,209 c	12 a	99.9	14,935.1 b	71.3 a	99.5
Low-Low	32,276 d	10,954 b	74.6	22,127.7 c	11,129.0 c	66.5

Means followed by the same letter in each column are not significantly different (P > 0.05, Tukey HSD test)

Table 17. The mean of emerged flies (emerged + deformed), partially emerged flies and non-emerged pupae, from the total of brown pupae produced from the different heat treatments of eggs of VIENNA 6-94.

	AUSTRIA 6-97				VIENNA 6-94
Treatment	% Emergence of Flies	Partially Emerged Flies	Deformed Flies	Brown Pupae not Emerged	% Emergence of Flies
High-High	78.9 a	3.3 a	3.0 a	14.8	75.2 a
High-Low	77.2 a	3.1 a	2.2 a	17.5	68.9 a
Low-High	88.0 b	2.9 a	1.8 a	7.3	74.4 a
Low-Low	64.9 c	6.3 b	2.6 a	26.2	57.6 b

Means followed by the same letter in each column are not significantly different (P > 0.05, Tukey HSD test)

expected that the difference is predominantly due to the production of triplication individuals.

Each of the emergence categories of **Table 17** can be separated into normal (balanced) and triplication individuals, as shown in **Table 18**. Un-emerged brown pupae were dissected to identify un-emerged normal and un-emerged triplication individuals. However, some un-emerged pupae could not be identified and they were classified as un-identified un-emerged (**Table 18**).

Brown pupae from heat-treated eggs yielded ca. 96% normal individuals, compared with 71% in pupae from untreated eggs. There was a small fraction of un-identifiable individuals (2.3%) which are assumed to be triplication individuals that failed to complete metamorphosis. There was only a small fraction of identifiable triplication individuals (1.7%) which represents less than 0.4% of the total number of emerged male flies and a quarter of these were deformed, higher than the proportion of deformed normal males. The majority of triplication individuals were partially emerged (ie failed to emerge from the pupae case) or did not emerge at all. Untreated eggs yielded a large fraction of triplication and unidentifiable individuals, which taken together represents nearly 30% of brown (male) pupae production.

However, the most striking result of this study is the low rate of emergence of normal flies from brown pupae produced from the early heat treatments (HH, HL). This implies that the early heat treatment of eggs not only kills some males, due to the maternal effect of the *tsl* mutation, but that it also impacts upon the survival of these males, later in development These results are very important for all generic heat treatments of *tsl* GSS. The LH generic heat treatment:

1. kills almost all female embryos,
2. eliminates the majority of triplication

Table 18. The mean of emerged flies (Em), partially emerged flies (PE), emerged deformed flies (D) and non-emerged pupae (NE), from the total of brown pupae produced from the different heat treatments of eggs of AUSTRIA 6-97.

Treatment	Normal				Triplication				Un-identified Un-emerged
High-High		**97.1**				**0.9**			**2.0**
	Em	PE	D	NE	Em	PE	D	NE	
	78.2	3.0	3.1	15.8	19.8	45.9	7.2	27.1	
High-Low		**94.8**				**1.7**			**3.5**
	Em	PE	D	NE	Em	PE	D	NE	
	79.3	2.5	2.2	16.0	25.0	43.2	5.7	26.0	
Low-High		**95.8**				**2.6**			**1.5**
	Em	PE	D	NE	Em	PE	D	NE	
	90.2	2.1	1.6	6.1	21.9	35.0	9.9	33.2	
Low-Low		**71.2**				**17.2**			**11.6**
	Em	PE	D	NE	Em	PE	D	NE	
	90.2	0.9	0.9	8.0	26.6	32.9	11.1	29.5	

individuals,

3. conserves production of brown pupae and
4. conserves normal (balanced) male flies.

Table 19 summarizes the average number of balanced males and triplication males produced in each treatment. Triplication males represent less than 1% of total males produced in the LH treatment of eggs, compared with 10% from untreated eggs. However, 25% of normal males are also lost following this treatment. This loss needs further investigation to optimize the heat-treatment protocol. This loss was less for VIENNA 7-97 (see 1997 Annual Report), and may point to a difference between the strains.

Table 19. The average numbers of balanced and triplication males produced from equal alliquots of eggs of AUSTRIA 6-97.

Treatment	Normal Males	Triplication Males	Total Males
High-High	1662	5	1667
High-Low	6578	45	6623
Low-High	15083	144	15227
Low-Low	20899	2089	22988

Now that GSS are becoming increasingly more stable, research into the number of generations to reach threshold levels of recombination is becoming less important than the conditions which give selective advantages to recombinants in the first place. This is difficult research and is, to some extent, negated by the implementation of the FRS. The FRS will remain a key to the successful use of GSS in large scale operations. Future research is directed toward properly harnessing the advantages of the FRS. AUSTRIA 6-97, as a tool in auditing triplication individuals during different rearing treatments, has been very useful and has highlighted a need to explore the optimization of the high temperature treatment of eggs of *tsl* strains. This work will be ongoing in 1999.

5. Behavioural Evaluation of Medfly Strains

THE ASSESSMENT *of behavioural quality represents the final evaluation of medfly GSS, which are synthesized and mass reared at Seibersdorf, before they are transferred to rearing facilities in Member States. During the development of this evaluation a field cage quality control test was devised (see 1997 Annual Report) which is now recognised as an integral part of the standard quality control procedure for fruit fly SIT programs.*

Data presented in the 1997 Annual Report documented the level of sexual compatibility among wild populations originating from 5 continents, and between wild populations and various GSS. In 1998, additional strains and populations were tested and documented.

During summer 1998, a series of field cage tests was carried out in Madeira using males from the GSS VIENNA 7-97 which were shipped as pupae from the large colony maintained at Seibersdorf. The VIENNA 7-97 strain was the proposed replacement strain for the facility in Madeira and had been previously successfully tested with wild flies from South Africa. However, the results from the test in Madeira demonstrated extremely low performance of the VIENNA 7-97 males with Madeiran flies. These tests were repeated in Seibersdorf to try to confirm the unexpected results in Madeira.

The Filter Rearing System (FRS) is based on the maintenance of a small isolated colony in which adult flies are maintained under relaxed rearing conditions (for example low fly density) and from which recombinant individuals can be removed to retain the integrity of GSS (see 1997 Annual Report). Material from this colony is used in a unidirectional production system that ensures that no insects are returned to the original small colony after going through the rigours of mass rearing. The FRS represents a much improved rearing system to prevent the accumulation of unsuitable behavioural traits and recombinant individuals and is currently under evaluation and development. Using the FRS concept, changes or improvement in the behaviour of mass reared insects can be investigated. In an attempt to develop this concept further, a colony of VIENNA 7-97 was maintained in an open room at low density. After 8 generations under such a regime, the behavioural quality of the strain was compared in field cage tests with the standard colony.

Through the support of a Co-ordinated Research Project (CRP) in collaboration with many scientists in Member States, a successful study of medfly mating behaviour is coming to an end in 1999. However, field cage studies on mating behaviour will continue to play an essential role in the evaluation of GSS at Seibersdorf. The main achievements of the programme and possible future research perspectives are summarised.

5.1 EVALUATION OF VIENNA 7-97

The Western Cape Region of the Republic of South Africa is embarking on an SIT programme to eradicate the medfly and the Natal fruit fly (*Ceratitis rosa* Karsch) from the Hex River Valley (**Fig. 22**). In May 1998, the sexual compatibility and mating competitiveness of VIENNA 7-97 flies with wild flies from South Africa were evaluated in green house tests in Seibersdorf.

Figure 22. View of the Hex River Valley (Western Cape, Republic of South Africa).

From August until October 1998, as a consequence of the dramatic results obtained from the field cage tests with this strain in Madeira, a second series of green house tests with this strain was conducted at Seibersdorf. In addition, a series of tests was carried out in November - December 1998 involving GS 7E and wild flies from Madeira. GS 7E is genetically identical to VIENNA 7-97 but has not been mass reared.

5.1.1 Biological material

Wild pupae from South Africa were collected from guava fruits and hand carried to Seibersdorf in May 1998. Wild pupae from Madeira were collected mainly from guava fruits and sent to Seibersdorf in several express courier shipments of about 4-500 individuals.

The VIENNA 7-97 individuals were obtained as pupae (from pupal collection 1 to 3) from the low density (LD) colony at Seibersdorf (see **Section 4.1**). When tested with South Africa, the strain was in its 17th generation, and in its 20th to 22nd generation when tested with Madeiran flies.

The GS 7E strain is the parental strain of VIENNA 7-97. Since the original strain was transferred into mass rearing (February 1997), the backup strain, GS 7E, has been maintained in the genetics laboratory in small rearing cages, at relatively low fly density. It was tested 23-24 generations after it was first constructed.

Following the standard protocol for behavioural tests, flies were sorted by sex on emergence and provided with food (sugar and yeast hydrolysate, proportion 3:1) and water. Male and female individuals were kept apart until testing.

5.1.2 The tests

Sexual compatibility and mating competitiveness of GSS flies were assessed following the protocol described in the 1997 Annual Report. The strains were compared in bisexual tests (involving males and females of both strains) done in a green house (**Fig. 23**), in which temperature is controlled and the relative humidity (RH) is monitored. Inside the green house there is a cage (15m^3) made of netting material containing 5 citrus trees.

30 flies of each sex and strain were released at dawn, and the test lasted for 7 consecutive hours. One replicate was done per day and all the flies were removed from the cage at the end of the day. During the test, the mated flies were not released back into the cage, neither replaced.

Figure 23. Green house used at Seibersdorf for behavioural tests of medfly strains.

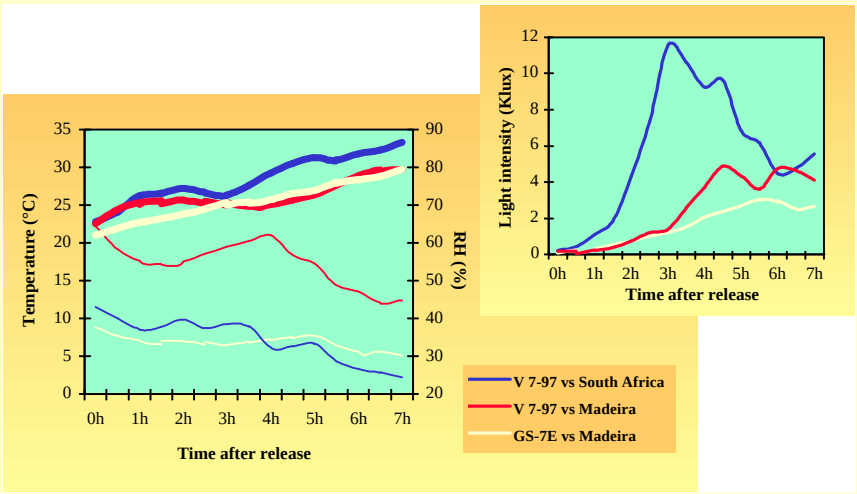
5.1.3 Environmental conditions

The environmental conditions which prevailed inside the cage during the tests are shown in **Fig. 24**.

For all tests, the conditions were suitable to initiate medfly sexual activities.

Apart from a higher light intensity during the tests of VIENNA 7-97 with South Africa, and a slightly higher RH when testing VIENNA 7-97 with Madeira, no other major differences have been noticed between the periods of testing. The barometric pressure ranged between 986 and 1000 mBar.

Figure 24. Environmental conditions recorded in the green house during the evaluation of VIENNA 7-97 and GS-7E genetic sexing strains.



Bold line: temperature; normal line: relative humidity

5.1.4 Overall mating activity

The overall mating activity as measured using the PM index (**Table 20**) revealed no significant difference between the three strains tested (F=3.252, df=2, P=0.056), as shown in **Table 21**.

This indicates that the testing conditions were suitable for the flies and were equivalent throughout the replicates and between the three tests.

5.1.5 Male calling activity

In order to be able to compete with wild males for mating, GSS males must exhibit the same calling behaviour pattern as their wild counterparts.

No significant difference was found when comparing the proportion of wild and GSS males calling throughout the day in the tests involving VIENNA 7-97 and Madeira, and GS 7E and Madeira (F=3.757, df=1, P=0.054 and F=3.794, df=1, P=0.053 respectively). However, in the comparison of VIENNA 7-97 and South Africa, significantly more GSS males than wild males were found calling (F=15.143, df=1, P=0.000).

In a subsequent step of the analysis, the calling activity pattern of GSS males during the three tests was compared. The proportions of GS 7E and VIENNA 7-97 (tested with Madeira) calling males were significantly higher than that of VIENNA 7-97 tested with South Africa (F=5.765, df=2, P=0.003) (**Fig. 25**).

The comparison of the calling activity pattern of wild males in the three tests shows that a significantly lower proportion of wild males was actively calling in the test involving South Africa and VIENNA 7-97 than in the two other tests (F=27.880, df=2, P=0.000) (**Fig. 25**).

In summary, the results indicate that: (i) VIENNA 7-97 and GS 7E males exhibit the

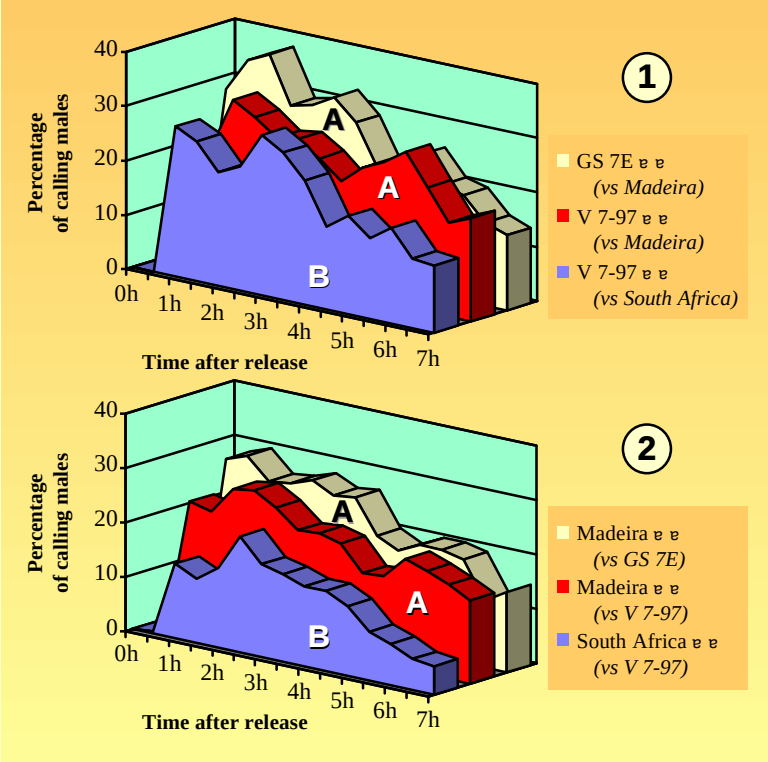
Table 20. Indices used to measure the sexual compatibility and mating competitiveness of medfly strains.

TO MEASURE	INDEX FORMULA	VALUES min.	equil.	max.
Participation in Mating	$PM = \frac{\text{No of pairs collected}}{\text{No of females released}}$	0	+0.5	+1
Sexual isolation	$ISI = \frac{(LL + WW) - (LW + WL)}{\text{Total No of matings}}$	-1	0	+1
Male Relative Performance	$RPI_{\varnothing} = \frac{(LL + LW) - (WW + WL)}{\text{Total No of matings}}$	-1	0	+1
Female Relative Performance	$RPI_{\varnothing} = \frac{(LL + WL) - (WW + LW)}{\text{Total No of matings}}$	-1	0	+1
Male Mating Competitiveness	$RSI^{(1)} = \frac{LW}{LW + WW}$	0	+0.5	+1

(1) After McInnis et al. (1996)
L and W stand for Laboratory and Wild strains respectively, first letter ♀, second letter ♂

same calling activity pattern, which is similar to that of wild flies from Madeira, and (ii) during the test of VIENNA 7-97 with South Africa, males of both types were significantly less active.

Figure 25. Calling activity pattern of GSS [1] and wild [2] males during the three green house tests.



Areas labelled with the same letter on the same graph do not differ significantly according to Tukey's HSD test (P<0.05)

5.1.6 Mating competitiveness of VIENNA 7-97 males

Mating competitiveness is measured using the Relative Sterility Index (RSI) (**Table 20**) although for GSS males it can also be assessed in a unisexual experiment.

There was no significant difference (F=12.686, df=2, P=0.000) between the mating competitiveness of VIENNA 7-97 males when competing with South African flies and that of GS 7E males when competing with Madeiran flies (**Table 21**).

However, there was a highly significant difference between those two experiments and the mating competitiveness of VIENNA 7-97 males when competing with Madeiran flies (**Table 21**).

This indicates that: (i) after nearly two years of small scale rearing, GS 7E retained its mating competitiveness with Madeiran flies (for information, the mean RSI obtained with various GSS is about 0.349, see 1997 Annual Report); (ii) VIENNA 7-97 males, when tested against South African flies in May 98, were as competitive as any other GSS males (RSI=0.30); (iii) VIENNA 7-97 males when tested against Madeiran flies in August-October 98, achieved only 6% of wild female mates (RSI=0.06).

5.1.7 Sexual compatibility of VIENNA 7-97 GSS

The sexual compatibility between two populations is measured using the Isolation Index (ISI) (**Table 20**).

There is no significant difference between the ISI value for GS 7E vs Madeira and VIENNA 7-97 vs South Africa. The ISI of VIENNA 7-97 vs Madeira is however significantly different from both. (F=26.123, df=2, P=0.000) (**Table 21**).

The ISI values of GS 7E vs Madeira and VIENNA 7-97 vs South Africa are close to the values usually obtained when comparing any GSS with a wild population (see 1997 Annual Report) whereas the value obtained with VIENNA 7-97 vs Madeira is about twice that usually observed (ISI=0.48±0.55).

This indicates that: (i) GS 7E is sexually compatible with wild flies from Madeira as is VIENNA 7-97 with flies from South Africa when tested in May 1998. However, when tested in August-October 1998 this strain showed very low compatibility with Madeiran flies.

5.1.8 Relative performance of VIENNA 7-97 males and females

Relative male and female performance is measured using ♀RPI and ♂RPI (**Table 20**).

For those two indices, results obtained with VIENNA 7-97 vs Madeira are also significantly

different from those obtained for the two other combinations (F=23.944, df=2, P=0.000 and F=6.340,df=2, P=0.006 for ɐRPI and ˛RPI respectively) (**Table 21**).

In previous comparisons of GSS and wild populations from different origins (see 1997 Annual Report), the ɐRPI value was usually close to zero, demonstrating a nearly equal mating performance of GSS and wild males. In such comparisons, the ˛RPI value is usually about +0.25, suggesting that GSS females are more prone to mate than are wild females. In the tests for GS 7E vs Madeira and VIENNA 7-97 vs South Africa, similar values were obtained. However, for VIENNA 7-97 vs Madeira there was a critical lack of performance of both males and females (**Table 21**).

5.1.9 Conclusions on the evaluation of VIENNA 7-97

The assessment of the sexual compatibility and the mating competitiveness of VIENNA 7-97 GSS has shown that:

1. the overall mating activity of the flies in the three tests was similar. Consequently any recorded differences between the strains are unlikely to result from a difference in the conditions of the tests.
2. GS 7E males and VIENNA 7-97 males (vs Madeira) follow the same calling activity pattern and in the same proportion as wild males. Consequently any differences in mating between the two strains must be concerned with a subsequent step of the male courtship behaviour.
3. the VIENNA 7-97 males were not competitive for mating with wild Madeira females, even though the parental strain, GS 7E was as competitive as any other GSS with the same wild population.
4. the VIENNA 7-97 population tested in August-October 1998 was almost completely sexually isolated from the wild Madeira population, even though GS 7E was compatible. In addition, both VIENNA 7-97 males and females when tested in August-October 1998 showed a critical lack of performance, which was not found with GS 7E flies.
5. The overall performance of GS 7E flies with Madeiran flies and VIENNA 7-97 flies with South African flies was similar. It should also be noted that the VIENNA 7-97 GSS tested in May 1997 against wild flies from Israel also gave good overall results (see 1997 Annual Report).

On the basis of the above, it is concluded that an event occurred between May and August which affected the biological fitness/mating behaviour of VIENNA 7-97 flies. As the VIENNA 7-97 males called in the same way as wild males, two hypotheses are possible: either those males did not attract the wild females, or they did not perform the courtship in a suitable way for wild females. There is also the possibility that the overall quality of the flies tested was low.

The GS 7E performed well and could therefore be introduced into mass rearing. The observed differences between the GS 7E and VIENNA 7-97 strengthen the concept of the FRS, which is based on a small colony

Table 21. Comparison of the mating competitiveness and sexual compatibility of VIENNA 7-97 and GS 7E genetic sexing strains.

	Month tested	No. reps	No. pairs	Indices				
				PM	RSI	ISI	ɐ RPI	˛ RPI
V 7-97 vs Madeira	08-10/98	10	256	0.38 ± 0.10 a	0.06 ± 0.09 b	0.48 ± 0.55 b	-0.80 ± 0.21 b	-0.42 ± 0.75 b
V 7-97 vs South Africa	05/98	7	245	0.48 ± 0.11 a	0.30 ± 0.22 a	0.26 ± 0.17 a	-0.03 ± 0.28 a	0.26 ± 0.49 a
GS 7E vs Madeira	11-12/98	10	311	0.52 ± 0.16 a	0.34 ± 0.12 a	0.07 ± 0.17 a	-0.20 ± 0.19 a	0.14 ± 0.11 a

Data presented as mean ± sd

Numbers in the same column followed by the same letter do not differ significantly according to Tukey's HSD test (P<0.05)

maintained under relaxed conditions.

The following three hypotheses are relevant to the differences found between VIENNA 7-97 with Madeira and South Africa wild populations:

1. Wild populations from South Africa and Madeira exhibit different sexual behaviour and one strain can therefore accept flies for mating which are rejected by the other strain. This hypothesis would contradict the recent findings on the sexual compatibility of medfly populations world-wide. In addition, the GS 7E flies (representing the same original strain) would also be rejected by Madeiran flies, if the hypothesis is true.

2. The three different samples of VIENNA 7-97 flies tested at Seibersdorf (plus the samples tested on Madeira) were of poor quality. However, the routine QC parameters for these samples did not reveal any deviation from the normal profile.

3. During the period between May and August-October 1998, the VIENNA 7-97 GSS experienced a selection change in the mass-rearing which led to some behavioural differences accumulating. According to the mass rearing team, no obvious event was observed during this period although the QC data did show an increasing level of recombination (see **Section 4.1** for details).

Further tests using this strain will be carried out in order to shed more light on the different observations.

It is important to note that the VIENNA 7-97 strain used in these tests was being evaluated at Seibersdorf to study its performance under mass rearing conditions and was not to be used in operational programmes. It had not been outcrossed with any field material as would normally be the case for a strain to be transferred to an operational facility.

5.2 EFFECT OF OPEN FILTER REARING ON THE BEHAVIOUR OF VIENNA 7-97 FLIES

The FRS represents an unique opportunity to investigate the effects of different rearing regimes on insect behaviour. A sample of the low-density (LD) VIENNA 7-97 colony was utilised to establish a smaller colony for the open filter experiment (see **Section 4.2**). As from the 7^{th} generation, a total of ±25,000 flies/generation (sex ratio 1:1) were used in the open filter colony (see **Section 4.2**).

The open filter colony, in its 8^{th} to 9^{th} generation, was then tested against the LD colony strain in green house experiments as described in the previous section. The objective of the test was to assess the relative mating competitiveness and sexual compatibility of the two colonies.

5.2.1 Conditions of the tests

Tests were run in September and October 1998.

Since the objective was to assess the relative behavioural quality of the open filter and LD colonies, the comparison was done in the absence of wild flies.

The environmental conditions encountered during the testing period were suitable to initiate medfly sexual activities as shown in **Fig. 26** and the overall mating activity was satisfactory (PM=0.38±0.10, **Table 22**).

5.2.2 Male calling activity

The calling activity pattern of open filter and LD colony males is shown in **Fig. 27**. and no significant difference was found between the two types of male (F=0.391, df=1, P=0.532). The open filter and LD colony males calling patterns are similar.

5.2.3 Relative mating competitiveness of open filter and LD colony males

The relative mating competitiveness of the open filter and LD colony males is assessed using two RSI formulas.

The RSI_{LD} measures the proportion of open filter (F) females mated with LD colony (LD) males:

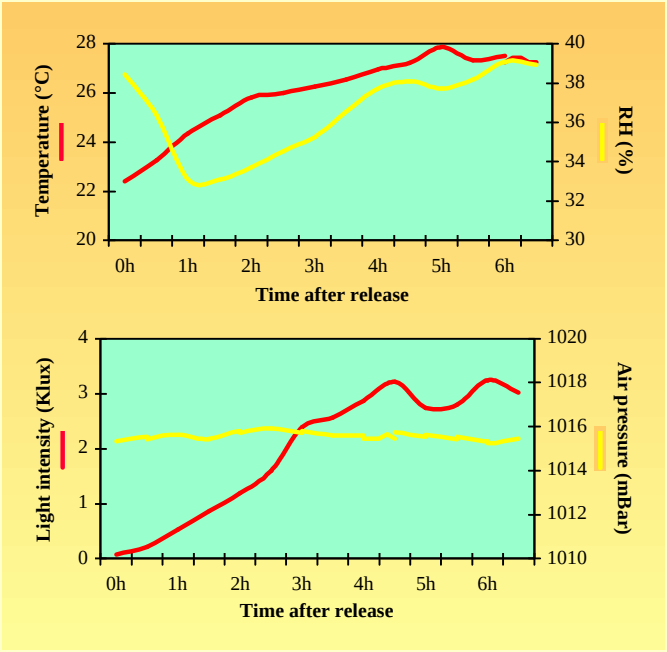
$$RSI_{LD} = \frac{LD♀\ F♂}{LD♀\ F♂ + F♀\ F♂}$$

The RSI_{Filter} measures the proportion of LD females mated with open filter males:

$$RSI_{Filter} = \frac{F♀\ LD♂}{F♀\ LD♂ + LD♀\ LD♂}$$

The RSI_{Filter} is significantly higher than the RSI_{LD} (F=4.45, df=1, P=0.04) (**Table 22**) which indicates that open filter males were more competitive than LD colony males for LD colony female mates, than were LD colony males competing with open filter males for open filter female mates.

Figure 26. Environmental conditions recorded in the green house during the evaluation of open filter VIENNA 7-97 GSS.



5.2.4 Sexual compatibility of open filter and LD colony VIENNA 7-97 and relative male and female performance

As expected, the two colonies of VIENNA 7-97 are sexually compatible (ISI=0.00±0.17, **Table 22)**

The ♀RPI value (**Table 22**) indicates a slight relative advantage for open filter male matings, and the ♂RPI indicates a slight relative advantage for LD colony female matings.

5.2.5 Duration and location of open filter and LD colony males mating

The duration of mating, starting when the pair was collected and ending with the separation of the partners, was monitored for all four types of mating. On average, matings lasted for 3 to 4 hours and some differences have been found between the four types of mating pairs (F=6.683, df=3, P=0.000) (**Fig. 28**).

Under green house or field cage conditions, it has been shown that most matings involving wild males occur on the tree and very little, if any, on the cage screen (see 1997 Annual Report). As shown in **Fig. 29**, significantly more matings occurred on the tree than on the cage screen for both types of males (F=128.736, df=1, P=0.000). However, no significant difference was found between open filter and LD colony males (F=2.998, df=1, P=0.099).

Most of the successful wild males usually call and consequently mate in the upper part of the tree (see 1997 Annual Report). As shown in **Fig. 30**, about 50 to 60% of open filter and LD colony males mated in the upper part of the tree. No significant difference was found between the proportion of open filter and LD colony males mating on the upper (F=1.515, df=1, P=0.233), the middle (F=0.262, df=1, P=0.614), or the lower (F=0.259, df=1, P=0.616) parts of the tree.

Figure 27. Calling activity pattern of open filter and LD colony males, throughout the day.

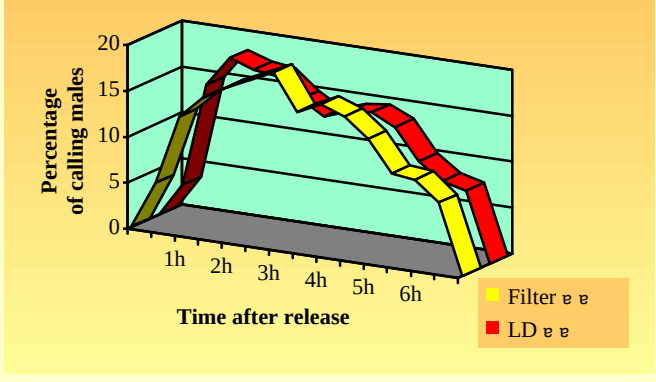


Table 22. Evaluation of the relative mating competitiveness and sexual compatibility of open filter and LD colony VIENNA 7-97 GSS.

	Month tested	No. reps	No. pairs	Indices PM	RSI "LD"	RSI "Filter"	ISI	♀ RPI	♂ RPI
Filter vs Colony	09-10/98	11	290	0.38 ± 0.10	0.37 ± 0.20 **a**	0.55 ± 0.18 **b**	0.00 ± 0.17	-0.14 ± 0.23	0.11 ± 0.55

Data presented as mean ± sd

The two RSI values are significantly different according to Tukey's HSD test (P<0.05)

5.2.6 Conclusions on the evaluation of the Open FRS

Based on data presented above, the open FRS seems to result in an improvement of male mating competitiveness.

No significant change of the fly behaviour was noticed regarding mating duration, or mating location. In the absence of wild flies, both LD colony and open filter flies preferentially mate on the upper part of the tree, as do wild flies under similar conditions.

As expected, the open FRS did not affect the sexual compatibility between the two colonies.

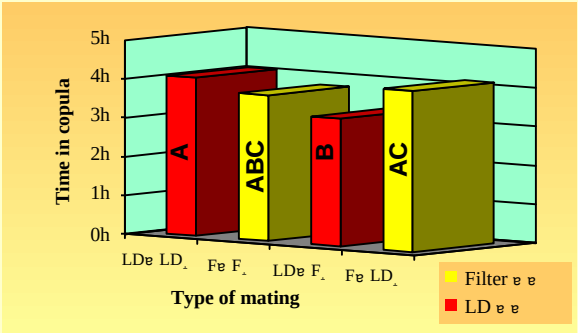
When comparing wild and GSS flies , the ♀RPI usually reflects a slight advantage for wild males, and the ♂RPI, a slight advantage for mass-reared females (see 1997 Annual Report). The ♀RPI and ♂RPI values obtained in the current experiments might reflect a slight tendency for the open filter flies to "play the role" of wild flies. However, without a control test of each of the two colonies with a wild population, this hypothesis cannot be tested.

These data, obtained during the time that LD colony VIENNA 7-97 flies proved to be of very poor mating quality when tested against Madeira wild flies, showed that individuals of the same colony were able to successfully obtain mates when competing with laboratory flies.

These results give some encouragement to the potential impact that an open FRS could have on the behaviour of mass reared flies. However, some changes in its operation are probably required, for example:

1. initiate the open FRS from a colony which has not yet been kept under mass-rearing conditions. It would be easier for an open FRS to maintain behavioural quality than to improve quality following long term mass-rearing,
2. maintain the open FRS for a longer number of generations,
3. assess the relative competitiveness of open filter and LD colony flies with a wild population.

Figure 28. Mean duration of mating by open filter and LD colony males and females.



Columns labelled with the same letter do not differ significantly according to Tukey's HSD test (P<0.05)

Figure 29. Location of open filter and LD colony males matings on the tree and on the cage screen.

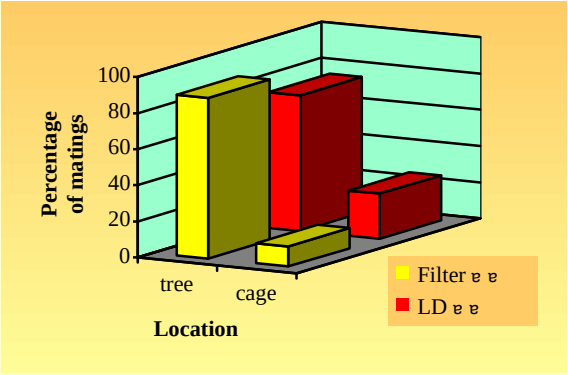
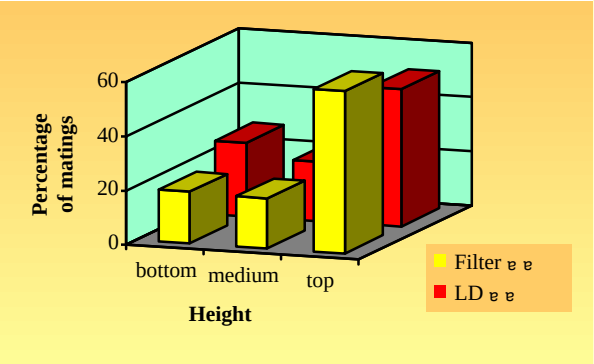


Figure 30. Location of open filter and LD colony males matings in relation to the height of the tree.



5.3 RESEARCH PROGRAMME ON MEDFLY MATING BEHAVIOUR: OVERVIEW OF THE MAIN ACHIEVEMENTS 1995 - 1998

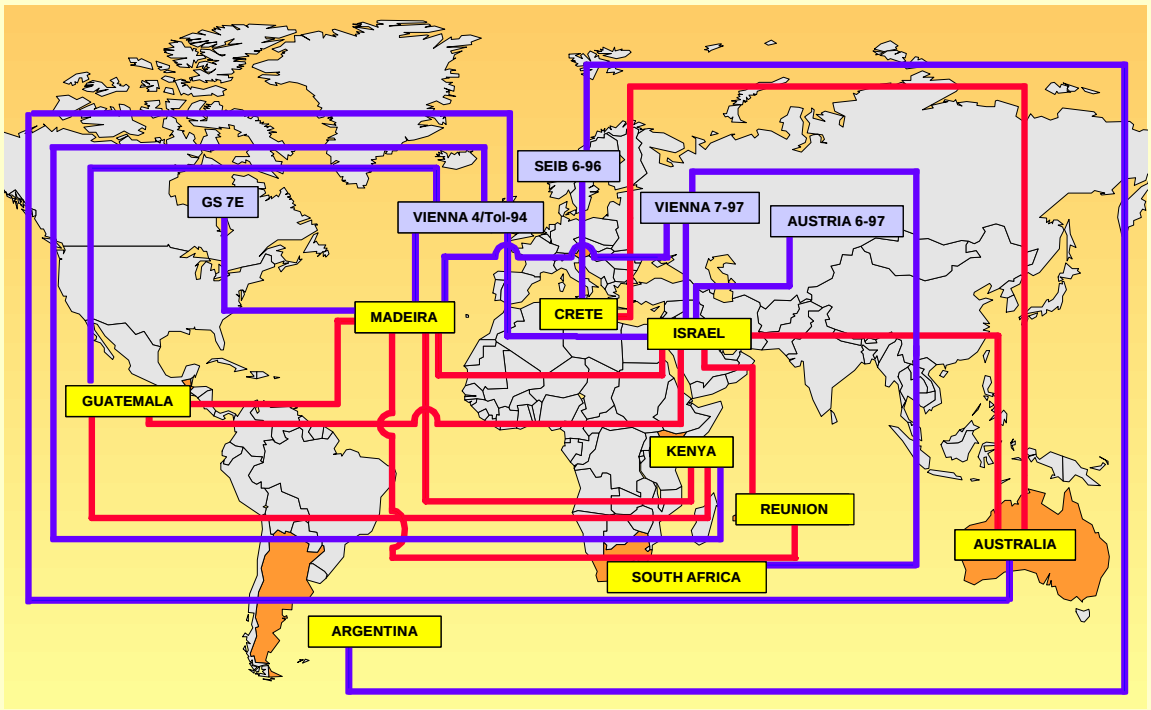
The research programme on medfly mating behaviour was initiated in 1995 at the Entomology Unit. This programme utilized both in-house research and the activities of a network of about 10 scientists working in various Member States organised as a Co-ordinated Research Project (CRP). The aims were (i) to analyse the mating compatibility of medfly populations from different origins and (ii) to study in detail the courtship behaviour of wild and laboratory flies. The CRP will be ending in 1999; given below is an overview of the major achievements.

5.3.1 Assessment of the mating compatibility of medfly populations from various origins

The most important practical finding of the programme has been the demonstration that there is no sexual incompatibility between wild medfly populations from any geographic origin and mass-reared GSS. A total of 9 wild populations and 5 GSS were tested in various combinations (**Fig. 31**). With the exception of the data reported here for VIENNA 7-97 tests with Madeira, which seems to be due more to a problem of rearing than one of the genotype of the strain, all tested wild flies and GSS mated successfully with each other (see 1997 Annual Report). The same experiments also provided a characterisation of the behaviour of flies from GSS (see 1997 Annual Report).

The results of the mating comparison represented a major step forward since (i) it shows that wild medfly populations world-wide have not yet developed pre-mating isolation mechanisms, and (ii) it allows mass-rearing

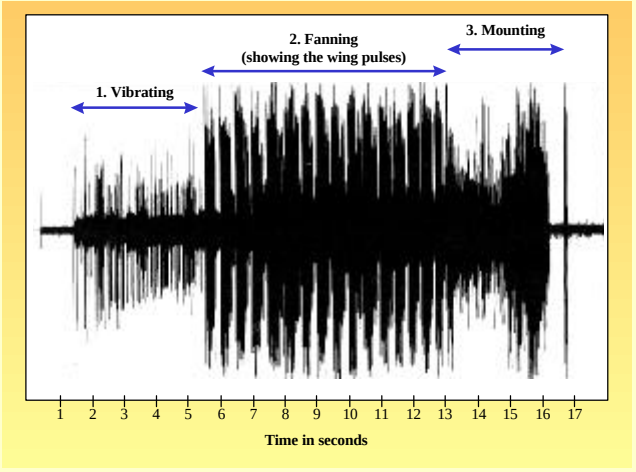
Figure 31. Overview of the combinations of wild populations and GSS tested during the research programme (1995-1998).



Yellow box: wild population *light blue box: GSS* *blue line: wild vs GSS* *red line: wild vs wild*

facilities and field programme managers to export/import sterile flies from/to any country/continent, as long as the insects fulfil the established QC requirements.

Figure 32. Typical sound track of medfly male love songs produced during the courtship.



5.3.2 Video analysis of medfly courtship behaviour

The courtship behaviour of single pairs of wild and mass-reared flies have been recorded, using slow motion video. Though part of the comparative analysis is still pending, no qualitative differences have been found between wild populations world-wide.

However, some differences have been found between wild flies and long-term mass-reared flies. It was shown that mass-reared males have a tendency to shorten the duration of the courtship, and even sometimes to omit some of the steps of the courtship. This is probably due to an adaptation to the overcrowding conditions which prevail in the rearing cages and this could be the key or one of the keys used by wild females to discriminate between mass-reared and wild males.

5.3.3 Sound analysis of medfly love songs

During courtship, the male medfly produces sounds (also known as "love songs") by vibrating and/or fanning its wings (**Fig. 32**) when in close contact with the female.

It was found that the mass-reared males produce significantly longer wing buzzes than do wild males. This could result from the adaptation to the rearing cages, in which flies are subject to high levels of sound interference.

Observations have also been made on the effects of irradiation on the male love songs. The video and sound recordings have been completed but the data have not yet been analysed.

5.3.4 Support to Member States

The field cage protocol which was defined for the mating compatibility studies has been recognised by the international fruit fly community and is now available as part of the standard quality control for fruit flies release programs.

In addition, several fruit fly quality control teams in the Member States have been provided with training in this methodology (**Fig. 33**).

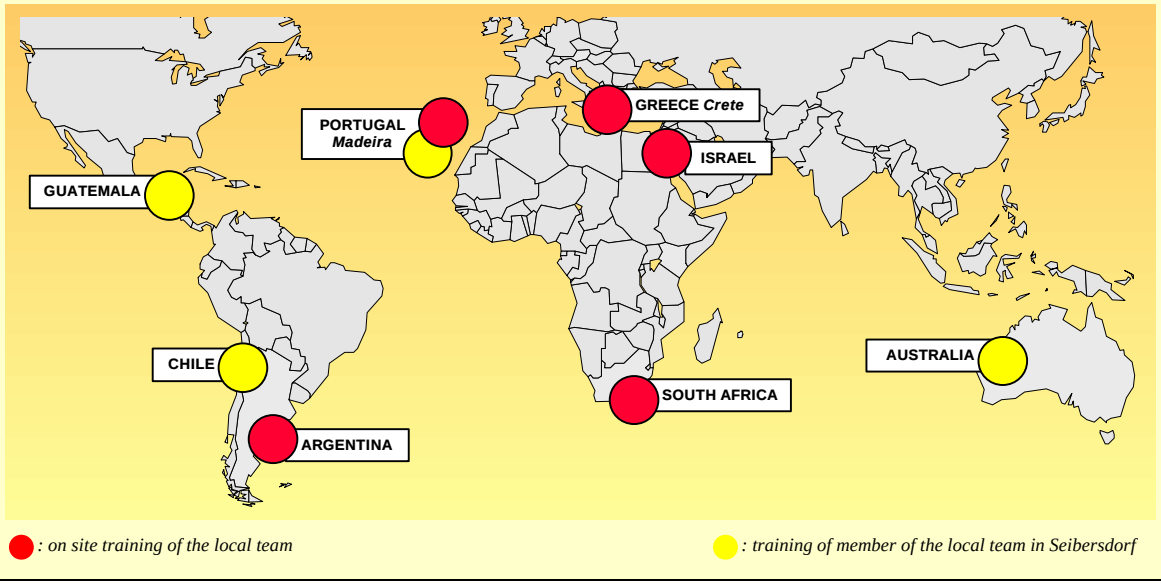
5.3.5 Future research perspectives on the behaviour of mass-reared fruit flies

Using the FRS concept, various aspects of fruit fly behaviour could be investigated with the aim of improving the quality of mass reared flies. These could include (i) using flies of different ages and sizes in the same cage; (ii) increasing the surface area available for the flies; (iii) designing new rearing cages to take into account the field behaviour of fruit flies. (in the field, feeding, courtship, mating and oviposition often occur in different locations, which is not easily provided in mass rearing cages); (iv) using a greenhouse with host trees to maintain the FRS colony.

The FRS is a major breakthrough in the amelioration of some of the negative effects of mass rearing on fruit fly behaviour but has highlighted the need for better quality control tests to assess the behaviour of mass reared fruit flies relative to that of wild individuals. The updated quality control manual for fruit fly SIT programs, which recommends that periodic mating compatibility tests be carried out in field cages, is a step in the right direction.

The field cage mating tests represent, to date, the best compromise between open field and laboratory tests to efficiently assess the mating competitiveness and sexual compatibility of mass reared medflies. However, do field cage mating tests answer all the questions related to the behavioural quality of mass reared fruit flies? The answer is obviously no for the

Figure 33. Member States for which training in field cage evaluation of medfly mating behaviour was provided, in support to on going medfly SIT programmes.



following reasons: (i) the tests can only be used to evaluate the relative quality of a mass reared strain but cannot be used to predict field results; (ii) the size of the standard field cage usually allows a single tree to be placed inside. This may not be representative of an open-field situation, especially in the case of lekking species; (iii) the flies tested are virgin and often of the same age; (iv) the same flies are tested for one day only and remating response is not measured; (v) the egg fertility is not measured; (vi) the tree often needs to be pruned to facilitate the observations, which may affect the response of the flies to various stimuli (e.g., light intensity).

These limitations of the present protocol indicate the direction that future research should follow to formulate a test protocol which can accurately predict the effectiveness of different fruit fly strains once they are sterilised and released as part of an operational SIT programme.

Mass reared fruit flies will always be inferior to wild flies. Rearing facility managers are developing strategies to overcome this and maintain a defined level of quality. One simple option is to replace the strain on a regular basis. It is easier to include such preventive actions than to face disastrous field results, and their consequences.

6. Appendices

6.1 PUBLICATIONS

Caceres, C., K. Fisher and P. Rendon. in press**.** Mass rearing of the medfly temperature sensitive lethal genetic sexing strain. *In* Tan, K. H. [ed.], Area-Wide Management of Fruit Flies and Other Major Insect Pests, Universiti Sains Malaysia Press, Penang.

Cayol, J. P. in press**.** Changes in sexual behavior and some life traits of Tephritid species caused by mass-rearing processes. *In* Aluja, M. and A. Norrbom [ed.], Phylogeny and evolution of Tephritid behavior. CRC Press. Boca Raton, Florida.

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

Cayol, J. P., J. Vilardi, E. Rial and M. T. Vera. in press**.** New indices and method to measure the sexual compatibility and mating performance of medfly (Diptera, Tephritidae) laboratory reared strains under field cage conditions. J. Econ. Entomol. 92(1).

Cayol, J. P. and M. Zaraï. in press**.** 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.

Fisher, K. 1998. Genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae): optimizing high temperature treatment of mass-reared temperature-sensitive lethal strains. J. Econ. Entomol. 91(6): 1406-1414.

Fisher, K. and C. Caceres. in press**.** A filter rearing system for mass reared medfly. *In* Tan, K. H. [ed.], Area-Wide Management of Fruit Flies and Other Major Insect Pests, Universiti Sains Malaysia Press, Penang.

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

Gariou-Paplexiou, A., G. Yannopoulos, A. S. Robinson and A. Zacharopoulou. in press**.** Polytene chromosome maps and RAPD polymorphisms in Glossina austeni. *In* Tan, K. H. [ed.], Area-Wide Management of Fruit Flies and Other Major Insect Pests, Universiti Sains Malaysia Press, Penang.

Katsoyannos, B. I., N. T. Papadopoulos, J. Hendrichs and V. Wornoayporn. in press**.** 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.

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

Robinson, A. S. in press**.** Genetic control of insect pests. *In* Rechcigl, J. [ed.], Environmentally Sound Approaches to Insect Pest Management. Ann Arbor Press Inc. Chelsea, Michigan.

Robinson, A. S. and G. Franz. in press**.** The applications of transgenic insect technology in the sterile insect technique. *In* Handler, A. M. and A. A. James [ed.], The Use of Transgenic Insects for Biological Control. CRC Press. Boca Raton, Florida.

Willhoeft, U. and G. Franz. 1998. Analysis of the Ceratitis capitata Y chromosome using *in situ* hybridization to mitotic chromosomes. 47-51. *In* IAEA [ed.], Genetic engineering technology for the improvement of the sterile insect technique. IAEA. Vienna, Austria.

Willhoeft, U., J. Mueller-Navia and G. Franz. 1998. Analysis of the sex chromosomes of the Mediterranean fruit fly by microdissected DNA probes. Genome. 41: 74-78.

6.2 TRAVEL

Staff member	Destination	Period of absence	Purpose of travel
Robinson, A.S.	New Haven, CT, USA	98-04-29 - 98-05-05	External review of tsetse programme
	Penang, MAL	98-05-26 - 98-06-07	Int. Conf. Area-wide control of insect pest and Int. Fruit Fly Symposium
	Oxford, UK	98-09-05 - 98-09-11	4th Int. Cong. of Dipterology, Chairman Section "Genetics of Diptera"
	Riverside, USA	98-09-12 - 98-09-19	3rd Annual Exotic Fruit Fly Symp and visit Dr. Atkinson's lab.
	Heraklion, Crete, GRE	98-11-20 - 98-11-29	Lecture at a graduate course "Applied Ecology"
Cayol, J.-P.	Xalapa, MEX	98-02-15 - 98-02-23	Symposium "Evolution of fruit fly behaviour"
	Cape Town, SAF	98-04-18 - 98-05-03	TC mission SAF/5/002
	Penang, MAL	98-05-27 - 98-06-09	Int. Conf. Area-wide control of insect pest and Int. Fruit Fly Symposium
Fisher, K.	Honolulu, HI, USA	98-01-15 - 98-01-25	USDA meeting on medfly
	Madeira, POR	98-02-28 - 98-03-07	TC mission POR/5/005
	Penang, MAL	98-05-23 - 98-06-07	Int. Conf. Area-wide control of insect pest and Int. Fruit Fly Symposium
	Arica, CHI Lima, PER	98-06-26 - 98-07-10	TC mission RLA/5/039
	Funchal, POR	98-11-08 - 98-11-13	TC mission POR/5/005
Franz, G.	Penang, MAL	98-05-23 - 98-06-05	Int. Conf. Area-wide control of insect pest and Int. Fruit Fly Symposium
Opiyo, E.	Penang, Kluang, MAL	98-05-26 - 98-06-05	Int. Conf. Area-wide control of insect pest Visit Old World Screwworm facility

6.3 FELLOWS

Name	Field of Training	Duration of Training	Fellowship Period
Kitwika, W. (URT)	Tsetse	12 months	98-02-06 - 99-02-05
Lyaruu, E.A. (URT)	Tsetse	6 months	98-03-02 - 98-09-01
Nyingilili, H.S. (URT)	Tsetse	6 months	98-08-03 - 99-02-02
Masaba Gidudu, A. (UGA)	Tsetse	6 months	98-10-01 - 99-06-30
Fogliani, R. (AUS)	Medfly	4 months	98-11-02 - 99-02-26

6.4 SHIPMENTS OF TSETSE FLY PUPAE

1.Pupae of Glossina austeni (Colony size 11,000 females)

Dr. D. Moloo	27,000	*ILRI , Nairobi , Kenya*
Dr. Maudlin	10,835	*University of Glasgow , UK*
Dr. Underwood	2,600	*University of Southhampton , UK*
Dr. S. Aksoy	2,050	*Yale University , New Haven , USA*
Dr. Zacharopoulou	400	*University of Patras , Greece*
Dr. Gariou-Papalexiou	200	*University of Patras , Greece*
Dr. K. Bourtzis	200	*Yale University , New Haven , USA*
Prof. Townson	150	*School of Trop. Medicine , Liverpool , UK*
Prof. H. Wetzel	150	*Vet. University ,Hannover , Germany*
Dr. G. Liebisch	100	*Vet. University ,Hannover , Germany*
	43,685	

2.Pupae of Glossina palpalis (Colony size 1,900 females)

Dr. Gomez	7,300	*Department of Biology , Legon , Ghana*
Dr. Maudlin	5,550	*University of Glasgow , UK*
Prof . Molyneux	4,750	*University of Salford , UK*
Dr. S. Aksoy	950	*Yale University , New Haven , USA*
Dr. Garms	350	*Tropical Institute , Hamburg , Germany*
Dr. G. Liebisch	100	*Vet. University ,Hannover , Germany*
	11,600	

4.Pupae of Glossina brevipalpis (Colony size 1,000 females)

Dr. Guerrin	10,600	*University Neuchatel Switzerland*
Dr. Garms	200	*Tropical Institute , Hamburg , Germany*
Prof. H. Wetzel	200	*Vet. University ,Hannover , Germany*
Dr. S. Aksoy	200	*Yale University , New Haven , USA*
	11,000	

5.Pupae of Glossina fuscipes (Colony size 1,500 females)

Dr. Melaku-Girma	8,700	*ICIPE , Nairobi , Kenya*
Dr. Auerswald	1,700	*University of Cape Town , RSA*
Dr. S. Aksoy	400	*Yale University , New Haven , USA*
Prof. Townson	210	*School ofTrop. Medicine , Liverpool , UK*
Prof. H. Wetzel	100	*Vet. University ,Hannover , Germany*
	11,010	

6. Pupae of Glossina pallidipes (Uganda colony size 16,000 females)

Dr. Gariou-Papalexiou	500	*University of Patras , Greece*
Dr. S. Aksoy	200	*Yale University , New Haven , USA*
	700	

7. Pupae of Glossina morsitans (Colony size 4,000 females)

Dr. Maudlin	5,520	*University of Glasgow , UK*
Dr. Zollner	5,300	*National Resources Inst. , Chatham , UK*
Prof. A. Tait	5,300	*University of Glasgow , UK*
Dr. Gibson	4,150	*School of Biological Sciences , Bristol , UK*
Dr. R. Brun	3,810	*Tropical Institute , Basel , Switzerland*
Dr. Turner	2,600	*University of Glasgow , UK*
Dr. S. Aksoy	1,850	*Yale Schoool of Medicine,New Haven,USA*
Mr. Gikonyo	400	*ICIPE , Nairobi , Kenya*
Dr. Underwood	200	*University of Southhampton , UK*
	28,930	

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