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ENTOMOLOGY UNIT
FAO/IAEA Agriculture and Biotechnology Laboratory
Seibersdorf

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1. Introduction

Medfly genetic sexing strains (GSS) are now being used for all operational SIT programmes. In 2002, a new strain, VIENNA 8, was evaluated for stability, quality and production characteristics. The strain carries the inversion as described in the 2001 Annual Report and it can produce ca 10% more males for irradiation and release than could previous GSS. This strain will be transferred to several facilities in 2003. As a further improvement to the strain the visible marker, Sergeant, has been introduced into the strain following the successful field cage evaluations reported in the 2001 Annual Report. In 2003 this strain will be further analysed to assess the stability of the marker.

There is considerable interest in the field of medfly SIT in developing procedures whereby eggs can be shipped between different rearing facilities. This could be especially relevant for GSS as a central facility could hold and maintain a specific strain and eggs could be sent to satellite facilities where only males would be reared for sterilization and release. During 2002 a series of egg shipments was received from the medfly mass rearing facility at El Pino, Guatemala and the effect of shipment on the quality parameters of the resultant flies was assessed. It was concluded that although there was some reduction in egg viability, especially for long shipments, it will be possible to develop an effective shipment protocol.

In 2002 colonies of Bactrocera oleae *and* Anastrepha fraterculus *were established, the former after some initial problems associated with the unsuitability of the larval diet. In 2003 these colonies will be used to develop improved mass rearing techniques for these species and for* A. fraterculus *a series of field cage evaluations will be carried out to assess the degree of mating compatibility of populations from the whole geographic range of this species in South and Central America. Dr. Teresa Vera, Argentina, was recruited as a consultant at the end of 2002 to carry out these field cage studies.*

The development of tsetse mass rearing facilities in Africa is expanding and in order to support this activity a prototype container rearing system was designed and constructed. The successful development of the TPU3 system for tsetse rearing enables this approach to be evaluated. The container has now been placed at Seibersdorf and it will be used for rearing tsetse in 2003. For areawide tsetse SIT programmes it will be essential that an aerial fly release system is developed and a prototype release mechanism has been designed and built. However, for such a system to be operational tsetse will have to be immobilized for some time at low temperature and a suitable temperature regime is being identified.

During 2002, a start was made on the renovation of part of the Unit to provide a rearing facility for mosquitoes and Mark Benedict joined the Unit to supervise the activities related to a feasibility study for mosquito SIT. The renovations will be completed in the first half of 2003 following which a colony of Anopheles arabiensis *will be established.*

1.1 Cryopreservation Techniques for Medfly Embryos

The ability to cryopreserve medfly embryos has two possible applications. Firstly it would enable mass rearing facilities to preserve a colony of a genetic sexing strain if there was a serious problem with the colony being maintained in the filter rearing system. Secondly, it would enable mutant strains to be maintained without the need for continuous rearing. During a consultancy, Dr. R. Leopold, USDA/ARS was able to successfully cryopreserve medfly eggs from the medfly GSS, VIENNA 7 with egg hatch being about 30% and larval to adult survival being 53%. This technique is repeatable and will lead to substantial savings in strain maintenance.

1.2 Liquid Diet for Medfly Genetic Sexing Strains

Brewer's yeast is an expensive component of the larval diet for medfly genetic sexing strains. Through a consultancy by Ms. Stella Chang, Hawaii, a replacement protein source, papaya-soy protein (PSP) was evaluated for the GSS VIENNA 7/D53-MIX 2002 strain. Pupal production, flight ability and adult emergence, egg production and egg hatch did not seem to be affected when brewer's yeast was replaced by PSP at various concentrations but proportionally more PSP was needed. PSP can only replace brewer's yeast in larval diet when used in higher concentrations. This study may provide some direction as to the possibility of developing a liquid diet for medfly GSS.

1.3 DNA Markers for Tsetse Hybrids

Certain species of tsetse hybridise readily in the laboratory and there are anecdotal reports and a few published reports that this also occurs in the field. The difficulty in the field is the recognition of the hybrids. During a consultancy, Dr. P. Olet (Kenya) analysed hybrids between *Glossina morsitans centralis* and *G. swynnertoni* and between *G. m. centralis* and *G. m. morsitans* using RAPD markers. For both hybrid crosses, she was able to identify specific markers that differentiated the hybrids from the parental types. These markers will be very useful in the field to assess the degree of hybridisation in areas where the two species overlap

1.4 Mating Behaviour of Tsetse

SIT relies on effective mating between the released sterile males and wild females. In tsetse very little is known about courtship between males and females and Dr. D. Briceno, Costa Rica made the first video recordings of this behaviour in tsetse. The courtship behaviour is more complex than appears and in *G. pallidipes* sound production by males appears to play some sort of role in communication between the sexes. Collaboration is also going on with the University of Vienna regarding sound production in tsetse (Dr. H. Kratochvil). It is planned to continue the work and to use it in the evaluation of the quality of mass reared male tsetse.

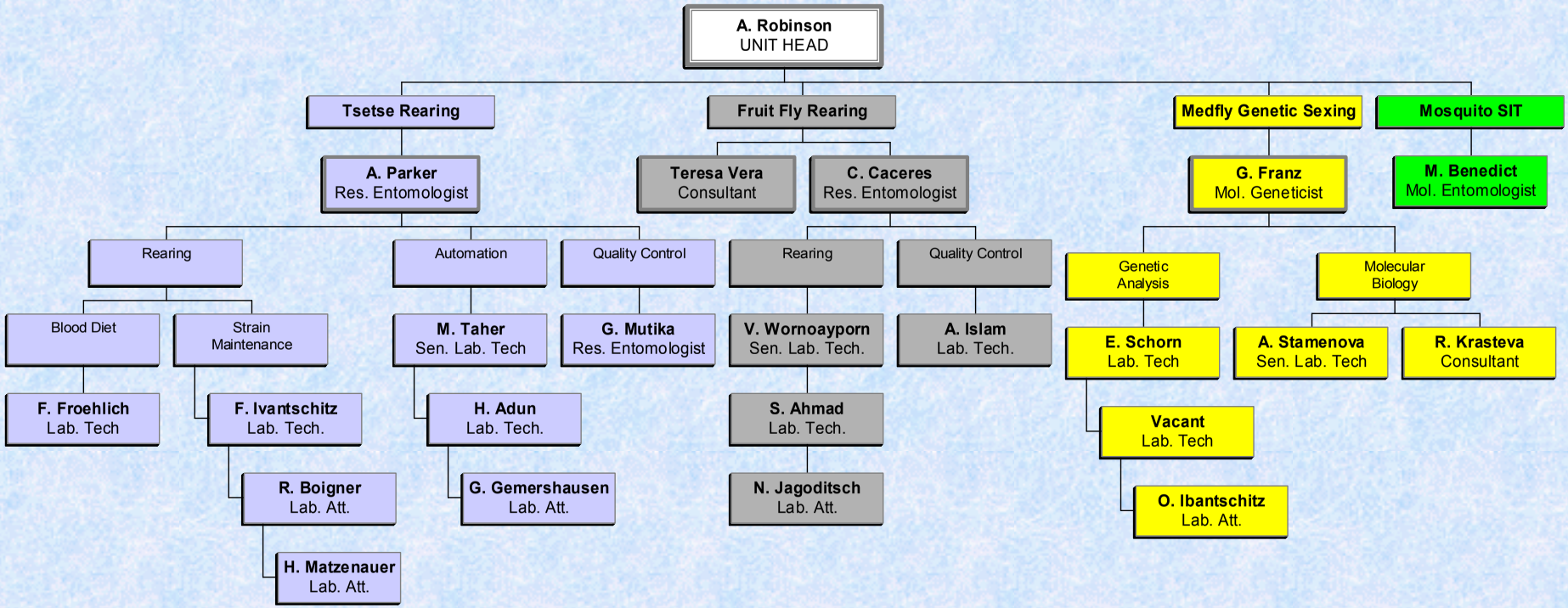
1.5 Support for a Medfly SIT Programme in the Middle East

A medfly SIT programme is being carried out in the Arava Valley, Israel and during the year sterile male pupae were provided to the programme from the Unit. The pupae were produced from VIENNA 7/D53-MIX 2002 and approximately 5 million/week were shipped to Israel. This amount of pupae is probably the maximum that the Unit can produce and the support required a 7-day workweek for the medfly group and the fellows. The exposure of the staff and fellows to the strict schedule required for fly production in an operational programme was very valuable.

1.6 Nomenclature of Medfly Genetic Sexing Strains

It is essential that a system is in place for strain identification and with more complex GSS being developed naming strains can become very cumbersome. A case in point is VIENNA 8, the number identifies the translocation but this strain includes inversion D53, it has a particular mitochondrial marker, is backcrossed into a mixed genetic background and was first mass reared in 2002. Including all this information in the name of the strain would be confusing and we propose that VIENNA 8 be the name used to cover all these attributes of the strain as it is maintained in the Unit. Once supplied to operational programmes, it is likely that collaborators will introduce other genetic backgrounds into the strain e.g. VIENNA 8/TOL or VIENNA 8/PERU.

ENTOMOLOGY UNIT ORGANIZATIONAL CHART



2. Quality Control Studies in Tsetse

The quality of mass reared insects for SIT is a major factor in determining the success of any subsequent release programme and in tsetse a field cage evaluation system continues to provide a very valuable measure of this parameter. In 2002 the system

was used to measure the mating competitiveness of three new species of tsetse and to evaluate the mating compatibility of two populations of Glossina pallidipes*, one colonized from Uganda many years ago and a second that is being reared in Ethiopia. No mating incompatibility was demonstrated. In relation to a proposed SIT programme for* G. palpalis *in West Africa the field cage system was tested in Burkina Faso. The field cage system is now used routinely for the evaluation of any treatment given to the adult male tsetse.*

Aerial release of tsetse using a chilled adult release system will require that male tsetse be held for some time at an immobilizing temperature. As described in the 2001 report, male tsetse flies are quite susceptible to low temperatures. Further work has been carried out in this area but as yet a viable temperature regime for holding male tsetse at a low temperature has not been identified. A prototype release unit has been constructed and the development of an appropriate sterile male holding and release protocol will have a high priority in 2003. These studies will include an assessment of the importance of male "exercise" for the full development of wing musculature.

There are numerous reports in the literature that irradiation in an atmosphere of nitrogen can improve the quality of irradiated insects and in tsetse this has also been shown to be beneficial. The use of nitrogen may also enable mature male pupae from the self-stocking system to be irradiated instead of adults and so simplify the logistics of the radiation procedure.

2.1 Sterility of *G. pallidipes* Irradiated in Nitrogen

Irradiation in the absence of oxygen reduces the amount of induced biological damage and in some insects, a net advantage has been demonstrated when using a nitrogen atmosphere during irradiation. Anoxia induced by sealing puparia in a plastic bag before irradiation is routinely used when irradiating medfly puparia and nitrogen has been used experimentally for *Glossina morsitans* and *G. palpalis*. Exposing flies to cold temperature before irradiation also reduces the deleterious effect of gamma irradiation. Chilling tsetse flies before irradiation was done during the eradication campaign in Zanzibar. The work outlined below is intended to provide some insight into quality of males that are released in an SIT programme.

Groups of *G. pallidipes* males were collected and irradiated thirteen days after emergence in a nitrogen atmosphere at 70, 85, 100, 120, 145, 170, 180 and 210Gy. Another two groups were irradiated in air at 110 and 120Gy plus one control group of males that was not irradiated. The flies were kept in normal colony conditions before and after irradiation. Equal numbers of fourteen-day-old males and eight days old females were introduced into 11cm diameter cages for mating and observed for two hours the day after irradiation. Mating pairs were removed and placed in separate tubes to calculate duration of mating. At the end of copulation, the males were removed and placed in separate tubes. The females mated with males exposed to each radiation dose were kept in individual tubes for 42 days. Males were also kept in individual tubes and their survival in standard colony conditions followed.

Abortions, egg and pupal production were recorded every working day. Pupae that were produced were incubated in normal colony conditions. Females were dissected at the end of the experiment to determine whether they had been inseminated and the uterine contents examined. Unirradiated males of the same age were used as the control.

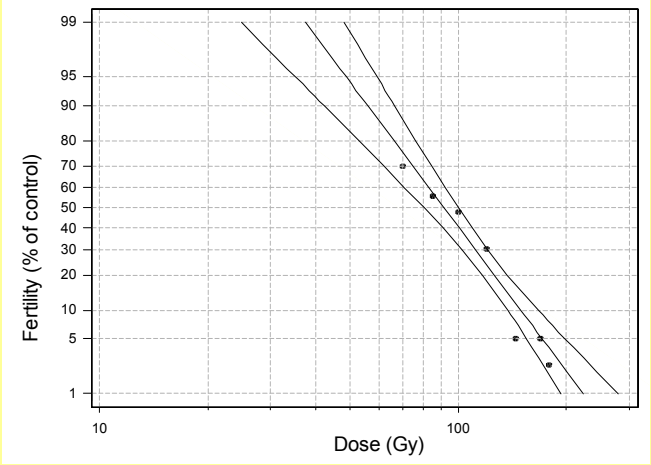
The duration of copulation varied from 22-32 minutes for all treatments of males (**Table 1**). Less than five percent of the flies did not have sperm in the spermathecae at the end of the experiment; all the rest had a mean spermathecal value of 25-100%. There was a large number of expelled eggs by females that mated with irradiated males (**Table 1**) and chances of successful pupation reduced as the radiation dose increased. However there were a few pupae produced at very high radiation doses. Taking the pupae production of females that mated with males that were not irradiated as 100% fertility it was shown that to reach

Table 1. The duration of copulation when fertile female *G. pallidipes* mated with males irradiated at different doses in air or nitrogen

Dose (Gy)	N	Duration (min)	Production/female			Total ± s.d.	% Fert.	% Prod.
			Egg	La.	Pup.			
0	78	26.8±8.4	0.3	0.2	1.9	2.5±1.1	100.0	100.0
70:N2	11	27.0±11.6	2.0	0.1	1.0	3.1±1.1	52.5	126.4
85:N2	25	24.6±5.5	2.0	0.1	0.9	3.0±1.5	48.3	123.9
100:N2	26	25.3±4.3	2.5	0.1	0.7	3.3±1.1	35.7	133.7
110air	23	31.4±11.2	2.8	0.1	0.2	3.1±1.4	11.4	125.8
120air	52	26.8±8.6	2.9	0.1	0.2	3.1±1.1	10.5	128.0
120:N2	36	25.6±5.0	2.9	0.0	0.4	3.2±1.2	18.9	132.5
145:N2	50	26.2±7.9	3.0	0.0	0.0	3.1±1.2	2.1	126.4
170:N2	50	29.9±5.7	3.0	0.0	0.1	3.1±1.1	4.3	127.3
180:N2	20	22.8±5.0	2.8	0.0	0.0	2.91.6±	0.0	118.2
210:N2	60	31.0±16.2	2.9	0.0	0.0	2.9±1.3	0.9	117.4

95% induced sterility males needed to be irradiated at about 145Gy in nitrogen atmosphere (**Figure 1**).

Figure 1 Residual fertility of females mated with male *G.pallidipes* irradiated in nitrogen atmosphere (bands are 95% C.I.)



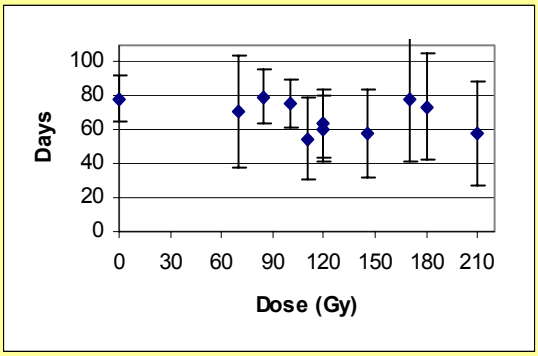
The egg production cycles were shortened in females mated with irradiated males leading to higher total production (**Table 1**). Females mated with irradiated males also ovulated earlier around day 14 after emergence compared to day 19-21 for females that mated with unirradiated males. Pupae produced by females mated with irradiated males from different doses were retained, in order to observe the emergence rate and sex ratio of the F1 adults. Emergence rate and numbers of females were lower for females that mated with irradiated males (**Table 2),** however the numbers of pupae were small so a definitive conclusion cannot be derived.

Table 2. Emergence rate and sex ratio of *G. pallidipes* adults from females mated with males irradiated in nitrogen.

Dose	# Females	# Males	# Pupae	% EM	% Females
0	58	59	121	96.7	49.6
70	2	7	10	90	22.2
85	8	11	23	82.6	42.1
100	4	8	17	70.6	33.3
110	0	2	5	40	0
120	8	11	23	82.6	42.1
210	0	1	2	50	0

Males that were irradiated showed a wide variation in survival that was slightly lower than the control (**Figure 2**) with the exception of males irradiated in nitrogen atmosphere at the very high doses of 170 and 180 Gy that also survived as long as the control males. Irradiation in air and also in nitrogen at 210Gy resulted in the least survival.

Figure 2. Mean survival of male *Glossina pallidipes* after irradiation and mating



G. pallidipes male pupae were irradiated at 120Gy in anoxic atmosphere. Male pupae were sealed in plastic pouches and placed in normal colony conditions for six hours before irradiation so that most of the oxygen was used up. The emergence rate (72-87%) compared favourably with male pupae that remained in normal colony conditions (over 80%). The survival of the flies that emerged was similar (over 90 days) under normal colony conditions (temperature, humidity, light and feeding).

2.2 Mating competitiveness tests of *G. f. fuscipes*, *G. p. palpalis* and *G. brevipalpis* in the field cage

The mating competitiveness of males of different ages of *G. f. fuscipes*, *G. p. palpalis* and *G. brevipalpis* was assessed in a field cage. It is important that the released males are of high quality and sexually competitive with the wild males. Age is one parameter that affects male tsetse mating competitiveness. Fifteen males of each age group and thirty females were released at the beginning of each test. Activity in the cage was then observed for two hours. Temperature, humidity and light intensity were measured at the start of the test (10:00h local time) and every thirty minutes thereafter. The time when a male successfully engaged genitalia with a female was recorded as well as the time when each pair separated (**Table 3**). The mating pairs were collected into separate tubes to determine duration of mating. The mating propensity (**Table 4**) was low for *G. brevipalpis* probably because observations were only carried out late morning while field data indicate that the species has crepuscular activity. The older males (8 and 13 days) dominated mating opportunities followed by 5-day-old males and the one-day-old males had the least chance of successfully engaging genitalia (**Table 3**). There were significantly more older males than the younger (5 and 1 day old) that successfully engaged genitalia. Flies that managed to engage genitalia successfully transferred sperm with duration of mating varying from just under an hour in *G. f. fuscipes*, to more than two hours in *G. brevipalpis*.

Table 3. Age differences as a factor in mating competitiveness in tsetse flies (*G.b.=G. brevipalpis, G.f.f.=G. fuscipes fuscipes, G.p.p.=G. palpalis palpalis*)

Age	Relative mating index (±s.e.)			Mating duration (minutes) (±s.e.)			Mean spermathecal value (±s.e.)		
	G.b.	*G.f.f.*	*G.p.p.*	*G.b.*	*G.f.f.*	*G.p.p*	*G.b.*	*G.f.f.*	*G.p.p.*
1	0	0.04±0.01	0.04±0.02	0	42±18	42±38	0	0.8±0	0.2±0.2
5	0.14±0.08	0.06±0.06	0.17±0.02	135±17	57±10	84±9	0.9±0.3	0.7±0.1	0.5±0.1
8	0.35±0.13	0.42±0.06	0.26±0.04	110±14	40±4	79±10	0.7±0.2	0.7±0.1	0.7±0.1
13	0.51±0.07	0.48±0.04	0.53±0.01	145±13	36±3	87±5	1.4±0.3	0.7±0.1	0.8±0

Table 4. Mating propensity of laboratory reared tsetse flies (*G .b.=G. brevipalpis, G.f.f.=G. fuscipes fuscipes, G.p.p.=G. palpalis palpalis*)

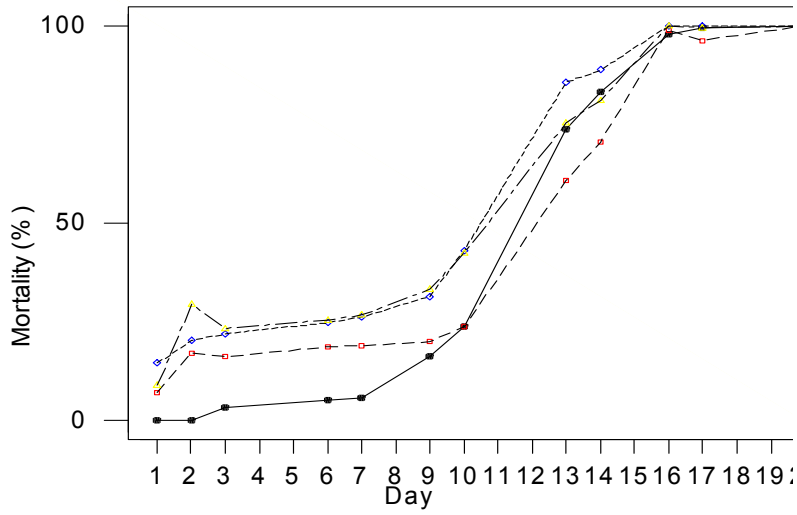
Day	# females	# males	Number mating pairs			Mating propensity (PM)		
			G.b.	*G.f.f.*	*G.p.p*	*G.b.*	*G.f.f.*	*G.p.p*
1	30	60	10	17	23	0.33	0.57	0.77
2	30	60	15	17	13	0.50	0.57	0.43
3	30	60	16	22	23	0.53	0.73	0.77
4	30	60	15	14	24	0.50	0.47	0.80
Total	120	240	56	70	83	0.47	0.59	0.69

2.3 Chilling of male *G. pallidipes*

Tsetse are relatively sensitive to the low temperatures that would be required for aerial release systems. In many insect species it is known that a cold or heat shock can induce protein synthesis for subsequent protection to exposure to low temperatures. In order to see if this approach would be useful for tsetse in relation to an aerial release system a series of experiments has been undertaken. Seven days old male *G. pallidipes* and *G. f. fuscipes* were cold shocked at 4°C for 30 minutes or one hour then returned to normal colony holding conditions for one hour prior to chilling for six hours at 4°C. Humidity was kept at 70%. The flies were then returned to normal colony holding conditions to observe survival without feeding. Mortality was initially

recorded on the morning after return to normal colony conditions. Mortality was variable about eighteen hours (overnight) after removal from low temperature for both *G .pallidipes* (0-15%) and *G. f. fuscipes* (0-25 %).

Figure 3. Mortality of *G. pallidipes* males after exposure to low temperature (control-black circles, 6h chill-red squares, 30m+6h chill-blue diamonds, 60m+6h chill-yellow triangles).



The data for *G. pallidipes* is shown on **Figure 3**. When the flies were removed from the chilling chamber they regained mobility after more than thirty minutes. Most of the flies died within fourteen days with a few surviving longer The mortality trend was similar for all treatments and no improvement could be seen following the cold shock. The experiments were carried out at two levels of humidity with no difference in the overall trend.

2.4 Mating compatibility tests of *G. pallidipes* (Ethiopia strain & Uganda strain)

The mating tests were conducted in a walk-in field cage erected inside a greenhouse under natural light. Temperature, atmospheric pressure and humidity were monitored using a digital Meteoscope™ and light intensity at three different levels inside the cage was measured by a TES-1334 Lightmeter™. Measurements were made at 10:00h and every thirty minutes thereafter until 12:00h. The *G. pallidipes* strain from Uganda was originally colonized in 1975 while colonization of the Ethiopian strain started in 2000. The Ethiopia strain flies emerged from pupae shipped by air from Ethiopia for the needs of this test. The adult flies from the Ethiopia strain were chilled as tenerals during sex separation and when marking males whereas the Uganda strain was only chilled when marking. Six cohorts of adult male flies from the Uganda strain were collected and all the females and males that emerged from the pupae from Ethiopia were also collected and sexes separated daily. The flies were kept in normal colony holding conditions until the day of the test. Males from one strain were marked with a dot of polymer paint on the thoracic notum. Males and females were eight days old on the day of the test except where flies from the Ethiopia strain that emerged on consecutive days were pooled to increase sample size. Twenty males from each of the two strains were released into the field cage immediately followed by twenty virgin females starting at 10:00h local time. Alternatively, if the number of females was insufficient the number of males was adjusted accordingly such that the male: female ratio remained 2:1. The release process lasted about five minutes. Any fly that dropped to the ground at the time of release was considered a non-flier and replaced. Mating pairs were immediately collected into single tubes and the duration of mating was recorded in minutes. The flies that mated were kept

overnight and females dissected to determine insemination rate as well as spermathecal fill. Both males and females were also checked for salivary gland hypertrophy regardless of mating status. Mean spermathecal value, pre-mating period and duration of copulation were analysed in Excel using z-test for means with known variances, this tool is used to test hypotheses about the difference between two population means. Mean environmental conditions in the cage were: temperature, 23.9°±0.5°C to 28.5°±2.2°C; humidity, 51.2±7.0% to 72.4±2.8% and Pressure 991 to 998 hPa. Pressure remained constant through the two hours of the test but varied negligibly with day, humidity dropped from start to end of each test and temperature rose from start to end.

Emergence of flies from the 500 pupae shipped from Ethiopia started five weeks after larviposition. Overall, emergence rate was 64% and close to one-third of all flies that emerged (both males and females) died within eight days after emergence. A similar trend was observed for another batch of pupae sent from Ethiopia at the end of November 2002. However, the flies that were used in the mating tests were actively flying in the cage just like the Uganda strain. From the Ethiopia strain, only two females and six males were classified as non-flyers out of the total survivors available for the mating tests. Mating propensity (PM) ranged from 0 to 0.43 on each day the test was run. The relative mating index (RMI) was also in the same range. Several unsuccessful mating attempts (8.3±6.2) during any one test were recorded without any attempt made to differentiate between strains. The female flexed her abdomen, closed the wings or flew away to avoid mating. Both females and males made short frequent flights in the cage. Spermathecal values for females mated with either strain were homogeneous but duration of mating was significantly longer for males from the Ethiopia strain (z-Test: two sample for means) (**Table 5**).

Table 5. Pre-mating period (min.), mating duration (min.) and mean spermathecal values of two strains of *G. pallidipes*.

	Pre-mating period		Mating Duration		Spermathecal Value	
	Eth.	Ug.	Eth.	Ug.	Eth.	Ug.
No.	4	12	4	12	4	12
Mean	65	68.7	44	25.4	1.3	1.3
s.e.	19.9	9	6.6	2.3	0.4	0.2
Min.	23	32	26	19	0.3	0.5
Max.	112	120	55	35	2	1.5

Out of the eleven replicates there were no successful mating attempts on three days, twice all the successful mating pairs were from the Ethiopia strain and for the remaining tests, males from the Uganda strain dominated the mating pairs

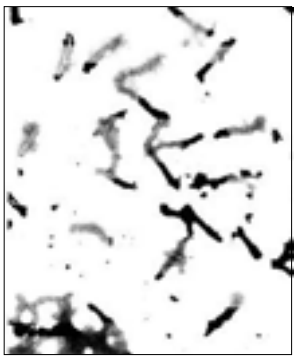
Table 6 The numbers of males of each strain that mated with females of the Ethiopia strain

Replicate	Number of flies used			% females mated	# males mated	
	Eth. female	Eth. male	Ug. male		Ug male	Eth. male
1	13	11	11	23.1	3	0
2	20	20	20	25	3	2
3	16	16	16	12.5	2	0
4	20	15	15	10	0	2
5	14	14	14	0	0	0
6	11	11	11	36.2	4	0
7	30	30	30	17	2	3
8	30	30	30	43	10	3
9	30	30	30	0	0	0
10	30	30	30	13	0	4
11	30	30	30	0	0	0

(**Table 6**). The incidence of salivary gland hyperplasia among males and females from both strains was similar. All males and females of both species that engaged in mating had normal salivary glands whereas 3.75% and 3.29% of the Ethiopia and Uganda strains respectively, that did not mate had salivary gland hyperplasia. Of the females that mated, two had salivary gland hyperplasia and a small proportion (4.30%) of those that did not mate were infected.

The proportion of types of males that mated is similar to an earlier experiment (45 Uganda strain males and 13 Ethiopia strain male mated with Ethiopia strain females in ten days of experiments) carried out using the Ethiopia strain maintained at Seibersdorf in early 2001 although the absolute numbers are lower in the current experiment. There was also a lower incidence of salivary gland hyperplasia although mate rejection by females was still very high. The incidence of salivary gland hyperplasia has been linked to male mating inadequacy. However, correlation between incidence of salivary gland hyperplasia and mating behaviour cannot be conclusively established using this data. Despite the high incidence of mate rejection by females there was successful sperm transfer among those few that copulated (only three females that mated with Uganda strain males were not inseminated). Flies from both strains made short flights in the cage and females rejected both types of males. The effect, if any, of delayed eclosion from the pupae that were transported by courier is unknown but it would be prudent to build up information on performance of flies that emerge from pupae transported over long distances. It would also be ideal to incorporate some aspects of flight ability in the competitiveness indices. It can thus be concluded that there is no absolute barrier to mating between males of the Uganda strain and females of the Ethiopia strain. This gives rise to the possibility of using Uganda strain males as a back-up colony for the sterile insect technique programme in Ethiopia.

3. Mass Rearing Studies in Tsetse



The G. pallidipes *colony from Ethiopia that was established in the Unit in 1996 reached a maximum size of about 10,000 producing females by the middle of 2000, thereafter there was a rapid decline and the colony had almost disappeared by the end of 2002. The reason for this drastic decline is most probably due to the infection of the colony by a virus that was brought in during the collection of field material. The virus is well known and there has been considerable work carried out on the effects of the virus on the fly. Through collaboration with Max Bergoin, Montpellier, work has been initiated to develop PCR based primers that can be used to identify insects carrying the virus. These primers will be used to try to establish a virus free strain of* G. pallidipes.

The supply of blood to large field projects in Africa remains a concern due to the limited availability of large irradiation sources for blood decontamination. In collaboration with Dr. M. Gemeiner, University of Vienna, a PhD student is revisiting the possibility of developing an artificial diet. Commercial blood substitutes are being used as a basis to develop the diet. In related work, the use of sodium citrate has been shown to be a much cheaper and easier way to prevent clotting of the blood during collection than heparin and a start has been made on developing a pasteurisation protocol for blood decontamination.

The TPU3 system for tsetse rearing has been evaluated and is now being transferred to counterparts in Africa for operational use. During the year several improvements have been made in the system to reduce costs and improve operational efficiency and a new plastic cage has been designed. Prototype cages will be produced in 2003 for a full evaluation. The successful development of the TPU3 system has made possible the containerization of tsetse rearing with all the associated advantages. As proposed in the 2001 report a container has now been constructed and placed in Seibersdorf. The environmental conditions will first be stabilized and then a colony of tsetse will be reared and production evaluated during 2003.

3.1 Cage Development.

The standard tsetse holding cages in use in the Entomology Unit and various projects in Africa are fabricated from a 50mm length of PVC drainage pipe, 200mm in diameter, to which polyester netting is glued. A 25mm diameter hole in the side allows access to the cage, which is closed with a natural rubber stopper. This design has been in use for 15 years, and the PVC tubing has proved extremely durable. The netting however is vulnerable to damage, and there is much labour involved in removal and replacement of the old netting. The cages cost about $5 each, without the cost of gluing on the netting.

As part of our continuing development of tsetse rearing, we have developed the TPU3 rearing system (see Annual Report 2000). In this system nine cages are held together in a metal frame as the basic holding unit. This gives us a defined size of 600 x 600mm. As a result a new cage system was developed that would fit in the same frame, but as previous experience had shown that a cage as large as 600 x 600mm gave problems with fly distribution and crowding, the new cage is 300 x 300mm, with four fitting in the frame.

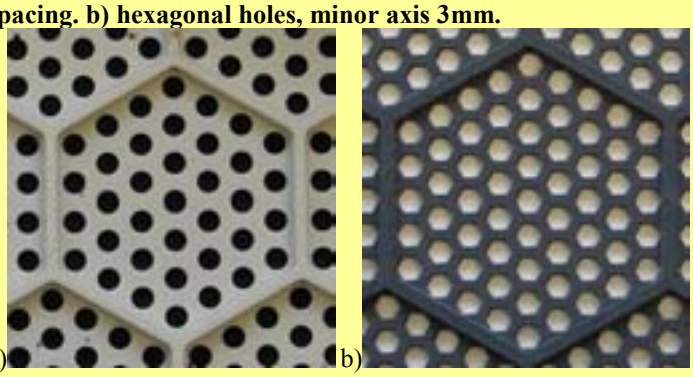
The objective was to produce a one piece, durable cage, that would not require net to be affixed or replaced, and which was cheaper than the previous cage, but that would satisfy the following requirements of tsetse rearing

- newly emerged flies can enter the cage through the lower surface but do not escape from the upper surface
- flies can feed easily through the lower surface
- larvae can crawl out of the cage to pupate.

The requirement for low cost, but large numbers, makes several manufacturing techniques practicable, but the method of choice was injection moulding.

The initial design concept was of two similar halves that clip together to form the cage. The two components would have different hole sizes to provide for the requirements listed above, and a system of catches to keep the parts together. The initial design had round holes and clips at diagonally opposed corners. From the design drawings the engineers produced a digital model, which they used to produce a stereolithography master. This first master however proved both too fragile to test properly, and also the flies failed to feed through the holes, but would emerge into the cage and the larvae would escape. The feeding problem was thought to be due to the quantity of plastic between the holes. The holes were round, 3mm in diameter, spaced 2mm apart in a hexagonal array, and it was felt that the remaining plastic was masking the feeding membrane, such that the flies were not responding to the heat from the heating mat. The problem was resolved by changing the hole

Fig. 4. Moulded cage design a) round holes, 3mm, 2mm spacing. b) hexagonal holes, minor axis 3mm.



a) b)

design from round to hexagonal, 3mm on the minor axis, and reducing the spacing between holes. This resulted in a decrease in solid of about 35% (**Figure 4**). A stereolithographic model of a 145 x 145mm section of this new pattern was produced and 8 samples cast for testing. New feeding tests showed a good response. The new cage design is now complete, and as soon as the digital model is prepared a new master will be produced of a complete cage and 15 samples cast for testing.

3.2 Diet

Tsetse flies feed only on vertebrate blood, but the collection and processing of blood is expensive, it must be stored at -20°C requiring costly storage rooms and reliable electricity, and it must be irradiated to reduce bacterial contamination. For smaller colonies this is tolerable, but as colony sizes increase to service large-scale programmes, the supply and processing of blood is going to become critical.

The issues involved in blood supply for tsetse colonies are:

1. Blood is normally collected from cattle at slaughter. This process is necessarily not aseptic, and large-scale collection is only possible where the animals are suspended for bleeding.
2. Large slaughter facilities with the necessary throughput of animals for slaughter and appropriate equipment for suspension are not common in Africa.
3. The blood must be prevented from coagulating, which is normally achieved by mechanical defibrination. This is laborious and potentially removes valuable nutrients from the blood. Heparin has been used as an alternative, but it is both expensive and thermolabile, making it extremely difficult to deliver to destinations in Africa.
4. Because collection is not aseptic, bacterial contamination must be reduced, which is currently achieved by gamma irradiation. Large irradiators are available in very few locations in Africa, and the lack of suitable legislative control prohibits the installation and use of any form of irradiator in several countries.
5. The collected, defibrinated blood must be stored frozen. The lifespan of frozen blood may be limited to a few years. Commercial cold storage is often not available, and the installation and running of dedicated storage is expensive.

To address some of these issues a number of different alternatives are being investigated.

3.2.1. Freeze dried blood

The Entomology Unit has conducted trials with freeze dried blood over many years. The freeze drying of blood is a slow process, but the product is shelf storable over an extended period. Both cow and pig blood have been freeze dried. Survival on freeze dried blood diet is acceptable, but fecundity is significantly lower than on fresh frozen blood; it may therefore be suitable for short term emergency use to prevent complete loss of a colony when fresh blood is not available, but it cannot currently be used for long term colony maintenance. Recent tests on freeze dried blood stored at ambient conditions for more than 10 years indicate no change in nutritive quality with storage.

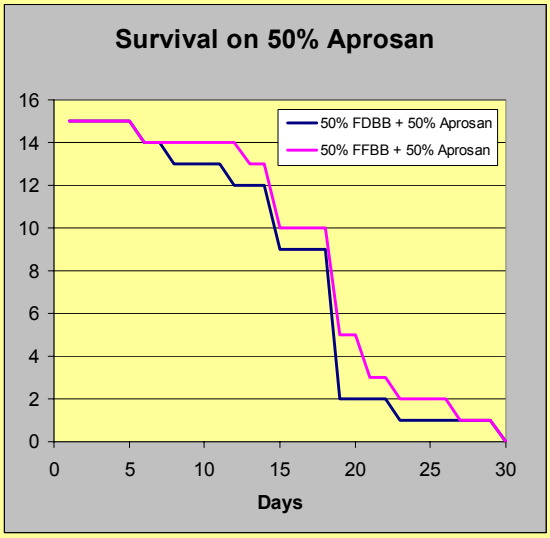
3.2.2. Commercial dried blood products

In an attempt to find a commercial product to serve as an alternative for the use of defibrinated bovine blood, Aprosan ® (Proliant) was tested. The product is a very

fine spray dried powder composed of 75% bovine and 25% porcine blood, which is claimed to be 100% water soluble. The anticoagulant used was pentasodium tripolyphosphate. Many mixtures of Aprosan with fresh frozen bovine blood and Aprosan with freeze dried bovine blood as well as controls of each product were compared in feeding tests on male *Glossina pallidipes* flies. All the conditions of feeding and maintenance were the same as the ones used for the main colony.

The results showed that the most successful mixture of Aprosan with either fresh bovine blood or freeze dried bovine blood contained 10, 20 and 30 % Aprosan. The survival data for the 50% Aprosan mixtures are shown in **Figure 5**; no flies survived beyond day 30 on the 50% mixture. Aprosan was difficult to dissolve, leaving a significant residue that had to be removed by filtration. As these tests showed that Aprosan cannot be used to replace a significant proportion of the diet, no further tests have been conducted on it. Tests will be conducted on the anticoagulant to check its toxicity (see next section).

Figure 5. Survival of *Glossina pallidipes* males on 50% mixtures of Aprosan ®



FDBB = Freeze dried bovine blood
FFBB = Fresh frozen bovine blood

3.2.3. Anticoagulants

The process of mechanical defibrination is laborious, and during hot weather may not be sufficient to prevent subsequent clotting. As the volume of blood collected increases this problem will increase. In the past tests were done on various anticoagulants, which indicated that heparin was acceptable, giving very similar survival and fecundity to defibrinated blood. Heparin however is expensive and thermo labile and so must be stored under refrigeration, making shipping to locations in Africa very difficult. Experience in Nigeria indicated that many batches of heparin arrived unusable.

Figure 6. Collecting blood for anticoagulant tests.

In order to standardise the experimental protocol to evaluate the anticoagulants, the blood for the tests was collected from animals on an experimental farm (**Figure 6**). Sodium citrate may be used as an anticoagulant, acting by sequestering the calcium ions necessary for several steps of the clotting cascade. It is stable under normal storage conditions, and about 40x cheaper than heparin. The original tests done to compare various anticoagulants indicated a slightly worse

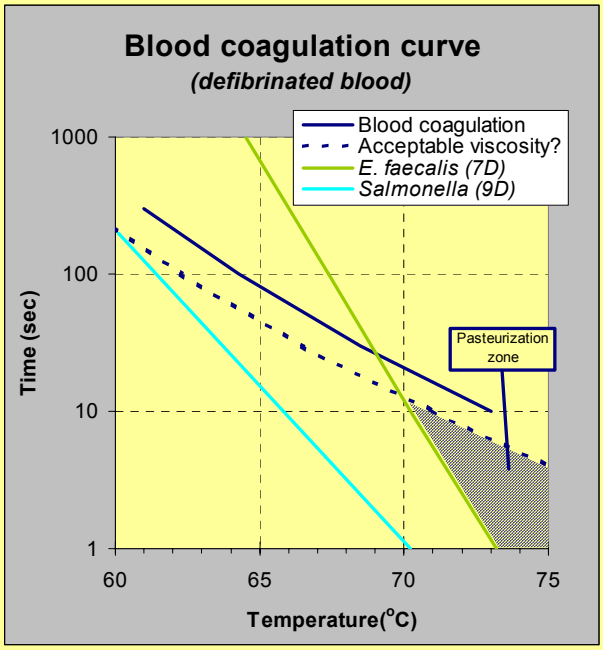
performance for citrate than heparin, so no further tests were done on it at that time, but the new need for alternatives to defibrination prompted us to look again at citrate. The new trials indicate that the performance is not significantly different from that of defibrinated blood, but the sample size is small. The inherent variability in fly performance gives a very high variance, so the trial will be repeated with more replicates to reduce the error. A second anticoagulant, sodium tripolyphosphate, will also be investigated. It is used in the preparation of commercial blood products such as Aprosan®, and is similar in cost to citrate.

3.2.4. Pasteurization

Blood has to be collected at slaughter but the conditions in the abattoirs inevitably lead to contamination of the blood. Further contamination of the blood is also possible during the subsequent handling, mixing and partitioning into storage bottles; the storage bottles are polythene and can only be heated to 80–90°C, which gives imperfect sterilization. Tsetse can only tolerate very limited bacterial contamination of the blood feed, less than 10 spores ml^{-1}, so it is usually necessary to reduce the bacterial load. This has been done utilizing a gamma irradiator, a dose of 1–1.5kGy giving about 99% reduction in viable spores. However large commercial irradiators capable of irradiating large volumes of blood are very few in Africa, and even small self-contained research irradiators capable of irradiating batches of 1–2 litres are not common.

The food processing industry uses pasteurization to reduce bacterial load in a wide range of products. The equipment required for pasteurization is simple in concept, and available to process quantities from a few litres for experimental work, a few hundred litres as used on individual farms, to very large-scale plants for the commercial processing of dairy and other products. Considerable work has been done on egg pasteurization, and this work has been taken as a starting point for investigating pasteurization of blood as a possible alternative to irradiation.

Figure 7. Blood coagulation curve and representative bacterial reduction curves.



The first step was to determine the coagulation curve for blood. 200µl samples were heated in a thermal cycler, and the physical characteristics examined by eye to determine a crude coagulation curve. Coagulation was taken as the point at which a solid clot was first observed. A noticeable increase in viscosity can also be detected from about 2°C below the coagulation temperature. **Figure 7** shows the approximate time/temperature relationship for the coagulation of defibrinated blood, and the line two degrees below which may be an acceptable viscosity for blood feeding. Also shown are two

typical bacterial reduction curves, for *Salmonella* and *Enterococcus faecalis*, a soil bacterium that is a common food spoilage contaminant. The two bacterial reduction lines are for 7 log and 9 log reductions, compared to a reduction achieved by irradiation of about 2 log.

There is a substantial difference in the gradient of the blood and bacterial lines, as also observed in egg pasteurization, suggesting that a time/temperature combination close to 10 seconds at 70°C would give an adequate bacterial reduction with acceptable blood characteristics. Higher temperatures, up to 75°C for less than 1 second may give even better bacterial reduction with improved blood quality. The possible range for pasteurization is shaded on **Figure 7**. If a lower reduction of 2–3 log proves acceptable, the time or temperature could be reduced.

Changes in the blood viscosity on heating are related to the denaturation of proteins and are a useful measure of the extent of denaturation. Tsetse feeding will certainly be affected by viscosity directly, and probably also be sensitive to protein changes. Measurements of viscosity will be taken to construct a time/ temperature/ viscosity diagram, and then feeding tests will be used to determine the maximum acceptable viscosity. This will then guide us to appropriate maximum time/temperature combinations that can be tested for bacterial reduction.

3.2.5. Synthetic (defined) diet

Another approach to the problem of diet storage is to identify a defined diet that can be stored as dry constituents on the shelf. Efforts some ten years ago fractionating blood in various ways to identify the essential components ended before any useful, commercially available, cheap substitutes were identified. This effort has now been renewed, starting with defined amino acid and lipid mixtures used in medical parenteral drip feeding solutions. The hope is to identify the important components so as to be able to assess the suitability of commercially available products.

The test mixture is Nutriflex from Braun. This mixture is designed for drip feeding patients, and contains all components essential for humans. The product is a clear or milky watery solution, supplied in a three compartment plastic drip bag for mixing just before use. The three compartments contain the amino acids, lipids and glucose respectively. The pH of the final mixture is 5.6 and osmotic potential 1100 mOsm (normal blood values are about pH7.41, 300 mOsm). Much of the osmotic potential comes from the glucose component; as whole peripheral blood normally contains little glucose this component was omitted from the feeding tests which reduced the osmotic potential to about 900 mOsm. The mixture was further diluted with saline to a final osmotic potential of about 300 mOsm.

Tsetse flies will feed readily on this mixture over several days, and are able to accomplish the primary excretion of water. Survival however is poor with all the flies dying by day 20. In comparison, flies fed nothing die in about 10–12 days, and flies fed normal Ringers saline solution died by day 8. The survival past day 10 therefore indicates that the flies were able to derive some nutrition from the mixture, and were not simply benefiting from the water or salt uptake. Mixtures of Nutriflex with blood containing at least 50% blood permitted survival and a varying degree of reproduction. Mixtures with less than 50% blood did not support reproduction. Tests with further additives and components are continuing.

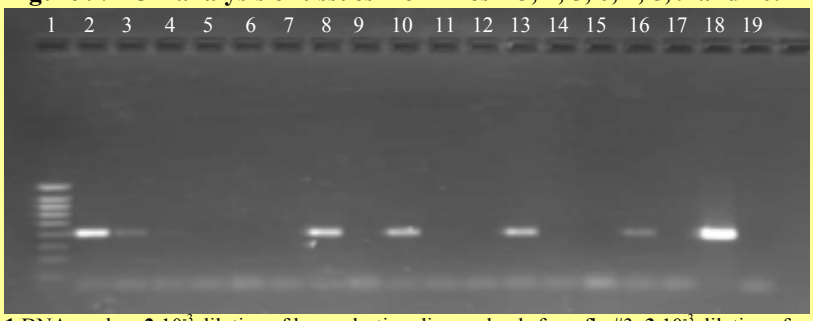
3.3 Salivary Gland Hyperplasia

The existence of a virus that causes salivary gland hyperplasia has been known for some time. The virus is usually reported from *G. pallidipes* but has also been seen in several other species. The virus, apart from causing salivary gland hyperplasia, causes complete sterility in males and reduced fertility of varying degrees in females to virtual sterility. In the wild the hyperplasia is seen in a few percent of flies only, but flies brought from the field to Seibersdorf show greatly raised hyperplasia levels after a few generations, resulting in a drastic reduction in fertility and crash in the population. The laboratory adapted strain held in Seibersdorf also harbours the virus, but the infection rate (as determined by patent hyperplasia) remains below 5% and the effect on overall fecundity is negligible. In an initial visit Dr Max Bergoin (Univ. Montpellier) collected hyperplastic salivary glands, from which he was able to purify the virus. Electronmicroscopy shows the virus to be very large, up to almost 1µm in length (**Fig. 8**). Sections of the viral DNA were cloned and sequenced. Among the sequences obtained, one showed interesting sequence homologies with a 79 kDa envelope polypeptide from various baculoviruses.

Fig. 8. Salivary gland hyperplasia virus

Primers were designed to amplify a 400bp sequence of this protein and they could be used to detect viral DNA in the fraction of the gradient containing viral particles, thus confirming that the DNA cloned is viral DNA and not tsetse fly DNA. Francois Cousserans (Univ. Montpellier) came to the Unit to verify the efficacy of the primers and to devise a protocol for detecting the virus in our colonies. Salivary glands and other tissue from fresh flies were dissected and tested with the primers, but initial tests revealed no amplification of viral DNA, even from known infected flies (with hyperplastic salivary glands). This however turned out to be due to inhibition of the reaction by tissue components, and by testing various dilutions amplification was achieved with 3–4 log dilutions (**Figure 9).** Virus was detectable in all hyperplastic salivary glands at appropriate dilution, and could also be identified in certain other tissues. In only one case was virus identified in salivary glands not exhibiting

Figure 9. PCR analysis of tissues from flies # 3, 4, 5, 6, 7, 8, 9 and 10.



1:DNA marker, **2**:10^{-3} dilution of hyperplastic salivary glands from fly #3, **3**:10^{-3} dilution of midgut from fly #3, **4**:10^{-3} dilution of uterus from fly #3, **5 & 6**:10^{-3} dilution of normal salivary glands from fly #4 & 5, **7**:Negative control, **8**:10^{-3} dilution of hyperplastic salivary glands from fly #6, **9 & 10**: 10^{-3} and 10^{-4} dilutions of hyperplastic salivary glands from fly #7, **11 & 12**:10^{-3} and 10^{-4} dilutions of faeces from fly #7, **13**: 10^{-3} dilution of hyperplastic salivary glands from fly #8, **14**:Negative control, **15**:10^{-3} dilution of normal salivary glands from fly #9, **16**:10^{-3} dilution of normal salivary glands from fly #10, **17**:Negative control, **18**:Positive control, **19**:Negative control

hyperplasia (**Figure 9**, lane 16, fly number 10). In an attempt to identify the presence of the virus in flies without killing them saliva, faeces and haemolymph were examined. Saliva was collected by inducing the flies to probe through a small piece of silicone membrane, but the volume obtained this way was extremely small. Virus was detected in only one faecal sample, but none of the flies from which these samples were taken except fly 7 showed salivary gland hyperplasia on subsequent dissection. Fly 7 was hyperplastic, but the faecal sample was negative (**Figure 6**, lanes 11 & 12). More work needs to be done on this aspect to develop a reliable assay.

3.4 Other Information

3.4.1. Pupal Sorter Calibration

One of the routine quality control measures used in tsetse production is the size of pupae. Originally size was measured by weighing the pupae 24 hours after larviposition, but now pupal size is normally measured by a mechanical sorter that separates the pupae based on diameter. The sorter consists of a pair of inclined, counter rotating rollers set to diverge by a defined amount. Pupae are fed onto the top of the rollers at the narrower end, and fall through into collecting containers when they have travelled along the rollers to the point where their diameter matches the roller spacing. The standard system has 5 collection chutes labelled A (smallest) to E (largest); the length of the collection area for each has been adjusted to correspond to the five weight classes previously defined for tsetse pupae.

The weight ranges for the five size classes for the species of tsetse held in the Unit currently or in the past is shown in **Table 7**, together with the roller spacings used to calibrate the sorting machine for those species where it has been determined. Calibration can be done with a standard set of feeler gauges, or more conveniently with a set of calibrated cylindrical probes.

Table 7. Pupal weight class definitions for various tsetse species.

Species	A	B	C	D	E	Roller calibration	
G. austeni	≤16	16–19	19–21	21–23	≥23	2.30	3.00
G. tachinoides	≤14	14–17	17–19	19–21	≥21		
G. p. palpalis	≤22	22–28	28–32	32–36	≥36	2.50	3.60
G. pallidipes	≤23	23–29	29–33	33–37	≥37	2.50	3.60
G. f. fuscipes	≤22	22–28	28–32	32–36	≥36	2.60	3.25
G. brevipalpis	≤56	56–68	68–76	76–84	≥84	3.50	4.25
G. m. morsitans	≤18	18–22	22–26	26–30	≥30	2.20	3.20

3.4.2 Video and sound recording of mating behaviour in tsetse

In relation to improving the quality of mass reared tsetse, studies have been initiated on an anlaysis of mating behaviour. Almost nothing is known of the courtship and mating behaviour of tsetse in the wild and the only time mating is observed in the field is in the "following swarm" seen in *G. morsitans*, but there is no reason to believe that this is representative of other tsetse species, as the other species do not form following swarms. Field cage tests do not provide sufficiently close observation of behaviour for detailed analysis, and are still an artificial situation.

With so little information available, it is not known how males locate females, and it is not know if there is any significant courtship, if females express choice, and if males can influence the outcome of mating by their behaviour. As the tsetse

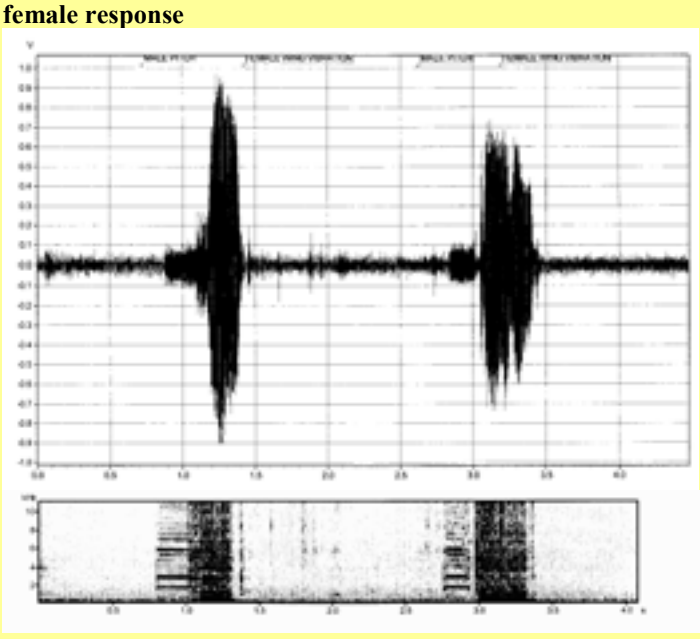
eradication programme in Ethiopia is targeting *G. pallidipes*, we have initiated a study of the mating behaviour of this species.

In field cage tests to measure the competitiveness of colony males against wild males, and the influence of irradiation on competitiveness, it was observed that males would take flight in the cage, fly directly to a female resting on the opposite side of the cage (about 2.2m) and attempt to mate. There were no cues obvious to a human observer to initiate flight or to locate the female. As no distant acting pheromones have been identified despite considerable work, we started to consider other possibilities. The only one that seemed likely was a sound signal too high for humans to perceive.

The University of Vienna, Department of Bioacoustics, has been making recordings of the sounds produced by tsetse both before and during mating. This work is ongoing, and a report is expected early in 2003. They have also identified the presence of a putative thoracic sound production organ.

At the same time a consultant made a preliminary visit to Seibersdorf to take slow motion video and sound recordings of tsetse mating behaviour. This was done in a small PMMA box, so the issue of long distance location of females could not be address, but the video footage of mating has already provided interesting information.

Figure 10. Male pitch and wing stroke during copulation and female response



In the past tsetse mating has appeared to be very simple, and largely controlled by the male. The video recording has shown however that the mating process is complex, approaching that of medfly, and the females are not simply passive. Males are unable to mate without the cooperation of the female, and the mating behaviour involves a sequence of complex patterns, which are not yet fully elucidated. Many of the movements produce sound (**Figure 10**) involving both male and female. The complexity of the behaviour observed clearly demonstrates the need to assess the behavioural responses of released flies to ensure their compatibility and competitiveness with wild flies. This work will be continued, with the objective of developing appropriate quality control protocols.

4. Genetic Sexing Strain Development in Medfly

All medfly SIT rearing facilities are currently using GSS based on VIENNA 7. This strain provides facilities with a predictable performance attributes and high degree of stability. As in all GSS that have been developed for medfly, the genetic basis is a male-linked translocation that links a selectable marker to the males. One disadvantage of using translocations to develop GSS is that they are associated with reduced fertility and hence they lower productivity in the mass rearing facilities. The reduced fertility is a consequence of the genetic behaviour of the translocation during meiosis and in general GSS are 50% as productive as bisexual strains. In 2002 activities were restarted on a GSS that was isolated many years ago but was not considered suitable as it broke down very rapidly during mass rearing. However, the introduction of inversion D53 into the strain has led to its stabilization. Extensive work is reported on the genetic and productivity analysis of this strain. It has been shown that this GSS has a significantly higher fertility than previous strains due to its specific genetic behaviour and this results in an increased production of male insects. The strain is named VIENNA 8. During 2002 the phenotypic marker, Sergeant, has now been introduced into the strain and this new strain will be evaluated in 2003.

The development of GSS for other pest species using the approach followed for medfly would be a very long process and the use of transgenic technology may enable sexing systems to be transferred between closely related species. In addition, the introduction of a fluorescent protein would provide an effective way to mark insects for release. The Unit has been developing this technology, in collaboration with other laboratories, for several years and a large number of transgenic strains have been studied. Technical concerns relating to the stability of transgenes and the biological fitness of strains carrying transgenes are probably of the most important. In relation to developing marker strains, injections have been carried out on the inversion strain in an attempt to isolate a strain with an insertion within the inversion. So far several lines have been isolated with an insertion on the chromosome that carries the inversion but so far they have all been outside the inversion. There is a striking range of phenotypes in the different transgenic strains.

The current debate concerning GMO's obviously has an impact on any eventual use of transgenic strains in field programmes. SIT is obviously the safest approach as there would be no vertical transmission of the transgene. In the coming year activities related to risk assessment for transgenic medfly will be carried out.

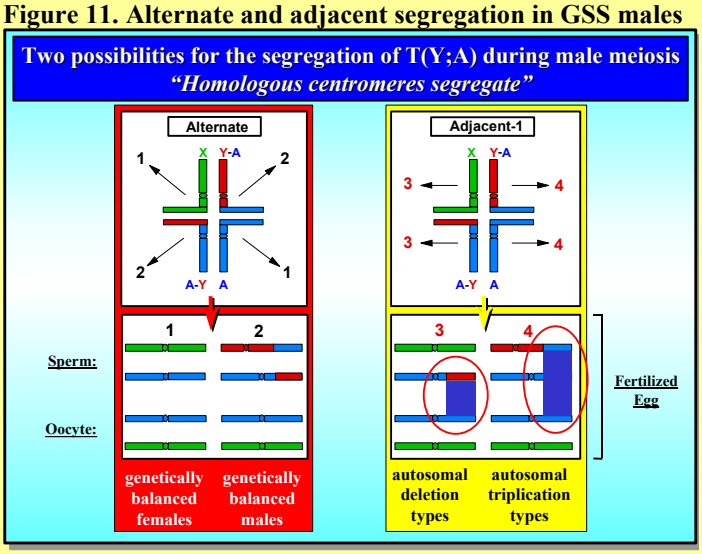
4.1 Productivity of Genetic Sexing Strains

Stability and productivity are two important criteria for the evaluation of GSS. In the 2001 Annual Report the improvement of stability was described and this report will focus on productivity of GSS reared at 25ºC, i.e. the temperature for colony maintenance. Both stability and productivity of a GSS are intimately linked to the structure of the translocation, i.e. to the position of the translocation breakpoint on the autosome in combination with the position of the Y-chromosomal breakpoint. The chromosomal behaviour of the translocation chromosomes in GSS males determines the number of genetically balanced, fully viable offspring and thereby the productivity of the strain and its quality with respect to the standard QC parameters.

4.1.1. Genetic principles

During male meiosis the two reciprocal translocation chromosomes, Y-A and A-Y (the order denotes which part contributes the centromere) can segregate in two ways, alternate and adjacent-1. **Figure 11** shows these two types of segregation and the consequences for the developing embryo.

Figure 11. Alternate and adjacent segregation in GSS males



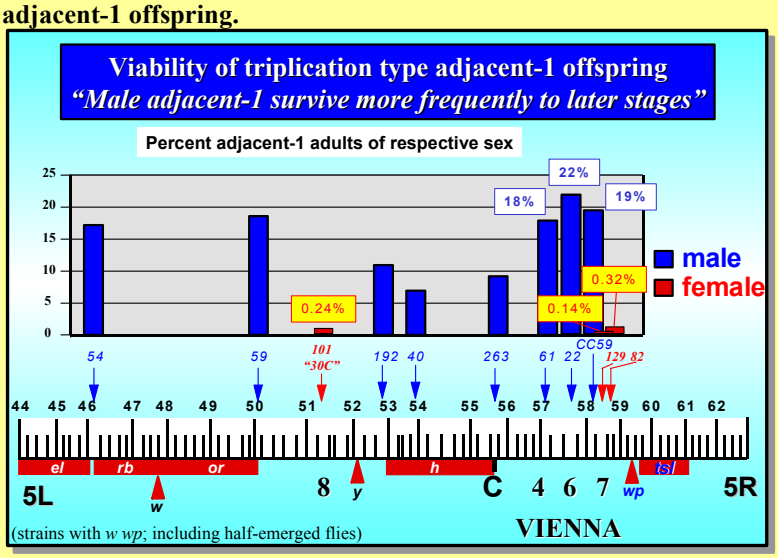
Only alternate segregation produces the genetically balanced parental genotypes, adjacent-1 segregation reduces the fertility/productivity of translocation carrying males and any survival of adjacent-1 zygotes to later developmental stages is unwanted because it impacts negatively on the quality control (QC) data of a strain. For the productivity of a GSS the most important parameter is therefore the frequency of alternate segregation. For example, if alternate segregation occurs at a frequency of 50%, a male with a simple translocation, T(Y;A), would be 50% sterile and a male with a more complex translocation, T(Y;A;A), would be 75% sterile.

Alternate segregation
The two reciprocal translocation chromosomes stay together while the free autosome segregates together with the X chromosome. The resulting sperm is genetically balanced, i.e. no duplications or deletions occur. After fertilization, males and females are produced that have the same genotype as their parents; the X/X females are homozygous for the free autosome and the males are heterozygous for the translocation.

Adjacent segregation
The X chromosome segregates with the translocation chromosome that carries the autosomal centromere (A-Y) while the free autosome stays with the translocation chromosome that carries the Y centromere (Y-A). Half the sperm carry an autosomal and a Y-chromosomal deletion (#3 in Fig. 1) and half carry an autosomal duplication and a deletion for part of the Y chromosome (#4 in Fig. 1). The zygotes carry a deletion of part of the Y and either an autosomal triplication or a deletion for this region.

Unfortunately, adjacent-1 zygotes do not die immediately after fertilization and the viability of the two types is determined by a combination of two factors; i.e. the length of the triplicated or deleted chromosome region and by the sex of the individual.

Figure 12. Differential viability of male and female triplication adjacent-1 offspring.



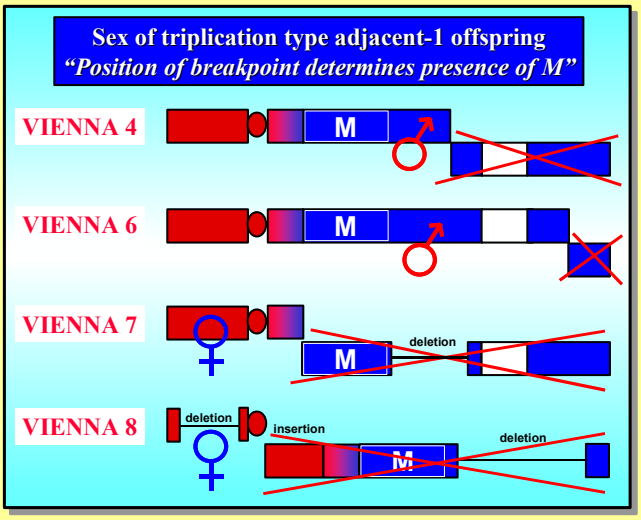
The length of the triplication/deletion is determined by the position of the translocation breakpoint on the autosome and it constitutes that part of the autosome between the breakpoint and it's tip. For example, VIENNA 4, 6 and 7 have breakpoints on the right arm of chromosome 5 (**Figure 12**) so that the triplication or the deletion consists of that part of chromosome 5 that is located between the breakpoint and right end of autosome 5.

In general, deletions reduce viability more significantly than triplications but in both cases the length of the affected chromosome is of importance, i.e. the shorter the region, the more viable the individuals. For triplications this is shown in **Figure 12**. To distinguish adjacent-1 flies from the balanced genotypes, two markers are required; one located outside of the triplication. The strains shown in blue produce male adjacent-1 adults of the triplication type. The closer the translocation breakpoint is to the centromere, the longer the triplication and the lower the proportion of adjacent-1 males that survive until adulthood.

The viability of adjacent-1 individuals also depends on their sex. The sex of the adjacent-1 offspring is determined by the position of the translocation breakpoint on the Y chromosome relative the centromere. As mentioned before both types of adjacent-1 have a deletion of part of the Y chromosome and it is known that major parts of the long arm can be deleted without any obvious consequences. The triplication type always contains the Y-A chromosome, i.e. that part of the Y that carries the centromere while the deletion type always contains the Y fragment without Y centromere (A-Y). The sex depends on whether the breakpoint is located in the region between the Y centromere and the male determining factor (M) or outside. In the former case centromere and M segregate together and the triplication type adjacent-1 individuals are male. This is shown in **Figure 13** for the four GSS described here. In VIENNA 4 and 6 the breakpoint is outside of the interval between breakpoint and M. Consequently, the triplication type adjacent-1 individuals are male. In VIENNA 7 and 8 the breakpoint is between the centromere and M; the resulting triplications type adjacent-1 individuals contain a Y fragment with the Y centromere

but without M and are, consequently, female. The reverse argument applies for the respective deletion type adjacent-1 individuals that carry those Y fragments that are crossed out in **Figure 13**. In **Figure 12** the proportion of surviving triplication type adjacent-1 female adults among all females is shown in red. It can be seen that females seem to be much more sensitive to a triplication. For example the triplication produced by VIENNA 7 is shorter than the ones from VIENNA 4 and 6 but never the less the survival of the triplication type adjacent-1 individuals to the adult stage is reduced significantly.

Figure 13. Sex of triplication adjacent-1 offspring



The viability of deletion or triplication carrying individuals is reduced in a gradual fashion. For example longer deletions usually cause lethality at the embryo stage, while shorter ones allow the egg to hatch but are lethal as early larvae. As mentioned earlier, triplications have less negative impact especially in males but the stage of death is dependent on the length of the triplication. In most cases they survive at least until the pupal stage. Lethality occurs at adult emergence, i.e. a large proportion does not emerge, of those that do a large proportion emerges only half or is crippled and those relatively few flies that look "normal" show a significant reduction in mobility (including reduced mating). The percentage of half emerged or crippled individuals is a good, although indirect, measure for the amount of triplication type adjacent-1 flies.

4.1.2. Assessment of productivity

A first assessment of the productivity of the different GSS can be made by using the same large data set that was used to determine their long-term stability (Table 6,

Table 8. Flies produced from 40ml samples of medfly pupae from different GSS (reared at 25ºC).

Strain	# of samples	# flies/ sample	% viable males	% viable females	# viable males/ liter	# viable females/ liter	% increase	
							males	females
VIENNA 4/*wp tsl*	147	1899	**88.4**	93.6	24200	18793	+13	+11
VIENNA 4/D53	18	2260	**80.1**	95.5	27225	21472	+27	+27
VIENNA 6/*wp tsl*	150	1788	**79.4**	95.4	21364	16947	**0**	**0**
VIENNA 6/D53	18	2203	**72.8**	93.5	25725	18465	+20	+9
VIENNA 6/*wp*	91	1945	**73.5**	96.5	21684	18200	+2	+7
VIENNA 7/*wp tsl*	134	2029	96.5	94.9	27434	21137	+28	+25
VIENNA 7/D53	101	2200	97.1	93.9	30728	21880	+44	+29
VIENNA 8/*wp tsl**	41	2354	96.7	95.5	31245	18226	+46	+8
VIENNA 8/D53	46	2423	93.3	92.3	33054	23197	+55	+37
VIENNA 8/*Sr*/D53	7	2385	93.9	92.0	30857	24607	+44	+45

* : Data are from strain that broke down during the rearing

Annual Report 2001). Here each generation is set up using 34ml of unselected pupae (i.e. no exceptional phenotypes removed) and a further 40ml is used to screen for recombinants. Four different GSS, VIENNA 4, 6, 7 and 8, have been tested using this scheme for up to 95 generations. **Table 8** shows the number of flies emerging from 40ml of pupae. However, a proportion of these flies is either crippled or only half emerged as they come from adjacent-1 segregation. The % of viable males is shown in **Table 8**. The recovery of viable females is fairly constant regardless of whether the strain contains only *wp*, *wp tsl* or the inversion D53, and varies from 92% to 96.5% with an average of 94.3%. These high values, especially for the *tsl*-containing strains, are only achieved under conditions of small scale rearing. In contrast, the recovery of viable males differs between strains and three levels can be distinguished: VIENNA 4 with an average of 84.3% viable males, VIENNA 6 with an average of 75.2% and VIENNA 7 and 8 with 95.5%. It can be concluded that VIENNA 4 and 6 produce considerable numbers of triplication type adjacent-1 male individuals. The final productivity, expressed as viable males or females per litre, is lowest in the strain VIENNA 6 carrying the *wp tsl* chromosome. However, the recovery is improved when this strain contains the D53 inversion. There is currently no plausible explanation for this effect but it seems to occur also in the other strains. VIENNA 8 in combination with D53 has the highest productivity.

A second set of data that can be used to determine productivity of GSS is based on the temperature tests that are conducted during routine rearing of the strains. Eggs are collected for 24h in water, batches of 100 eggs are placed on a strip of black filter paper (damaged eggs are removed) and the filter paper is placed on larval diet in a 9cm diameter Petri dish. The closed Petri dishes are incubated at the appropriate temperatures. The results for the 25ºC control are summarized in **Table 9**. For comparison, two additional strains are included that contain more complex

Table 9. Adults per 1000 eggs from different GSS in medfly (reared at 25ºC, eggs collected for 24h)

		Per 1000 eggs:				
Strain	**# eggs counted**	**# hatched**	**# *wp***	**# *wp*$^+$**	**# females**	**# males**
T(Y;3;5)1-56	4900	484±27	164±13	128±16	116±10	114±10
T(Y;2;5)2-82	5100	461±30	82±14	221±42	74±14	105±11
VIENNA 4	19840	654±41	187±24	372±43	175±28	227±31
VIENNA 6	14200	645±52	181±23	333±51	172±23	224±42
VIENNA 7	11700	713±50	192±24	292±41	182±21	196±22
VIENNA 8	4200	723±40	299±36	310±38	249±34	280±26
VIENNA 8/D53	3600	752±18	296±8	322±14	247±20	283±21
wp tsl	6900	817±94	763±118		345±67	345±67
EgII	5680	929±45		880±59	428±29	432±32

translocations, i.e. two autosomes linked to the Y chromosome. All translocation strains are compared to the homozygous *wp tsl* strain and to the wild type strain EgII. The results show that the complex translocations produce fewer flies, i.e. such males are more sterile than those with a simple translocation. Secondly, among the simple translocations VIENNA 8 stands out because it shows a significantly increased productivity. Based on the data in **Table 9** the fertility of the different strains was calculated (**Figure 14**). As expected, translocation carrying males show a significant level of sterility whereby the degree of sterility is directly correlated to the complexity of the translocation. Furthermore, these data can be used to estimate the frequency of alternate segregation. Considering that the translocation strains represent a

combination between the homozygous *tsl* strain and the wild type strain, one can calculate that a fully fertile strain without adjacent-1 segregation should produce 78 flies per 100 eggs, a simple translocation strain where there is 50% alternate segregation should produce 39 flies and a more complex translocation strain should produce ca 20 flies per 100 eggs. **Figure 14** shows that the observed values come very close to these estimates with the exception of VIENNA 8 where the males are clearly less sterile.

Figure 14. Fertility of different genetic sexing strains

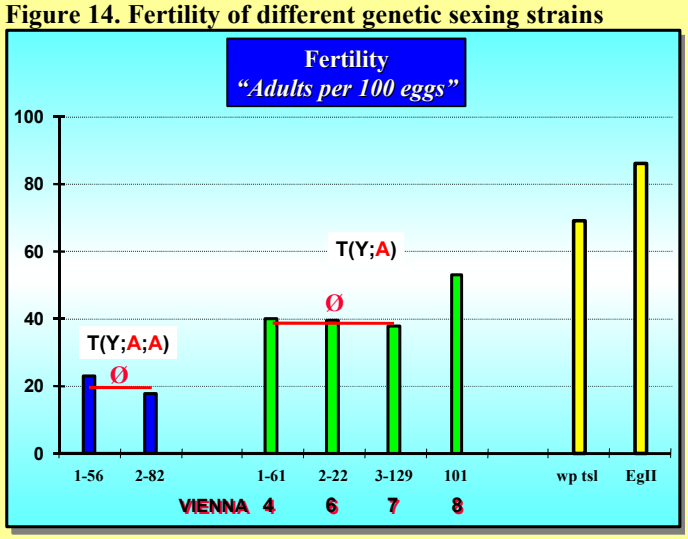


Table 10 allows a more accurate comparison of the viable and half emerged/crippled categories. Based on the values for the *tsl* homozygous strain one would predict that strains where adjacent-1 segregation occurs at 50% should produce 173 females per 1000 eggs. This appears to be the case for VIENNA 4, 6 and 7. The same calculation for the males, based on the EgII strain, would predict 216 males. The average for the strains VIENNA 4, 6 and 7 is 193 males, only slightly below that number. Therefore, it can be concluded that in the males of these three strains alternate and adjacent-1 segregation occur at equal frequency. It follows that such strains have a productivity of 50%, i.e. the theoretical maximum is 250 male offspring per 1000 eggs. The surviving adjacent-1 flies in VIENNA 4 and 6, represented here as half emerged males, add to these numbers but because of their poor quality this is an unwanted effect (see below). The strain VIENNA 7 represents an improvement compared to VIENNA 4 and 6 not because it is more productive but because the number of unwanted adjacent-1 zygotes surviving until later developmental stages is significantly reduced. As described above, this is due to the fact that in this strain the triplication type adjacent-1 individuals are female and die preferentially during earlier stages of development. **Table 10** also shows the numbers for VIENNA 8. This strain produces more adults than the other three GSS and it should be stressed that this is true for both males and females (41% and 44% more adults as compared to the average of VIENNA 4, 6 and7). This is a strong indication that this increase is not due to differences in viability of either males or females. It indicates however that the translocation in the males of this strain segregates more

Table 10: Adults per 1000 eggs from different GSS in medfly (reared at 25ºC, eggs collected for 24h)

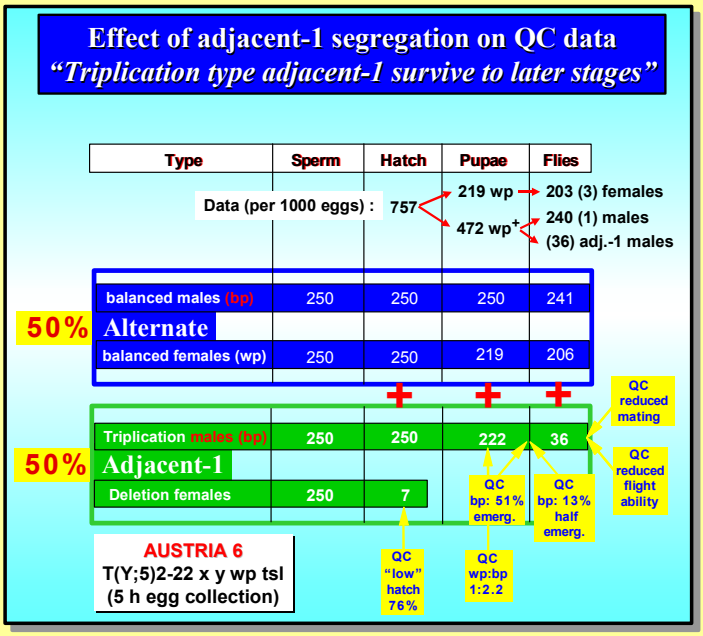
Strain	Per 1000 eggs		
	# females	# half emerged males	# fully emerged males
(25% of *tsl*)	173		
(25% of EgII)			216
VIENNA 4	175	28	199
VIENNA 6	172	39	185
VIENNA 7	182	2	194
Average	176		193
VIENNA 8	248 +41%	3	278 +44%

frequently following the alternate type. Based on the numbers in **Table 10** it can be estimated that alternate segregation occurs in VIENNA 8 with a frequency of ca 70%. The reason for this effect is unknown. It can only be speculated that it might be linked to the fact that the Y chromosome in this strain is more severely rearranged than the Y in the other strains (**Figure 13**).

4.1.3 Effect on quality control parameters

The occurrence und survival of adjacent-1 offspring does not increase the productivity of a strain. On the contrary, it affects many QC parameters negatively. To show this, the two strains AUSTRIA 6 and 7 were analysed. These strains differ from the corresponding VIENNA strains only by the presence of an additional marker (*yellow body*, *y*). This marker is located outside of the triplication and, therefore allows adjacent-1 adults to be discriminated from flies resulting from alternate segregation. To maximize the recovery, eggs were collected only for 5 h.

Figure 15. Effect of adjacent-1 survivors on quality control data for GSS AUSTRIA 6.

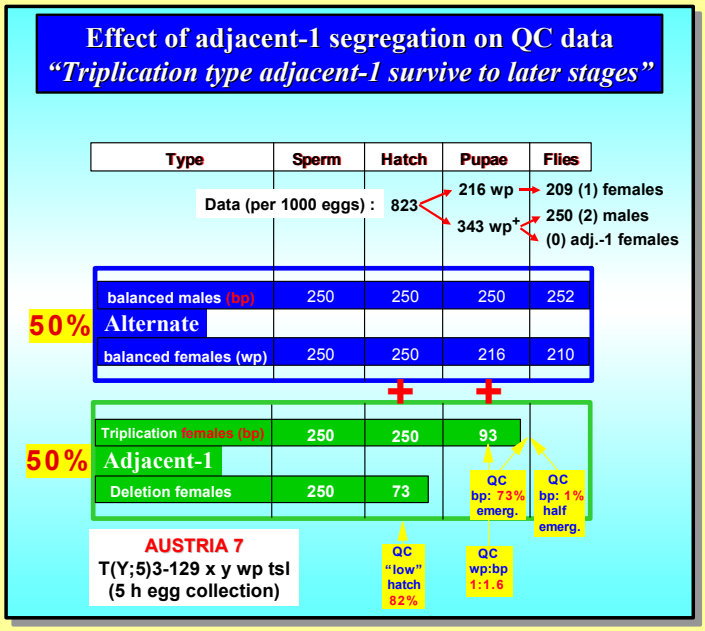


AUSTRIA 6 strain: In the upper part of **Figure 15** the actual data are shown and below are the numbers based on the assumption that alternate segregation occurs at a frequency of 50%. This means that 4 different types of sperm and, after fertilization, four different types of zygotes, are produced at equal frequency. The genotypes of these are as shown in **Figure 11**. The observed value for egg hatch is 757 per 1000 eggs. Only the deletion type adjacent-1 embryos are expected to die at this stage while all other genotypes should be viable. At the pupal stage no additional lethality is detected, i.e. the triplication adjacent-1 pupae, with wp^+ phenotype, are present in nearly the same numbers as the balanced male pupae. This leads to a highly distorted ratio between *wp* and wp^+ pupae, i.e. there are roughly twice as many brown as white pupae. The two classes with balanced genotypes emerge at normal rate. However, for triplication adjacent-1 pupae a significant level of lethality is observed that is manifested in a severely reduced emergence (36 of 222) and, at least in this experiment, all flies emerged either only partially or were deformed. Based on a larger data set, ca 6% of these males emerges completely and shows no obvious deformations. In a normal GSS, where alternate and adjacent-1 segregation products cannot be distinguished the overall emergence (i.e. the average of the emergence of the balanced males and the severely reduced emergence of adjacent-1 males) from brown pupae is as low as 51% plus 13% half emerged/crippled flies. Obviously, these low values are entirely caused by the low viability of the adjacent-1 pupae/flies.

Those triplication type adjacent-1 flies that emerge fully will reduce the average QC values for flight ability and mating, i.e. if only balanced males would be present these QC values would be normal. The numbers shown in **Figure 15** support the statement that in VIENNA 6 alternate and adjacent-1 segregation occur at equal frequency.

AUSTRIA 7 strain: The frequency of alternate versus adjacent-1 segregation is a fixed characteristic of each individual translocation and cannot be modified. The only way to reduce the adjacent-1 problem is to identify translocations where the structure is such that the adjacent-1 individuals die as early during development as possible. This is the case for the translocation T(Y;5)3-129 in VIENNA 7 and AUSTRIA 7 (**Figure 16**). The egg hatch is higher than in AUSTRIA 6 indicating that here more deletion type adjacent-1 eggs hatch. Significantly fewer wp^+ pupae are detected but the numbers indicate that this is not due to reduced productivity. On the contrary, the reduced number of wp^+ pupae indicates that fewer triplication type adjacent-1 individuals reach the pupal stage. No or only very few flies emerge from these pupae with the consequence that the value for emergence is relatively high (73%) and all the following QC values, the number of half emerged/crippled flies, flight ability and mating reach the same levels as in a normal strain. The numbers obtained with VIENNA 7 are very close to those that were predicted assuming an alternate segregation frequency of 50%

Figure 16. Effect of adjacent-1 survivors on quality control data for GSS AUSTRIA 7



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4.1.4. Conclusion

The structure of a translocation determines the ratio between alternate and adjacent-1 segregation. For high productivity the frequency of alternate segregation should be as high as possible. The only translocation detected so far that shows more than 50% alternate segregation is VIENNA 8. The structure of the translocation also determines the fate of the adjacent-1 individuals. For high productivity with optimal QC values these individuals should die as early as possible. In VIENNA 7 the lethality occurs to a large extent at the larval stage. In VIENNA 8 even fewer adjacent-1 larvae pupate resulting in a more or less equal ratio of white and brown pupae. VIENNA 8 represents therefore a significant improvement over earlier strains and it will provide mass rearing facilities with a significantly increased productivity.

4.2 Medfly Transgenics

4.2.1 Production and analyses of transgenic medfly strains

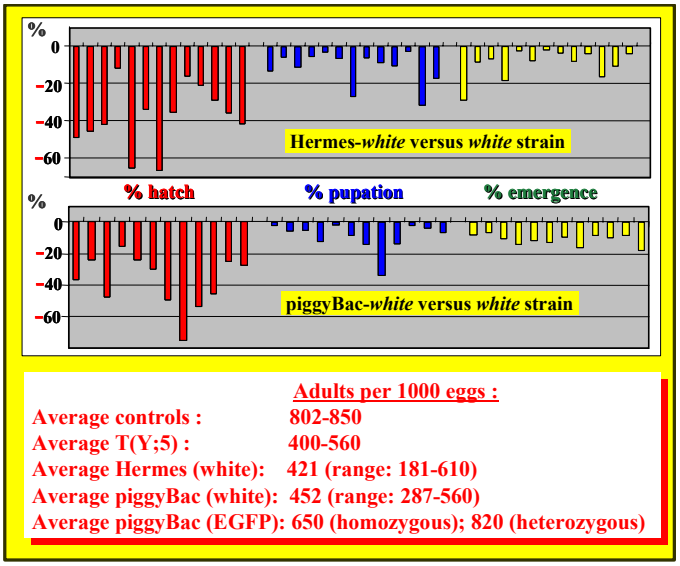
The generation of transgenic medflies has two goals: a) to incorporate a marker gene that would allow discrimination between released and wild flies, and b) the incorporation of genes that allow either the separation of the sexes, the elimination of the females or the conversion of the females into males. In the past the very first step, the genetic transformation of non-drosophild insects, was the practical bottleneck. This has been solved to a point where many different species have been transformed successfully. However, in many cases the efficiency of transformation or, more precisely, the number of transformants obtained was relatively low. This means that the procedure is still rather labour intensive and due to the small number of transgenics no general conclusion can be obtained with respect to the expression of the transgenes, their impact on the fitness (from viability to behavior) and their stability especially in large scale rearing. All these issues are, however, very important for the practical application of such strains within the SIT.

4.2.1.1 Transformation using the white gene as marker

In collaboration with Peter Atkinson (University of California) and Al Handler (USDA, Gainesville) two different transformation vectors were employed. One is based on the mobile element *Hermes* from *Musca domestica* and the other on the *piggyBac* element from *Trichoplusia ni*. Both vectors contain a medfly mini-white gene under the control of the *Drosophila melanogaster hsp* 70 promoter. These constructs were micro-injected into a *white* medfly strain together with a plasmid carrying the respective transposase controlled by the *D. melanogaster hsp* 70 promoter. Subsequent tests showed that no heat shock is required to induce the expression of the mini-white gene. The statistics of both experiments were reported in the Annual Report 1998. In total 13 and 16 transgenic strains were isolated from the two series of injections, some of which represent identical insertions.

In generation 43 to 44 the viability of 13 *Hermes* and 12 *piggyBac* strains was analyzed. All strains are homozygous for the particular insertion at the time of testing.

Figure 17. Fitness of medfly transgenic lines.



Nine to eighteen samples with 100 eggs each were collected and the resulting hatch, the number of pupae and the number of adults was measured. The results in **Figure 17** are expressed relative to the values obtained with the recipient white strain. A significant reduction in egg hatch is observed in all transgenic strains irrespective of whether they carry a *Hermes* or a *piggyBac* transgene. In some cases this reduction was considerable. Also the pupal and adult recovery is reduced although not as severely as the hatch. On average, the viability of the transgenic strains is reduced by ca 50%. As

the comparison in **Figure 17** shows the productivity is very similar to strains carrying a simple Y-autosome translocation as they are used in the current sexing strains and which are known to be 50% sterile. Preliminary results with some of the strains carrying the EGFP marker described below seem to suggest that the viability is higher although only if the strain is heterozygous. The results also show that each strain has a different fitness profile that is impossible to predict. It will therefore probably be necessary to induce many transgenic lines and from these select the one that has the right characteristics. It is considered that fitness will be a major concern when dealing with transgenic strains for use in SIT programmes. Clearly, further evaluations are needed to identify transgenic strains that show a better performance.

4.2.1.2. Transformation using fluorescent marker genes

The use of fluorescent markers to monitor transformation events is a now widely used in many areas of molecular biology. However for insects that are being transformed in relation to SIT these markers may also provide a means to identify sterile released insects in the field. To be of use for this purpose they need to have several characteristics, including stability of expression during rearing, minimal effects on fitness and the ability to be recognized in dead flies. It may be that a fluorescent maker strain could be the first type of strain to be evaluated for use in an SIT programme. So far three transformation experiments were conducted in collaboration with Al Handler (USDA, Gainesville) using the following vectors carrying fluorescent markers:

#257 *Drosophila melanogaster* polyubiquitin promoter, SV-40 T-antigen nuclear localization signal, enhanced Green Fluorescent Protein (EGFP) gene from *Aequorea victoria*

#319 *D. melanogaster* polyubiquitin promoter, SV-40 T-antigen nuclear localization signal, DsRed marker gene from *Discosoma striata*

#320 *D. melanogaster* UAS promoter, EGFP marker gene from *A. Victoria*

The results of the different transformation experiments are shown in **Table 11**.

Table 11. Overview of transformation experiments in medfly

		G0					G1		G8-G23		
		Number				(%)	Number		(%)	Number	
Strain	Vector	Injected	La.	Pup.	Ad.		Screened	Transf.		/1000 eggs	Single
Injection 2/01											
D53	**EGFP**	2376	170	46	32	1.3	5556	9	7 (78)	3	6
D53	**DsRed**	1728	276	48	8	0.5	708	0			
EgII	**EGFP**	1188	464	338	317	**26.7**	16476	61	50 (82	**42**	44
Injection 12/02											
D53	**EGFP**	not					1	8	5(63)		6
D53	**DsRed**	counted					2	31	12(39)		5
Injection 4/02											
w wp	**EGFP**	580	222	164	156	**26.9**		>20	15(75)	**26**	13
D53	**EGFP**	470	160	91	60	12.8		>4	2(50)	4	
D53	**DsRed**	1080	244	156	105	9.7		>17	3	3	1
EgII	**DsRed**	650	118	60	58	8.9		>12	7	11	1
Total		**>8072**						**>162**	**>101**		**>76**

In each case relatively high numbers of transformants were detected so that in the third set of injections not even all G1 flies were screened. It appears that usually only

few G0 flies produce transgenic offspring. However, these flies produce a large number transformants including independent ones. When the success rate of transformation is so high, two criteria become of practical importance, i.e. how many independent transformants were generated and how many strains carry multiple integrations. All strains have been analyzed by Southerns and for some strains *in situ* hybridizations were performed in collaboration with Antigone Zacharopoulou (University of Patras) (see below). Based on this information strains with apparently identical insertion can be discarded and further crosses are required to establish single insertion lines from those strains with multiple insertions.

Of the 61 strains obtained from injecting the wild type strain EgII in February 2001, 57 were analysed in more detail with respect to the pattern of fluorescence and the visibility of the fluorescence in living and dead flies. In strains where EGFP is used as marker, four distinct patterns can be distinguished (**Figure 18**):

Figure 18. EGFP patterns in transgenic medfly

A. Fluorescence of the head, thorax, legs and reproductive organs
B. Fluorescence of the head, thorax, legs (weak) and male reproductive organ
C. Fluorescence of the whole body. (Not shown in **Figure 18**.)
D. Fluorescence at the tip of the head, thorax, joints of the legs and the reproductive organs

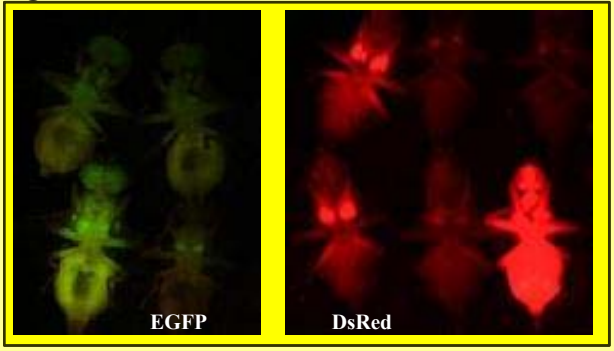
The same patterns were observed in all other transgenic strains. Within each pattern there are strains with stronger or weaker fluorescence. The different patters as well as the intensity of fluorescence appears to be inheritable, i.e. they do not represent simple variability of expression within a strain. It has to be noted that the set of strains analysed here may be exceptional and particularly suitable for this kind of analysis because a large proportion represents independent insertions (50 of 57) and most of them carry only a single insertion (44 of 57). In strains carrying several transgenes additional variability may be introduced by the segregation of the different insertions and, potentially, by an interaction of the transgenes affecting expression levels.

As **Table 12** shows that the visibility of the fluorescence declines in some strains in older flies. Roughly only half of the strains with pattern B still show fluorescence that is detectable by eye when the flies reach the age of 25 days. For the

Table 12. Fluorescence persistence in medfly transgenic strains

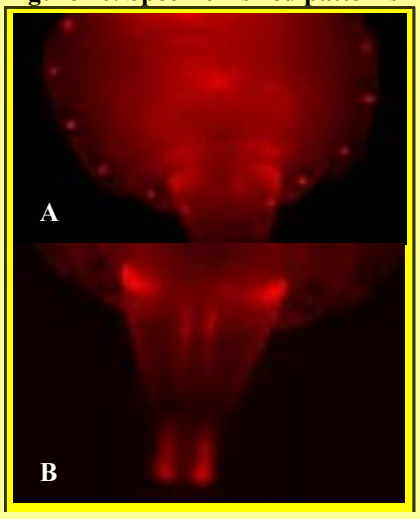
Pattern	Number of families with clearly visible fluorescence (EGFP)				
	After 1d	After 3d	After 25d	Killed 3d after emergence	
				After 1-3d	After >10d
A	10	10	9	7	7
B	43	43	20	11	11
C	3	3	3	3	3
D	1	1	1	0	0
TOTAL	**57**	**57**	**33**	**21**	**21**

Figure 19. Patterns of EGFP and DsRed fluorescence



practical application of fluorescence marker genes it is of course important if and how long the signal can be detected in dead flies. To test this, flies were killed 3 days after emergence and were kept in Petri dishes at room temperature. In 21 strains the fluorescence was still visible 1-3 days after death and there was very little decline in fluorescence 10 days or later after death. Some cases were re-examined after several months and the fluorescence was still clearly detectable. The viability of the fluorescence in dead flies appears to be linked to the original strength of the fluorescence in the living flies, i.e. the stronger the fluorescence is in the living flies the better detectable is the signal also in dead flies. In general, the DsRed marker shows more intense fluorescence than the EGFP marker (**Figure 19**). However, roughly the same types of patterns can be distinguished as with the EGFP marker. In addition and, probably due to the higher intensity of the fluorescence, in some strains very specific labelling of some tissues/organs can be detected (**Figure 20**). So far no obvious differences between the promoters used were observed.

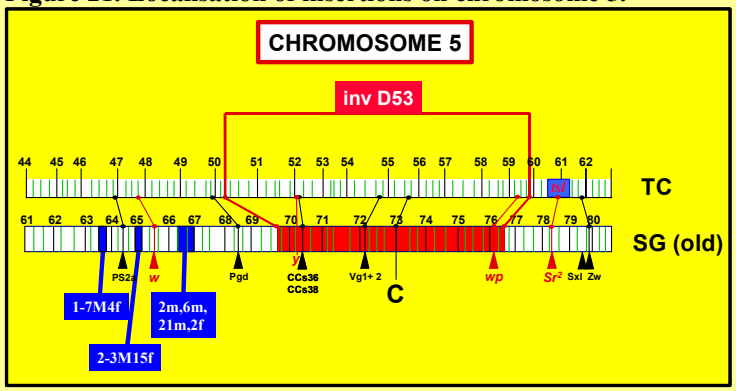
Figure 20. Specific DsRed patterns



4.2.2. Integrations on chromosome 5

For the practical application as a marker for released flies the transgene should be integrated on chromosome 5 that carries the mutations used for sexing. In the current sexing strains the chromosome 5 also carries an inversion (D53, *wp tsl*). With respect to the stability it would be ideal if the insertion would be located within the inverted region (the sexing strain males are heterozygous for the inversion chromosome). All transgenic strains derived from injecting D53 *wp tsl* were crossed with EgII followed by inbreeding to determine whether the transgene (i.e. the fluorescence) co-segregates with *wp*. In 8 strains the transgene appears to be inserted on chromosome 5 and of these 6 have been analyzed by *in situ* hybridization by Antigone Zacharopoulou (University of Patras). As corroborated by Southern analyses, the strains fall into three

Figure 21. Localisation of insertions on chromosome 5.



classes, i.e. three different integration sites were detected (**Figure 21**); four strains have an insertion at 66B/C, one insertion is at 65A and one at 63C. Unfortunately, all insertions are outside of the inversion.

It is now relatively easy to generate many transgenic strains. In many cases a single successfully injected embryo produces many independent transgenic strains. However, there are several labour intensive steps required to identify transgenics, to eliminate duplicates and to construct single integration strains. The issue of viability has to be addressed more thoroughly with well defined single insertions strains. In this context the resulting phenotypes have to be re-evaluated.

5. Quality Control of Medfly Genetic Sexing Strains

In support of TC project ISR/5/010, the Unit shipped 5 million sterile male pupae/week to Israel for an 8 month period in 2002. The flies were released in the Arava Valley as part of an on going project to suppress medfly populations in the area. The strain used to supply the flies was VIENNA 7 D53 (see Annual Report 2001). This strain carries an inversion to increase stability and has been reared at the Unit without the use of the Filter Rearing System (FRS) for over two years without showing any signs of breakdown. It is currently being used for the medfly programme in South Africa.

In relation to the possible development of medfly rearing facilities that may decide to purchase eggs from an existing facility and simply rear males for sterilization and release, trial shipments of eggs between Seibersdorf and the El Pino medfly rearing facility have been carried out. Initial results are promising but shipment logistics will need to be improved and standardized before this technology becomes a practical option. Based on the shipment data, detailed experiments have been carried out to assess the effects of storage time, heat treatment, transportation medium etc., and the results will be elaborated in 2003.

In 2000 a new GSS was constructed incorporating an "old" translocation and inversion D53 (Annual Report 2001). The genetics of the strain, VIENNA 8, is described in the previous section and an overview is given of the potential production benefits associated with this strain. In order to assess these potential benefits, the strain was mass reared and most of the expectations were confirmed. A new adult cage was also tested using this strain and it was shown that egg production was significantly increased. Reduced egg production is an unfortunate characteristic of medfly GSS necessitating the development of better cages. The strain will be transferred to mass rearing facilities during 2003.

During 2003 activities will be expanded on Anastrepha *and* Bactrocera *fruit fly species to develop and improve SIT technology. Colonies of* Anastrepha fraterculus *from several regions of Central and South America have been established and mating compatibility studies will be carried out in 2003. Further developments include improving oviposition techniques and developing a set of QC protocols. SIT for* Bactrocera oleae *has a long history and much work has been done. Key constraints that will be addressed include larval diet formulation and initial studies on the development of a GSS.*

5.1 Characterization of Mass Rearing of the VIENNA 8 Genetic Sexing Strain

As described in Section 4, the GSS VIENNA 8 demonstrated increased productivity under conditions of normal laboratory rearing. To verify this increased productivity the strain was characterized for production and important QC parameters following 4 generations of consecutive mass rearing.

VIENNA 8 was constructed using translocation T(Y:5)101 backcrossed with the *wp-tsl* D53-Mix strain that contains a diverse genetic background from populations of five different geographic areas (Chile, Argentina, Peru, South Africa and Tunisia) to increase its genetic variability. The strain also carries a chromosomal inversion on the autosome 5 to increase stability. As reported in Section 4, adjacents-1 segregants from this translocation are female and die during the early larval stage and the zygotes that carry the deletion die during the embryo stage. Basically during all the larval rearing cycle only balanced individuals are present for both sexes allowing the recovery of insects with normal quality.

5.1.1.Production and quality characteristics

5.1.1.1. Colony production

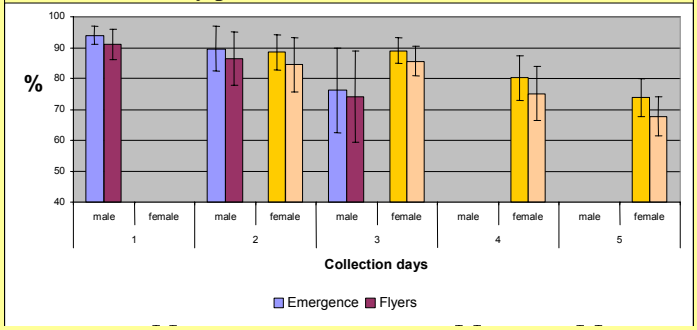
The data for colony production are shown in **Table 13**. Assuming that 50% of the individuals are genetically balanced then the expected overall efficiency from egg to pupae is 50% and with this strain a value of 49.6% was observed. This is very high for a strain under mass rearing conditions and thus confirms the data presented in Section 4 that in this translocation there is an excess of balanced individuals being produced above that expected. However when the data are analysed individually the observed efficiency egg to pupae for brown pupae (male) and white pupae (female) are different than the expected values. In the case of the brown pupae values higher than the expected (0.25) are observed (0.29±0.06) this value supports again the hypothesis that segregation is in favour of balanced zygotes. But in the case of the white pupae lower values (0.20±0.06) were observed than the expected (0.25). We assume that the main reason was due to the mortality of the white pupae during the larval stage. White pupae females are sensitive to high temperature and consequently more sensitive to the heat stress produced under mass rearing conditions. Notwithstanding this fact, VIENNA 8 demonstrates a better efficiency for white pupae than the other genetic sexing strains already evaluated in mass rearing. The values shown for VIENNA 8 for efficiency of eggs to males (0.25±0.05), eggs to females (0.18±0.05), eggs to flying males (0.23±0.05) and eggs to flying females (0.15±0.05) are very similar to those for other sexing strains. The percentage of adult emergence and flying flies calculated for

Table 13. Rearing efficiency for VIENNA 8 genetic sexing strain

Rearing Parameter	Colony production	Male only Production	Total
Egg hatch (%)	71.61±5.80	58.03±16.71	
Expected efficiency	0.25	0.25	0.5
Efficiency egg to pupae			0.496±0.06
Efficiency egg to white pupae	0.20±0.06		
Efficiency egg to brown pupae	0.29±0.06	0.21±0.03	
Efficiency egg to adult female	0.16±0.05		
Efficiency egg to adult male	0.25±0.05	0.19±0.04	
Efficiency egg to flying female	0.15±0.05		
Efficiency egg to flying male	0.23±0.05	0.18±0.05	
Overall % flying females	72.12±12.12		
Overall % flying males	83.12±4.27	82.00±6.90	
Flight ability index	86.77	94.70	
Sex ratio (male:female)	60:40	100:0	

each pupal collection showed that in VIENNA 8 the quality decreased progressively with each collection but not drastically as in other GSS where normally in the last collections there is an accumulation of individuals produced from adjacent-1 segregation (**Figure 22**).

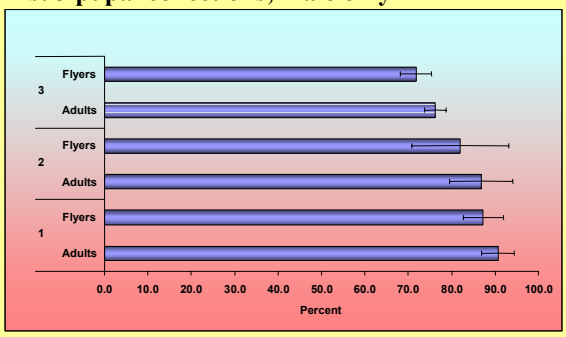
Figure 22. Emergence and flight ability from different pupal collections; colony production



5.1.1.2. Male only production

Male only production was carried out using the established thermal treatment protocol of 24h incubation at 24°C followed by 24h at 34°C. The efficiency of eggs to pupae (0.21±0.03) was lower than that found in colony production. The efficiency of eggs to males (0.19±0.04) and eggs to flying males (0.18 ± 0.05) were a little lower than in the colony production but values for all collections are acceptable (**Figure 23**). The overall percent of flying males after the thermal treatment for VIENNA 8 was 82.8±5 and the overall fly ability index (FAI) was 94.0% (**Table 14**). The thermal treatment is used to kill females and also it leads to the death of the adjacent-1 individuals in other GSS. However, it does have an effect on the males and as in the VIENNA 8 there are no adjacent individuals an improved thermal treatment regime could be developed.

Figure 23. Emergence and flight ability from the first 3 pupal collections; male only



During the four generations of mass rearing of VIENNA 8 no accumulation of recombinants individuals was detected even though a filter rearing system was not used. The strain will provide significant advantages to operational facilities when they use this strain. 1) The cost per million fly can be reduced because more pupae will be recovery from the same egg seeding density and same amount of diet per tray of diet, 2) The colony size can be smaller because the overall fertility of the VIENNA 8 is better than the current strains, 3) Larva collection for only male pupae can be easily automated because there are no differences of quality between the different days of larval

Table 14. Potential facilities for transfer of VIENNA 8 in 2003

Facility	Country	Production level (million)	Transfer dat
El Pino	Guatemala	2000	Feb.-March
CNSTN	Tunisia	8	Feb.-March
La Molina	Peru	160	May
Infrutec	South Africa	10	June
Km 8-Mendoza	Argentina	160	Not defined
Arica	Chile	60	Not define
Metapa	Mexico	300	Not defined

collection and 4) the cost per million of flying males released in the field should be cheaper because the percentage of flying males produced is at least 10% higher than the current GSS. It is planned to transfer VIENNA 8 to several facilities during 2003 (**Table 14.**).

5.2 Evaluation of Egg Shipments from Guatemala

The successful integration of genetic sexing technology into operational mass rearing facilities for medfly SIT can be further encouraged by developing procedures to ship eggs from a main production facility to satellite facilities where the eggs would be heat treated and males reared for sterilization and release. This system has many advantages but will depend on the feasibility of developing an efficient egg shipment protocol. The results reported here are a first step in that direction.

5.2.1 Shipment protocol

Six shipments of medfly eggs from the GSS VIENNA 7-TOL were air-freighted from the El Pino medfly mass rearing facility in Guatemala to the Unit. Prior to shipment the eggs were bubbled for 24hr in water. The eggs were then shipped either in water or in a 0.1% solution of agar to prevented settling. The length of the transportation time fluctuated between 48 and 82hr. Each shipment consisted of two metallic thermos flasks containing approximately 250ml of eggs in 500ml of the transportation medium. Upon arrival in the Unit, the eggs from each flask were divided in two aliquots with one aliquot being incubated at 24°C for 24hr whilst the other aliquot was incubated at 24°C for 12hr followed by 34°C for a further 12hr. The latter is the standard treatment necessary to kill the female embryos. Following each type of incubation, 5 larval trays with 5kg of wheat bran based larval diet were set up to assess production parameters. For the heat-treated eggs, 8ml of eggs were used and for the non heat-treated 3.2ml were used. Pupal yield and standard QC test were performed to determine any effect of the long distance shipment on the quality and quantity of insects recovered. In Guatemala a sample of the eggs was retained and used to provide control data.

5.2.2 Egg viability

Egg hatch was determined on a sample of eggs immediately upon arrival as well as on the eggs that were subsequently incubated with the two different egg incubation treatments. Egg hatch for those eggs sampled upon arrival and those incubated for 25°C for 24hr was reduced by about 15% in comparison with the expected value for the VIENNA 7-TOL strain. Eggs sampled after incubation at 34°C for 12 hours showed a similar value as the VIENNA 7-TOL strain. No significant differences in egg hatch were found between the egg shipments and there was no correlation between the egg hatch and the duration of the shipment (**Table 15**).

Table 15. % egg hatch following shipment in agar or water; upon arrival and after incubation and after heat treatment

Shipment Time (hrs)	Before incubation		After incubation		After heat treatment	
	Water	Agar	Water	Agar	Water	Agar
82	71.0	59.2	62.9	62.5	45.7	55.8
82	67.0	75.3	51.7	66.7	70.7	55.7
48	69.0	62.6	57.6	65.7	53.2	53.1
70	60.4	72.0	73.2	67.6	46.9	47.3
45	67.1	65.5	64.4	66.0	45.6	62.6
50	69.2	71.1	73.7	75.7	49.3	53.2
Mean	67.3	67.2	63.9	67.4	51.9	54.6
s.d.	3.7	6.2	8.6	4.4	9.6	5.0

5.2.3. Pupal yield following egg incubation

Table 16. Number and phenotype of pupae produced and egg to pupal efficiency in colony production

Shipment Time (hrs)	Agar			Water		
	Brown	White	Eff.	Brown	White	Eff.
82	1271	1339	0.35	1505	815	0.44
82	1222	2158	0.49	1188	2162	0.49
48	784	221	0.13	1966	1014	0.43
70	884	466	0.20	620	179	0.12
45	1540	720	0.33	1339	571	0.28
50	1505	815	0.32	1647	623	0.31
Mean			0.11			0.09
s.d.			0.06			0.05

The expected efficiency from egg to pupae under normal conditions of colony production is 0.35 to 0.40 for VIENNA 7 GSS. The egg to pupae efficiency from the transported eggs was highly variable (**Table 16**). For first and sixth shipment, efficiency was normal (0.35 in agar and 0.44 in water; 0.32 in agar and 0.31 in water respectively) while for second shipment efficiency was slightly higher than the expected value (0.49 in agar and 0.47 in water). For the third shipment efficiency was normal for eggs shipped in water (0.43) but lower for those shipped in agar (0.15) whist shipments 5 gave data in the opposite direction. Shipment 4 was uniformly bad. The reasons for these wide fluctuations have not been identified.

5.2.4 Pupal yield following heat treatment

Efficiency of male only production showed a similar pattern of efficiency as for colony production when the shipments were compared (**Table 17**) but the average efficiencies of around 0.1 were considerably below the expected (0.19). However in shipments 1 and 2, male only production was affected because of a high proportion of recombinant white pupae males; 22 and 29 % males emerged from white pupae respectively. These white males are fully fertile and a proportion of them carries the *tsl* mutation in a homozygous condition and is thus killed by the heat treatment. The reduction of male production will be directly correlated with the level of contamination of this type of male.

Table 17. Number and phenotype of pupae produced and egg to pupal efficiency following male only production

Shipment Time (hrs)	Agar			Water		
	Brown	White	Eff.	Brown	White	Eff.
82	2337	213	0.15	2391	99	0.14
82	2154	506	0.15	2093	467	0.15
48	879	1	0.05	1793	7	0.10
70	877	13	0.05	660	0	0.04
45	1088	2	0.06	800	0	0.05
50	2970	20	0.17	947	23	0.06
50	2418	2	0.14	1380	0	0.08
Mean			0.11			0.09
s.d.			0.06			0.05
Mean in Guatemala			0.15			0.31
s.d.			0.04			0.08

In shipments 1 and 2, 13 to 20% of the male population was lost corresponding to the presence of these recombinant individuals but the “normal” males survived as expected indicating that the reduced efficiency was attributed only to the presence of these recombinant types. This fact also explains the high efficiency values for the two first shipments for colony production, as the recombinant males are fully fertile. For the other shipments, even though the level of white pupae males was less than 2.5%,

male-only efficiency was affected for other unknown reasons during the shipment or during the larval rearing in Seibersdorf (**Table 17**). Control data from eggs stored for 48 hours in Guatemala simulating the shipment conditions showed the expected values for male only production (treated eggs) but not for colony production where the values were lower that expected (**Table 17**). Both sets of data did not show the wide fluctuations exhibited in the analysis of the shipped eggs.

5.2.5. Efficiency of egg to flying insects for colony and male only production

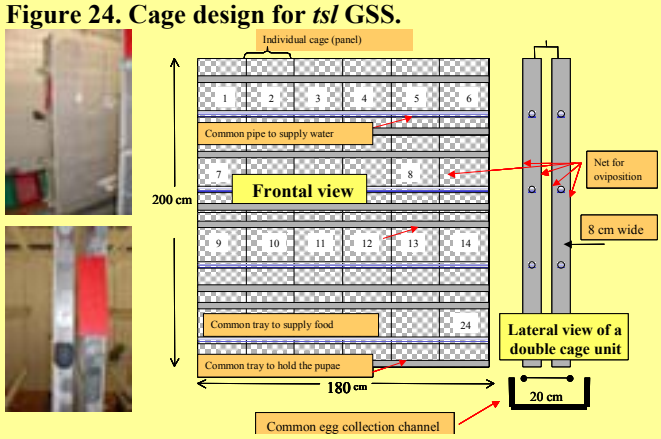
The values for this parameter are given in **Table 18**. The normal expected efficiency of eggs to flying adults is 0.15 for both sexes in the VIENNA 7 GSS. Following shipment, the average efficiency of the egg to female population was reduced to 0.09 for eggs in agar and 0.11 for eggs transported in water. For the male population the transport did not seem to affect this parameter and average values were near or equal to the expected, i.e. 0.13 for eggs transported in agar and 0.15 for eggs transported in water. For male only production efficiency of egg to flying males is also expected to be 0.15. The values for shipments 1, 2 and 6 were acceptable whereas for values for shipments 3, 4 and 5 were unacceptable (**Table 18**). It is not clear if the reasons for these low values are due to the shipment or to some problems during the rearing.

Table 18. Efficiency egg to flying adults in colony and male only production from six egg shipments.

Colony				Male only			
Agar		Water		Agar		Water	
Male	Female	Male	Female	Male	Female	Male	Female
0.14	0.13	0.16	0.19	0.11	0.01	0.11	0.00
0.14	0.23	0.13	0.23	0.10	0.01	0.10	0.02
0,09	0.02	0.24	0.08	0.04	0.00	0.08	0.00
0.09	0.05	0.06	0,01	0.04	0.00	0.02	0.00
0.17	0.07	0.15	0,06	0.05	0.00	0.04	0.00
0.17	0.07	0.19	0.06	0.13	0.00	0.09	0.00

In general these results are encouraging and they will be followed up in 2003. It appeared that there was a slight difference in pupal production depending on the transportation medium, the eggs transported in an agar suspension gave the better value. About half of the shipments produced acceptable values for pupal and adult production and the control data from Guatemala suggested that either the shipment or the rearing conditions in Seibersdorf were the cause. Egg hatch was reduced as consequence of the shipment but the total number of good flying adults was unaffected and larval production was not affected despite the use of a different larval diet in Seibersdorf.

Figure 24. Cage design for *tsl* GSS.



5.3 New Cage Design to Improve Mass Rearing System for *tsl* GSS.

In GSS using the *tsl* mutation, females are homozygous for the *wp* and *tsl* mutations and show some fitness reductions. This is especially so as regards their sensitivity to temperature as adults during the egg

production phase in the colony. Due to the higher temperature and crowding in the adult cages, there is increased mortality during the pre-oviposition period leading to a reduction in the amount of eggs produced per cage during the whole production cycle. This means that the space devoted to the egg producing colony has to be increased and the density of adults in the cages reduced by 25 % compared to bisexual strains. This, in addition to the fact that the strain is partially sterile, results in the area required for colony maintenance being 3 times larger than for bisexual strains.

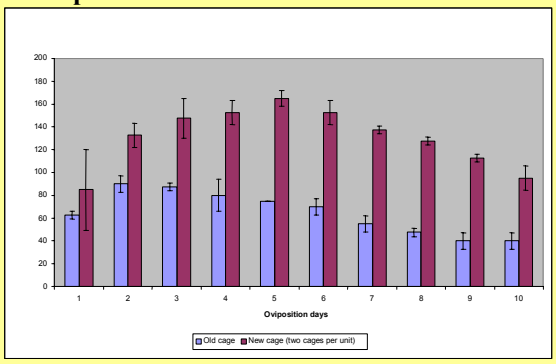
As the total number of insects required for the colony is fixed, the only way that the space required for the colony can be reduced is to use a cage that retains the same number of flies but uses a much smaller area of space. Some modifications to the conventional rectangular cage used in Seibesdorf (**Figure 24**) were introduced. The new cage has the same length and height but the width was reduced from 20 to 8cm, in addition the cage is divided into 24 separate compartments that constitute a single small cage to allow better distribution of adults after emergence. Common transverse tubes supply water and food to the adults. As the cage was reduced in width it is possible to place a second unit in the same space as the original cage (**Figure 24**). Even though the cage has half of the volume of the original cage, the relationship between ovipositor surface area and number of insects remains the same. The presence of the small compartments leads to a better distribution of the insects and removes the need for the need for cold light to aid distribution. According to the production capability of each facility the desired number of units can be calculated. When several units are used in parallel a more efficient egg collection system can be designed. The handling requirements of the new cage are almost the same as for the old cage.

Table 19. Egg production in the new and old cage.

Oviposition Day	Egg Production		Eggs/Female/Day	
	Old Cage	New cage	Old Cage	New Cage
1	63	85	15.3	10.4
2	90	133	22.0	16.2
3	88	148	21.4	18.0
4	80	153	19.6	18.6
5	75	165	18.3	20.2
6	70	153	17.1	18.7
7	55	138	13.4	16.8
8	48	128	11.6	15.6
9	40	113	9.8	13.8
10	40	95	9.8	11.6
Mean	64.7	130.7	15.8	16.0

Results of a comparison of egg production in the new and old cages are presented in **Table 19**. The new cage produces slightly more eggs per female per day as a consequence of a better fly distribution and as expected the total egg production in the new cage was twice that in the old cage. The clear advantage of the new cage can be seen in **Figure 25** where egg production/surface area is compared and the new cage is clearly superior. This improvement in egg production per unit area could have a major impact on facility design and space efficiency. The principal advantages should be a reduction in the space required to hold the colony and a reduction in labour and energy costs.

Figure 25. Number of eggs/ml for equivalent floor space.



5.4 Pupal Shipments to Israel

The SIT project to control medfly in the Middle East has been supplied with sterile males produced and shipped from the El Pino mass rearing facility in Guatemala. However due to an increase in Moscamed programme commitments the facility cannot supply to the project with all the flies it requires. In addition the long transit times between Guatemala and Israel are having a negative effect on the quality of the flies and some shipments do not even arrive. At the moment there are no other sources of sterile insects capable of supplying the project. To solve this problem the Unit was requested to supply flies on a regular basis to Israel.

Between July and August 2001 two samples of sterile males from the VIENNA 7/Mix-2001/D53 were sent to Israel to conduct competitiveness and compatibility tests between the sterile males and wild flies collected in Arava Valley in Israel as first step to establish an agreement to produce sterile male pupae for shipment to the region. The results are shown on **Table 20**. The tests were conduced by staff assigned to the SIT project in Israel and Citrus Marketing Board of Israel. The results indicated an acceptable degree of compatibility between sterile flies produced at the Unit and wild flies from the region. A contract to provide 5 million medfly sterile male pupae per week was agreed and 75 million sterile pupae were sent to Israel between November 2001 and July 2002.

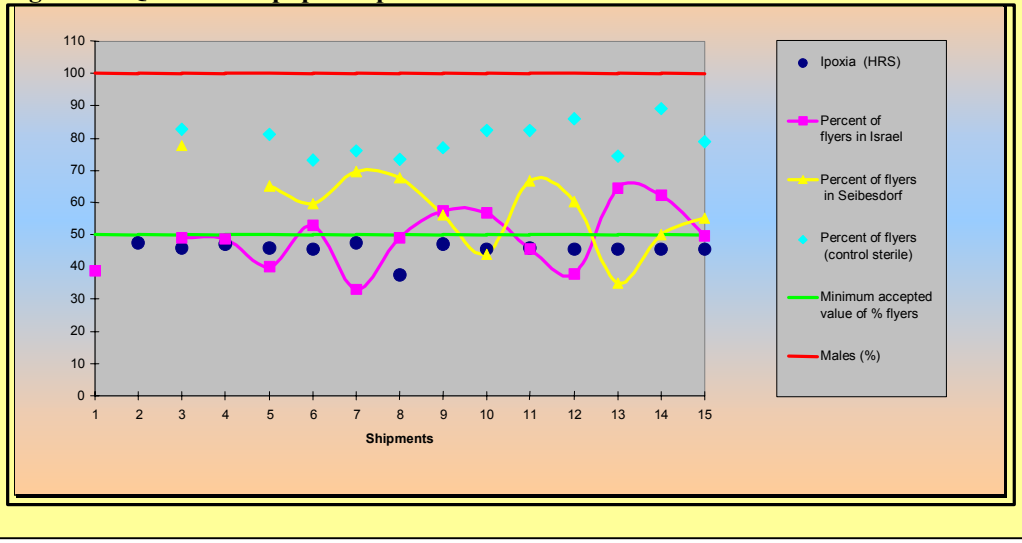
Table 20. Mating tests in Israel

Index		VIENNA 7/Mix2001/D53		
	Control	1st Shipment	2nd Shipment	Mean
Proportion Mating (PM)	0.46±0.15ab (n=6)	0.42±0.16a (n=24)	0.38±0.14a (n=12)	0.45±0.18b (n=12)
Relative Sterility Index (RSI)	0.49±0.13a (n=6)	0.33±0.21b (n=24)	0.34±0.21b (n=12)	0.31±0.21b (n=12)

Means with the same letter in the same row do not differ significantly according to Tuley-Kramer HDS test at P=0.05

The quality of sterile males produced in Seibesdorf was normal for all the QC parameters, however the quality of the flies that were received in Israel showed a

Figure 26. QC data for pupal shipment to Israel



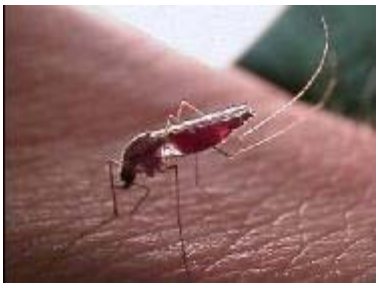
drastic reduction of quality due to the long period of anoxia during shipment. The values for the parameter percent of flying males was affected drastically and was reduced almost 30% in relationship to the control value in Seibesdorf (**Figure 26**). As can be seen from **Figure 26** there were also a wide variation in most of the parameters, even for the pupae that were not shipped to Israel. This is probably due to the fact that the number of pupae that had to be produced was at the limit of the physical and human resources available in the Unit.

The main reason for this long period of anoxia was due to quarantine and security restrictions imposed at the airport in Vienna. In addition when the shipment arrived in Israel there was no possibility to transport pupae directly to the release and emergence center in Arava Valley due to the customs schedule. The full data set for the Israel shipments is shown in **Table 21**. The period of anoxia averaged about 47 hours from closure of the bags in Seibersdorf to their opening at the release site in the Arava. The percentage of flying males recorded in the release center was 49% compared with 59% recorded for pupae kept in anoxia in Seibersdorf for the same amount of time. The reasons for this rather large reduction have not been identified. The colony used to produce the pupae for shipment was reared without a filter rearing system (FRS) and as can be seen by the % of males produced, it was extremely stable. This is probably due to the inclusion of the inversion and suggests that other strains being developed will also benefit from having the inversion. In conclusion, the exercise of having to rear pupae for an operational SIT programme was very useful training for the group but it did stretch resources to the maximum and it can only be undertaken in an emergency.

Table 21. Quality control data for medfly shipments

Shipment	Anoxia (hrs)	% Flyers Israel	% Flyers Seib.	% Flyers Control	% Males
1	◦	38.8	◦	◦	100
2	47.5	◦	◦	◦	100
3	46.0	49.2	77.7	82.7	100
4	47.0	48.8	◦	◦	100
5	46.0	40.0	65.0	81.0	100
6	45.5	53.0	59.7	73.0	100
7	47.5	33.0	69.7	76.0	99.9
8	37.5	49.2	67.7	73.3	99.9
9	47.0	57.4	56.2	77.0	100
10	45.5	56.9	43.9	82.3	100
11	45.7	45.4	66.7	82.3	100
12	45.5	38.0	60.3	86.0	99.9
13	45.5	64.4	34.8	74.3	100
14	45.5	62.2	50.0	89.0	100
15	45.5	49.8	55.06	78.8	100
Mean	45.5	49.0	58.9	79.6	99.9

6. Mosquito SIT



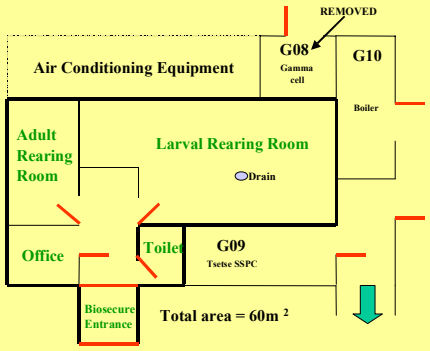
In last year's report, we described plans to revive a technology that has not been attempted for approximately 20 years – Anopheles *SIT. We believe that by investing adequate efforts in research and development of new molecular and quality control technologies and careful site selection, a more effective programme will result. All of the essential elements of* Anopheles *SIT have been demonstrated in various programmes at different times but with limited overall success. We hope to avoid past mistakes and combine the past successes and subsequent advances into a single programme that effectively controls an anopheline over an extended period. The target species for the project is the African malaria vector,* Anopheles arabiensis*. The Entomology Unit's efforts to develop a programme in mosquito SIT are now actively under way.*

A senior scientist, Mark Q. Benedict, is now working at Seibersdorf to begin the laboratory development of genetic sexing strains and sterilization methods. He is also beginning the efforts for release site development and colonization and has assumed technical responsibility for the completion of the mosquito insectary. Mr. Benedict has relevant expertise in anopheline classical and aberration genetics, molecular biology including germline transformation, and quality control in the Malaria Research and Reference Resource Center (MR4) repository setting. A second scientist will be recruited in 2003 to develop mass rearing and release technology.

6.1 Renovations of Insectary

The mosquito insectary plans are complete, construction is underway and should be completed in the spring of 2003. This building will provide a small office space as well as adult and larval rearing rooms, all of which will be physically isolated from other insect rearing areas (**Figure 27**). Structural changes have been completed, necessary measures for physical containment have been designed, and the final mechanical plans have been concluded. All the funds for the renovation have now been secured.

Figure 27. Plan of mosquito insectary



6.2 Release Site Development

In order to create sexing strains, sterilization, production and distribution methods, it is most desirable to begin with material from potential release sites. Furthermore, extensive entomological and epidemiological information about these sites will be necessary in order to plan releases. In order to compress the timeline for release to as short a period as possible, we are actively developing two sites concurrently with efforts to develop laboratory technologies. These sites are currently at different stages of development.

6.2.1. La Reunion

At the suggestion of Dr. Pierre Guillet of the World Health Organization who informed us of the potential of this site for SIT, an *Anopheles* expert was recruited during 2002 to assess the potential of the French department of La Réunion as a site for *A. arabiensis* SIT. This island, located southeast of Madagascar (**Figure 28**), may prove to be an ideal location in which to demonstrate SIT for further use on the African continent. The report of the expert, Dr. Harold Townson (Liverpool School of Tropical Medicine, UK), was very positive about the potential of SIT against the only malaria vector on La Réunion, *A. arabiensis*. While there are only rarely locally transmitted malaria cases on La Réunion, active medical surveillance and vector control programmes are conducted because of the great risk of return to the epidemics of the 18-1900s. Three factors make the resurgence of malaria likely: (1) increasing numbers of travellers entering from malaria-endemic locations (especially Madagascar, the Comoros Islands, and Mayotte), (2) the presence of a vector with a proven record of transmission on the island, and (3) a suitable climate for year-round breeding and transmission. Because there is excellent historical information about the both the vector and malaria transmission on the island, it has been possible to determine that *A. arabiensis* is the only vector, its distribution is restricted to the coastal plain, and its density is relatively low. However, the current anti-anopheline control program has not resulted in its eradication.

Figure 28. Satellite photo of Reunion

Much of this information was compiled and determined by Dr. Romain Girod (chargé de mission Prevéntion et Lutte contre les Endémies Vectorielles, Direction Regionale des Affaires Sanitaires et Sociales de La Rénion-DRASS) as part of his PhD research. The content has largely been confirmed by both Dr. Townson and by Mark Benedict in a return visit during Dec. 2002. Dr. Girod has offered his enthusiastic support to the research program and 30% of his efforts have been allocated to this project. Furthermore, the DRASS has agreed to renovate laboratory space in an existing facility for an insectary sufficient to support the colonization project. This activity will occur during the spring of 2003.

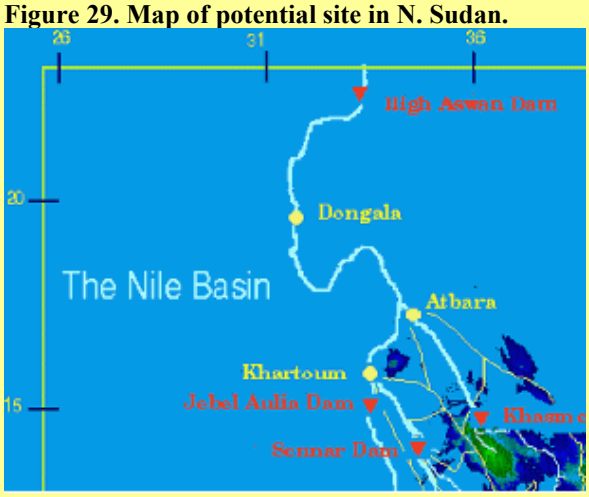
Support for the SIT research program on La Réunion has been forthcoming from both the French Ministry of Health, the Institute for Research and Development (IRD), the local Délégue Régional à la Recherche et à la Technologie, and DRASS. While significant written and verbal support has been obtained, The Agency is actively pursuing financial support from the French delegation to support the research activities specific for eventual release on the island. These will include population genetic surveys, studies of mating and dispersal behavior, and GIS mapping of breeding sites.

All information gathered to date indicates that La Réunion is an ideal site for *A. arabienis* SIT. As a programme exists for medical surveillance and to conduct anopheline surveillance and control, it is expected that eradication of *A. arabiensis* would eliminate the potential for malaria transmission and many current surveillance and control resources could be shifted to preventing reintroduction of malaria vectors.

6.2.2. Sudan

A second site has received considerable attention as an SIT site during 2002. Based on the recommendations of the scientific expert committees' recommendations from their 2001 meeting, the Nile River valley in the northern state of Sudan was visited by Dr. Colin Malcolm (Queen Mary U. of London) for consideration. His report concluded that in all likelihood, *A. arabiensis* is the only malaria vector – indeed, the only *Anopheles* - that occurs in this portion of the Nile River valley, and therefore must be responsible for transmission. Furthermore, he reported favourably on the potential for exploiting the existing artificial and natural barriers to the distribution and migration of this mosquito as improving the success of an SIT programme in the vicinity of Dongola (**Figure 29**). In contrast to the situation on La Réunion, considerable background must be obtained on the distribution and seasonal abundance of *A. arabiensis* in this region. Transportation and insectary infrastructure is also limited. We are very encouraged for the prospects for SIT in this region, however considerable investment will be necessary to create both the information and

Figure 29. Map of potential site in N. Sudan.



insectary infrastructure necessary for colonization and as a base for more detailed studies of *A. arabiensis* before the feasibility of SIT control/eradication can be concluded.

A return trip by Mark Benedict and Dr. Malcolm is planned for February of 2003. At the conclusion of this trip, we hope to have sufficient information and support to finalize specific plans for location of a minimal insectary and the scope of projects to be performed.

7. Appendices

7.1 Publications

CACERES, C. (2002). Mass rearing of temperature sensitive genetic sexing strains in the Mediterannean fruit fly (*Ceratitis capitata*). Genetica 116:107-116

FRANZ, G. (2002). Recombination between homologous autosomes in medfly (*Ceratitis capitata*) males: type-1 recombination and the implications for the stability of genetic sexing strains. Genetica 116:73-84.

HENDRICHS, J., A.S. ROBINSON, J.P. CAYOL and W. ENKERLIN (2002). Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: The importance of mating behavior studies. Florida Ent. 85:1-13

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7.2 Travel

Staff Member	Destination	Period	Purpose of Travel
Robinson, A.S.	Sao Paulo, BRA Tuxtla Gutierrez, MEX	26 Jan.–9 Feb.	Sci. Sec. screwworm RCM, discuss CRP participation in Tuxtla.
	Rome, ITA	7–12 April	Scientific Secretary, FAO Experts Mtg on Transgenic Arthropods.
	Capri, ITA	7–13 July	Consultants Meeting on Transgenic Technology and its Application in the SIT.
	Geneva, SWI	12–16 August	WHO Scientific WG Insect Disease Vectors & Human Health.
	Kelowna, CAN	17–23 August	Int. Training Course.
	Riverside, CA, USA	14–19 Sept.	Invited speaker, 7th Exotic Fruit Fly Symp.
	Beijing, CPR	9–17 Oct.	Invited speaker, 7th Int. Symp. on Biosafety of Genetically. Modified Organisms
	Oxford, UK	7–8 Nov.	Attend meeting in relation to NIH project on *G. pallidipes.*
	Ashford, UK	2–6 Dec.	Participate in consultation on FAO Code of Conduct for Import and Release of Exotic Biological Control Agents.
Caceres C.	Tel Aviv, ISR	16–22 Nov.	Review ISR/5/010.
Parker, A.	Bratislava, SLR	28 June	TPU3 tsetse rearing equipment for CIRDES, BKF
	Tororo, UGA	14–20 July	To assist with tsetse facility construction/upgrade.
	ETH/UGA/KEN	9–20 Sept.	To assist with tsetse facility construction/upgrade and tsetse rearing.
Franz, G.	Rome, ITA	7–12 April	Present paper at FAO Experts Mtg on Transgenic Arthropods.
	Capri, ITA	7–13 July	Scientific Secretary of RCM (CRP D4.10.12).
Mutika, G.	Bobo Dioulasso, BKF	6–18 Oct.	Assist counterparts in mating compatibility tests.
Benedict, M.	Atlanta, Denver, USA	6–15 Nov.	Attend American Soc. of Trop. Med. and Hyg. Nat. Meeting. Pick up research materials in Atlanta.
	Johannesburg and Durban, SAF St. Denis, REU	26 Nov.–9 Dec.	Discuss with counterparts aspects related to colony build-up and field experiments for Malaria SIT.
Wornoayporn, V.	Thailand	6–9 July	Assess current programme of *B. dorsalis* and assist on manual on mass-rearing and quality control THA.5.046.

7.3 Fellows

Name	Project	Fellowship Period
ZUBRIK, Mr. M.	SLR/01024	2001-09-24 - 2002-02-23
DAFFA, Mr. C.T.	URT/01025	2001-10-01 - 2002-05-31
MAMAN, Mr. E.	ISR/01004	2001-10-01 - 2002-11-30
LUKIKO, Mr. C.P.	URT/01053	2002-01-15 - 2002-07-14
KIENDREBEOGO, Mr. M.	BKF/01024	2002-01-17 - 2002-07-16
ABILA, Mr. P.P.O.	UGA/01017	2002-01-21 - 2002-09-20
M'SAAD, Ms. M.	TUN/01005	2002-01-31 - 2002-04-30
SHOMAN, Mr. A.A.	EGY/01043	2002-02-01 - 2002-07-31
MATEMBO, Mr. S.I.R.	URT/01013	2002-05-01 - 2002-10-31
ROSENBERG, Mr. MS.	SAF/02008	2002-07-15 - 2002-10-14
ARNOLDS, Mr. M.S.	SAF/02007	2002-07-15 - 2002-10-14
MASHENGA, Mr. G.R.	URT/02026	2002-08-05 - 2003-08-29
BAYALA, Mr. E.	BKF/01032	2002-08-19 - 2003-02-18
DEMBELE, Mr. K.I.	BKF/01023	2002-10-28 - 2003-04-26
PODA, Mr. A.B.	BKF/01030	2002-10-28 - 2003-04-26

Scientific Visitors

KIRAGU, Mr. J.M.	KEN/01008
OKEDI, Ms. L.M.A.	UGA/02001
WOLDEGHIORGIS, Mr. K.W.	ETH/01031

7.4 Insect Shipments

***Glossina m. morsitans*, (Colony size 3,600)**		
Dr. Maudlin	Univ. Edinburgh, UK	1,250
Dr. Gariou-Papalexiou	Univ. Patras, Greece	6,250
Dr. Turner		2,500
Dr. Tait	Univ. Glasgow, UK	3,750
Dr. Gibson	Univ. Bristol, UK	6,350
Dr. Bett		250
Dr. Brun	Swiss Tropical Inst.	6,800
Dr. Aksoy	Yale Univ., USA	6,200
		33,350
***Glossina pallidipes*, (Colony size 41,000)**		
Dr. Guerin	Univ. Neuchatel, Switzerland	18,800
Dr. Gariou-Papalexiou	Univ. Patras, Greece	7,700
Dr. Aksoy	Yale Univ., USA	8,700
Dr. Makumi	KETRI, Kenya	83,500
Dr. McCall	Univ. Liverpool, UK	100
Dr. Msangi	TTRI, Tanzania	134,000
		252,800
***Glossina palpalis*, (Colony size 5,300)**		
Dr. Maudlin	Univ. Edinburgh, UK	7,950
Dr. Molyneux	Univ. Salford, UK	7,950
Dr. Aksoy	Yale Univ., USA	10,250
Dr. Den Otter	Univ. Groningen, The Netherlands	1,000
Dr. Omoogun	NITR, Kaduna, Nigeria	3,800
		30,950
***Glossina austeni*, (Colony discontinued October 2002)**		
Dr. Maudlin	Univ. Edinburgh, UK	18,900
Dr. Aksoy	Yale Univ., USA	7,250
Dr. Kappmeier	OVI, South Africa	9,105
Dr. Gariou-Papalexiou	Univ. Patras, Greece	7,350
		42,605
***Glossina brevipalpis*, (Colony size 1,750)**		
Dr. Kappmeier	OVI, South Africa	10,300
Dr. Aksoy	Yale Univ., USA	6,750
Dr. Guerin	Univ. Neuchatel, Switzerland	7,100
		24,150
***Glossina fuscipes*, (Colony size 20,500)**		
Dr. Guerin	Univ. Neuchatel, Switzerland	8,650
Dr. Okedi	LIRI, Uganda	62,000
Dr. Averswald	Univ. Cape Town, South Africa	12,450
		85,060
Total		**468,915**

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