Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Entomology Unit

Activities Report 2006

FAO/IAEA Agriculture & Biotechnology Laboratory
Agency's Laboratories, Seibersdorf
International Atomic Energy Agency
Austria

Executive Summary

For many years isotopic irradiators have been used to sterilize flies for release in SIT programmes. The reliance on these types of machine is now becoming more and more doubtful as a major supplier has announced that they will no longer produce this equipment. The cessation in the production is related to heightened awareness concerning potential terrorism. This situation has caused problems for future SIT programmes and also for ongoing programmes when re-charging of equipment is required. Fortunately new developments in X-ray technology offer an alternative. Prototype machines are now available and late in 2006 such a prototype has been purchased for validation in the Unit. This will bring significant additional work in 2007 requiring both radiation and biological dosimetry protocols to be developed.

Mating compatibility between released sterile males and the wild females is the crucial interaction that determines success or failure of SIT programmes. For many years experimental work in the Unit has focused on this for several different insect pests e.g. medfly, codling moth and the South American fruit fly. The Unit has a comparative advantage in carrying out these studies based on the accumulated experience of the staff, the level of biosafety in the rearing facility, the geographical location of the laboratories and the year round availability of field cages. In 2006 these studies were continued with the olive fly and with other fruit flies which had been treated with juvenile hormone analogues.

Significant progress has been made in the identification of the virus that infects some tsetse species and which causes male and female reproductive abnormalities. A large amount of sequencing has been carried out and the complete annotated sequence will be published in 2007. The virus has been shown to be quite unique and in contrast to earlier assumptions it has been shown to have a circular genome. Quantitative PCR has also been used to try to determine virus levels in flies with salivary gland hyperplasia and those without. The sequence information will be used to identify key proteins against which antibodies can be raised. The effect of these antibodies on the tsetse infection process will then be assessed. Experiments have also been started on the effect of feeding anti-viral agents on virus infection.

Among the most important fruit fly species in the New World is Anastrepha fraterculus, the South American fruit fly which is distributed from Mexico to Argentina. Field cage work carried out in 2004 indicated that between populations from Peru and Argentina there existed a significant degree of mating incompatibility. Work carried out in the Unit in 2006 by Diego Segura confirmed these earlier studies and in addition chromosome analysis of the hybrids by Antigone Zacharopoulou has

demonstrated remarkable levels of chromosomal variation. Other observations on reproduction, pheromone analysis and Wolbachia typing will now be carried out to confirm the species status of these two populations.

The tsetse production unit TPU3.2 will be the cornerstone of tsetse mass-rearing. It provides a semi-automated system for holding and feeding flies and collecting pupae. Much of the work carried out on this system in 2006 was concerned with refinement and modification of the prototype. Several changes were made involving alignment of the feeding trays with the cages holding the flies and with ensuring an even temperature of the blood during feeding. During the year there were again unexplained difficulties in the Glossina pallidipes colony resulting in a serious drop in colony numbers. However as in the past, the colony has recovered. These unpredictable and unmanageable fluctuations in colony productivity would pose serious problems for an operational programme with this species. It is hoped that understanding more about the virus in the colony will help to manage this problem.

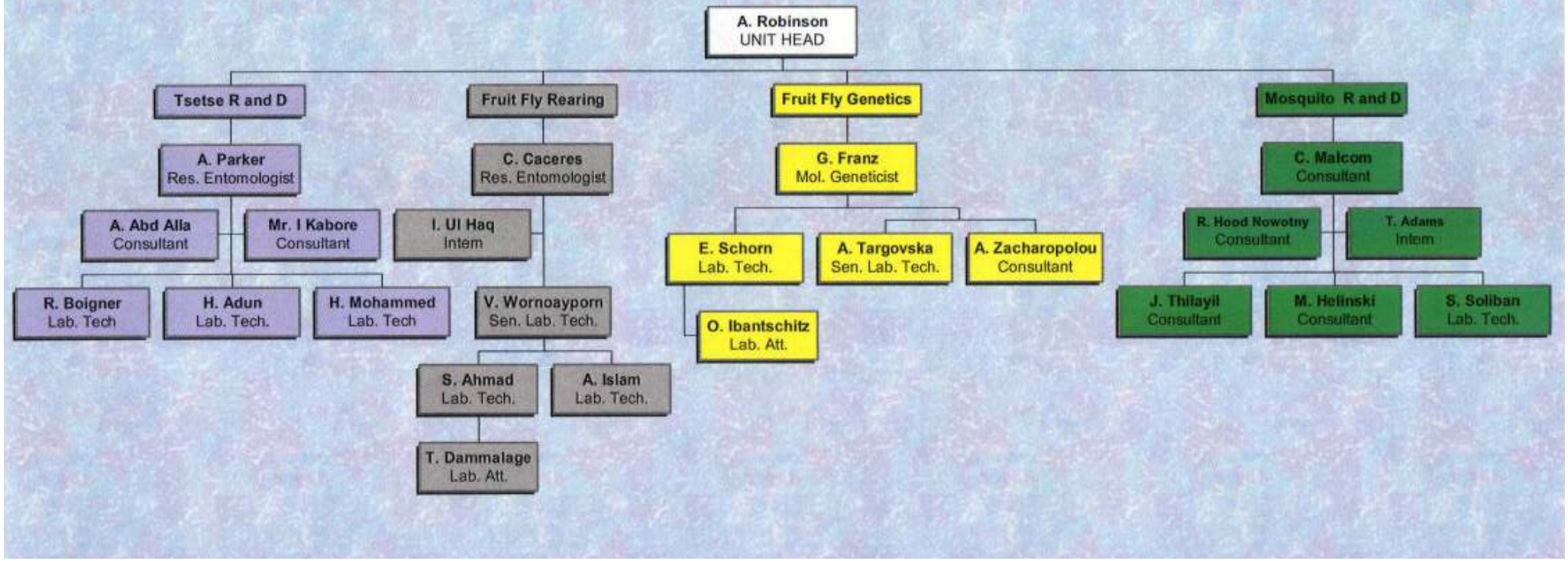
In medfly SIT it is very difficult to assess in the field if wild females have mated with sterile males. In other species, it is possible to assess this by monitoring the level of sterility in the wild females. A new transgenic strain has been isolated in medfly in which the testes of males are labeled with a fluorescent red protein. When females are mated with these males, it will be possible to observe the red fluorescence in the spermathecae of the females. The use of such strains in operational SIT programmes will require regulation and in 2006, significant progress was made in this with the development of a draft standard by the North American Plant Protection Organization. This standard would regulate the use of transgenic insect strains in plant protection programmes in Canada, USA and Mexico. The transgenic work could not have been carried out without very close collaboration with Dr. A. Handler, ARS, USDA, Florida.

Rearing large numbers of mosquitoes on a scale that will be required for a meaningful field feasibility study has never been done, with the exception of a programme in El Salvador in the 1970s where mass rearing only involved upscaling of the normal laboratory rearing. In 2005 a prototype adult rearing cage was developed and tested. Based on this, a new design evolved and a second cage was built. The cage enables pupae to be introduced, adults to be fed with sugar water, females to be provided with a blood meal and eggs to be collected. All these activities can be done without opening the cage. Crucially the cage provides ample area for mosquitoes to rest. This cage should enable a cycling adult population of about 10,000 females to be established from which eggs can be harvested on a regular basis.

In 2006, Mr Colin Malcolm joined the Unit to lead the mosquito SIT project and Ms. Antigone Zacharopoulou started a sabbatical period to support work on fruit fly genetic sexing and biotechnology. Mr. A. Toloza and Mr. Jagoditsch left the Unit to join the Soils Unit and Mr. T. Dammalage joined the medfly group. The mosquito group lost two key staff in 2006, Mr. B Knols took up a position at Wageningen University and Mr H. Bossin took up a position in a mosquito control programme in Tahiti.

ENTOMOLOGY UNIT ORGANIZATIONAL CHART

(as of December 31, 2006)



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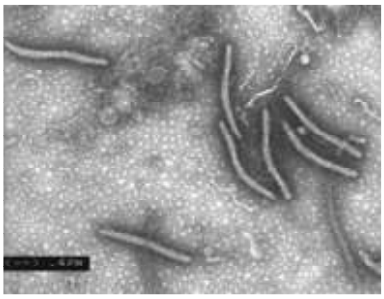
1. Tsetse Research and Development

In support of tsetse SIT activities in Member States, the Unit maintains several colonies of economically important species, in some cases, these colonies are not available elsewhere. They are therefore an important resource. There were two issues of concern in 2006 for colony maintenance. Firstly, the G. pallidipes *colony went through a serious decline, from which it is just recovering. The cause of this regular but infrequent fluctuation in the health of the colony cannot be traced to any environmental factor and is a serious concern to SIT programmes targeting this species. It is hoped that work on the virus which affects this colony will help solve this problem. Secondly, there was a major malfunction in the environmental controls in one of the tsetse holding rooms over a weekend and large numbers of flies were lost from many colonies. Fortunately all the species could be saved. The situation arose because the environmental control systems were not connected to the on-call system of the laboratory. This situation is now being remedied.*

Much progress has been made in the identification of the virus that causes salivary gland hyperplasia (SGH) in various tsetse species. Activities have focused on the sequencing of the genome, assessing the effects of anti-viral agents on tsetse infection and the use of quantitative PCR to assess the level of virus load in individual flies. The sequence is now almost complete and it has been confirmed that the virus has a circular DNA molecule. Several genes have been identified as candidates for the development of antibodies. The virus is quite unique based on sequence analysis. Using quantitative PCR it has been possible to demonstrate a difference in the viral load between individuals with SGH and those without. This difference was also evident in the haemolymph. Initial results feeding anti-viral agents to tsetse were encouraging but subsequent experiments gave equivocal results. This approach to manage the virus is very important and the work will be extended in 2007.



Microbial decontamination of blood used for feeding tsetse has conventionally been done using radiation. However isotopic radiation sources have some disadvantages and alternatives are required. During 2006 a full evaluation of a UV irradiator, obtained on loan from a company in Scotland, was carried out. Very encouraging results were obtained from this evaluation with very acceptable reductions in the bacterial load of treated blood being obtained. The use of UV as opposed to isotopic irradiation has many logistical advantages. To proceed further to an operational system, a full scale prototype will need to be designed and built.

1.1 UV Treatment of Blood

The reliance on gamma irradiation to control bacteria contamination severely restricts opportunities for blood collection and processing for tsetse diet in Africa, as large gamma irradiators are uncommon. An alternative method to minimize the bacterial contamination is to use UV radiation for the sterilization of tsetse blood diet. It is known that direct exposure to 254 nm UVC radiation will inactivate the DNA and RNA of micro-organisms such as bacteria, viruses, mould spores, yeast and protozoa, rendering them sterile and finally killing them. The average bacterium will, for example, be killed in ten seconds at a distance of 15.25 cm from the UV lamps. Based on an improvement of this system, a commercial UV blood product virus inactivation machine, constructed by IATROS, an engineering company in Dundee, Scotland, is being effectively used in the medical field for blood transfusion. Therefore, the possible use of this new technology as an alternative blood diet processing technique is being investigated. Following a successful proof of principle using an artificial feedstock of similar viscosity and absorbance to whole blood, tests were undertaken using defibrinated horse blood spiked with a representative bacterium (*E. coli 9481*) and virus (*PhiX 174*). The log reduction curves for both bacteria and virus were similar, indicating about a 5-log reduction in 15 minutes of recirculation at 1500ml/min. Further UV treatments of whole blood, using the IATROS Production Scale Prototype, have achieved three log reductions in virus/bacteria load in seven minutes at a flow rate of 1500 ml/min in recirculation mode for one litre. A measurable increase in met-haemoglobin was observed after 45 minutes treatment, the significance of which in terms of tsetse nutrition needed to be determined.

Investigations were pursued to test the effect of the UV irradiation on the nutritional quality of the blood, and to test the effect of the irradiation on the bacteria normally encountered during blood collection. The main objective was to confirm the efficiency of the IATROS Full Scale Laboratory Machine for the sterilization of tsetse blood diet by performing feeding tests to assess the effects of UV irradiation on the nutritional quality of the treated blood and to evaluate the level of reduction in bacterial load to confirm the efficacy of UV treatment on the bacteria most frequently encountered in blood collected for tsetse colony maintenance.

1.1.1 The UV irradiator and process

The IATROS production scale prototype is shown in **Figure 1**. The unique system ensures that all parts of the blood are exposed to the UV light. The efficiency of such a system is so high that optically dense fluids can be virally and bacterially inactivated to regulatory levels of log kill. The circulation of fluids is achieved using a peristaltic pump. At the Entomology Unit the system was operated with an IKA PA-SF pump.

Figure 1. UV irradiator for tsetse blood

For efficient use of the machine, only defibrinated blood is recommended. Another major factor is the proper cleanliness of the system. Plastic gloves should be used to manipulate the external parts such as the irradiation pipe and the UV lamps. If necessary, utilization of swabs with isopropyl alcohol is recommended for external removal of finger prints, grease and other dirt. Sanitization of the circuit after treatment of blood or infected feed-stocks is obtained using 0.25 M NaOH solution followed by several flushings with tap water and then flushing with demineralized water.

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1.1.2 Bacteria cultures

Before running the experiments, the defibrinated cattle blood was spiked with bacteria isolated from blood collected in a slaughterhouse located south of Vienna, Austria for the maintenance of tsetse colonies. Cultures of three different commonly found bacteria were used: *Acinetobacter sp* (BI06015), *Aerococcus viridans* (BI06016) and *Staphylococcus aureus* (BI06017). For each experiment, a fresh culture of the bacteria was made the day before in nutrient broth for microbiology (MERCK). Samples are titrated from 10^{-1} to 10^{-6} from which 200µl are poured onto sheep blood agar plates and incubated for 24 hours. The blood to be UV treated is then spiked at 10^{5} cfu ml^{-1} according to the bacteria load read from the plates.

1.1.3 UV Treatment of contaminated blood

For each experiment, the defibrinated cattle blood spiked with bacteria (10^{5} cfu ml^{-1}) was UV irradiated using both the single-pass and recirculation methods. Samples of the treated blood were collected to determine the related reduction in bacteria load.

1.1.3.1 Decontamination by single pass method

The machine was run at 6 different flow rates, 100, 200, 300, 400, 500 and 600 ml/min corresponding to 68.4; 34.2; 22.8; 17.1; 13.68 and 11.4 seconds residence time to determine the flow rate at which the treatment is most efficient. As recommended for infected feed stocks the irradiation was run starting from the lowest to the highest flow rate. The system was sanitised at the beginning and between the different flow rates by flushing with 0.25 M NaOH for 10 min. and then rinsing three times with distilled water. Samples of each blood treatment were taken after discarding twice the dead volume. The dead volume, about 262ml, is the required quantity of liquid to fill one circuit of the system (total volume of the irradiation pipes and the feed tubing).

1.1.3.2 Decontamination by recirculation method

Following a proper sanitisation of the irradiation circuit as above, 1 litre of the same defibrinated cattle blood spiked with bacteria was also treated using the recirculation method. The system was run at a flow rate of 1000 ml min^{-1} (6.84 seconds residence time per pass) and samples were collected after 15, 30, 45, 60, 90 and 120 minutes recirculation time and titrated 10^{-1} to 10^{-5}. All UV treated blood samples collected during the single pass method and each titrated sample from the recirculation method and their controls were poured in duplicate onto sheep blood agar plates, incubated at 35°C for 48 hours. The number of colony forming units (cfu) per ml was read for each plate.

1.1.4 Quality factors (QF) of UV treated blood

Feeding tests were performed for 30 days to evaluate the effects of the UV treatment on tsetse blood diet quality. Cattle blood already controlled as bacterially acceptable for colony feeding (less than 1 cfu ml^{-1} on normal agar plates) was UV irradiated under sanitized conditions, using the two techniques, single pass and recirculation methods.

For single pass method, six different flow rates of 100, 200, 300, 400, 500 and 600 ml min^{-1} were applied. These flow rates correspond to 68.4; 34.2; 22.8; 17.1; 13.68 and 11.4 seconds residence time. For each run three litres of blood was used from which about 1 litre of blood was taken before irradiation as a control. The corresponding irradiated sample was collected after discarding at least two times the dead volume. In recirculation mode, six blood batches, treated for 15, 30, 45, 60, 90 and 120 minutes at 1000 ml/min were used. The different treated blood batches and their controls were portioned into 15 ml containers under a laminar flow hood, labeled accordingly and immediately stored at -20°C.

Tests were undertaken with *Glossina m. centralis* females using day0 mating. The males were removed from the cages after one week. 360 females and 300 males *G. m. centralis*, emerged from the batch of pupae produced between 13 and 15 May 2006 were put into normal female holding cages for day 0 mating at a ratio of 0.83/1 (25 males/30 females). The fly groups were fed five days a week for ten minutes on the corresponding blood samples and controls. Dead flies were recorded and removed from the cages. The pupae produced were recorded and their individual weights determined.

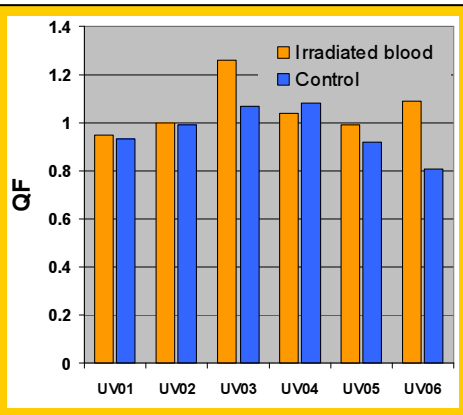


Figure 2. Effect of flow rates on QF of *G. m. centralis*

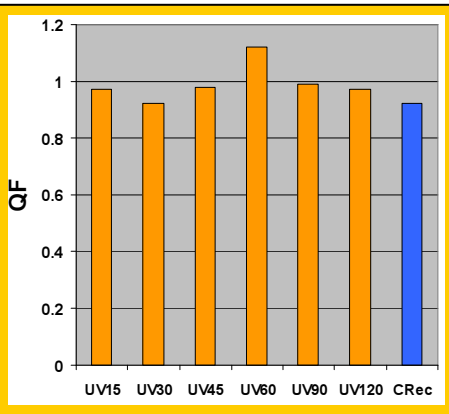


Figure 3. Effect of recirculation time on QF of *G. m. centralis*

1.1.5 Effects of UV treatment on blood diet quality

Effects of UV treatment on blood diet quality.

The results of feeding tests with *G. m. centralis* using blood treated by the single pass method at different flow rates indicated that QF is acceptable for all flow rates down to 100 ml min^{-1} and for the recirculation method for all times up to 120 min (**Figures 2 and 3**). These results were similar to the average QF values (1.09±0.12) recorded with *G. m. centralis* during routine screening of the blood quality at Seibersdorf in 2006 (**Table 1**). During dissection of the surviving flies, some cases of blockage in the oviduct were found from both control and experimental groups. Blockage (**Figure 4**) is a reproductive abnormality where eggs fail to develop in the uterus, and consequently

Table 1: QF Values from routine testing of bovine blood, March – November 2006 at Seibersdorf

		QF values	
Test date	**Blood Batch**	***G. pallidipes***	***G. m. centralis***
27/3	**BC06057**	1.32	0.92
05/5	**BC06076**	1.70	1.25
16/6	**BC06077**	1.23	1.12
14/8	**BC06162**	1.62	1.16
12/9	**BC06163**	1.41	1.11
05/10	**BC06218**	1.72	0.99
		1.50±0.21	1.09±0.12

Figure 4. Blockage of eggs in the uterus

remain in the oviduct, resulting in an accumulation of undeveloped eggs. The presence of individuals showing blockage reduces the QF value for the batch of blood.

1.1.6 Effects of UV irradiation on bacterial load

The results of the bacterial tests showed that with the recirculation method the level of bacterial reduction was in good agreement with the figures provided by IATROS from the preliminary tests. For all three of the bacterial species tested, the reduction was about three log at 15 minutes, increasing to almost four log at 60 minutes **(Figure 5)**.

In contrast, treatment using the single pass method at lower flow rates ranging from 100 to 600 ml min^{-1} achieved less than 1 log reduction in the same blood batches and bacteria load (10^5 cfu ml^{-1}). Calibration tests of the UV machine have confirmed an optimal efficiency of the system when run at a flow rate of 1500 ml/min but when the system is run at very low flow rates with viscous solutions containing significant amounts of viruses or bacteria, it can be expected that a significant proportion will be shielded from the effects of the UV radiation. Future work on single pass treatment should concentrate on achieving the necessary retention times at flow rates comparable to those employed in the recirculation treatment (1500 ml min^{-1} ≡ 0.22 m sec^{-1}).

Concerning the nutritional quality of the tsetse blood diet, the UV treatment has not shown any measurable effect on cattle blood quality. The experimental groups fed on blood treated either by single pass or recirculation methods have recorded QF values greater than those of the corresponding control groups. In the single pass method most of the QF values were just above 1.0, the acceptable value for blood to be used for fly feeding, with a maximum of 1.26 at a flow rate of 300 ml min^{-1}. In the recirculation method at a flow rate of 1000 ml min^{-1}, the QF values increased from 0.92 to a maximum of 1.12 after 60 minutes and then progressively decreased to 0.92 after 120 minutes. The control value was also 0.92.

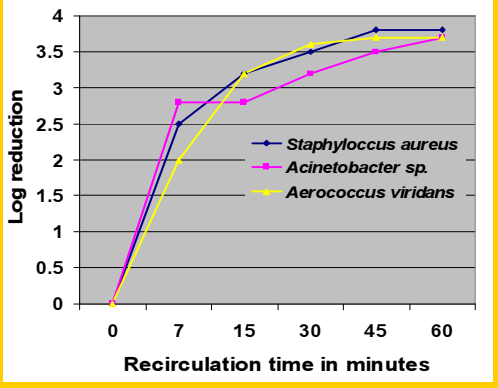


Figure 5. Load reduction for three species of bacteria in UV treated blood by the recirculation method.

The slightly low values of the QF (0.92–1.26) observed for some of the experimental groups and controls is not a side effect of the UV treatment, but is related to the tsetse species itself, *G. m. centralis*. This is confirmed by the results of the routine feeding tests performed for the Seibersdorf colonies from April to October 2006. The same blood batches at the same periods have shown QF values ranges between 0.92 and 1.25 with an average of 1.09 (±0.12) for *G. m. centralis* and values between 1.23 and 1.72 with an average of 1.5 (±0.21) for *G. pallidipes*. The defibrinated cattle blood tested during this period seemed to be more suitable for *G. pallidipes* than *G. m. centralis,* the species used to test the UV blood quality.

The QF values observed are on the other hand also related to the quality of the blood batch itself. Previous feeding tests performed in March 2006 with the same blood without UV treatment recorded low QF values of about 0.92 for both *G. m. centralis* and *G. pallidipes*. None of the 12 batches of UV treated blood using either recirculation or single pass method has shown a decrease in the QF values compared to the corresponding control groups. Thus, the nutritional quality of the blood was not impaired by the UV irradiation. These achievements are the result of the high efficiency of the technology developed by IATROS. These encouraging results also show the negligible significance

in terms of tsetse nutrition of the reported increase in met-haemoglobin previously observed after 45 minutes treatment of defibrinated horse blood.

1.2 Colony Status

The tsetse rearing group remains with one fewer established posts than previously, with the senior technician post unfilled. The consultant working on the salivary gland hypertrophy virus continues to work in the unit, and another consultant joined us, initially for one year, to work on the TPU3 and blood processing.

The Seibersdorf colonies have experience mixed success throughout the year (**Figure 6**). The *Glossina pallidipes* colony declined throughout the first part of the year from a high of 19,882 to a low of 7,275 at week 35, but then recovered somewhat to finish at 11,928. The decline, particularly the sharp drop early in the year, was thought to be due to blood quality problems, but no specific problem could be identified and test flies kept on the blood thrived. The recovery of the colony seems to be related to the availability of a new batch of blood from week 30.

The *G. brevipalpis* and *G. palpalis* colonies remained stable, but the *G. m. centralis* colony declined in the second half of the year because of the quantity of material taken for testing the TPU3. These three colonies are maintained together in a single holding room and suffered a major mortality in weeks 47/8 due to the failure of a humidistat, which resulted in the humidifier running continuously from Saturday afternoon until the arrival of the staff on Monday morning. Between 80 and 90% of the adults were lost, but fortunately the pupae, also in the same room, survived. New emergence from these pupae mean that the colonies all recovered to at least 50% of their previous level by the end of the year, but the full productivity of the colonies will not be restored until well into 2007 when the ageage structure of the colonies is re-established. As a result all pupal shipments had to be suspended.

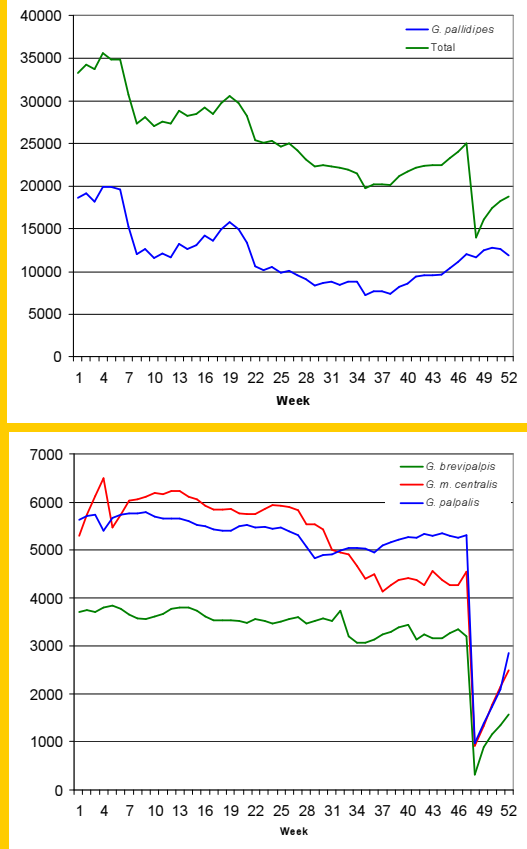


Figure 6. Tsetse colony status through 2006

The shipment of pupae has continued the decline seen in 2005. The total number shipped was 101,734, down 44% from 2005. Part of this drop can be accounted for by the cessation of pupal shipments in December due to the high mortality in the colony, but it is mostly due to moving the *Glossina morsitans*, *G. f. fuscipes* and part of the *G. pallidipes* colonies to the Institute of Zoology, Bratislava and the continued low demand from TC projects.

1.3 Pupal Sorting by Near-Infrared Spectroscopy

The emergence of tsetse fly adults from the pupa shows a consistent pattern, with females emerging two to three days before males. When pupae are incubated in a controlled chamber at 24°C and 75% RH a characteristic pattern appears for each species (**Figure 7**). The degree of overlap between the

females and males varies with species and other factors. This sex-specific difference has been used as the basis of a sex separation system for a number of years, the Self Stocking of Production Cage (SSPC) system. SSPC has proved useful, but has two main drawbacks. The first is that emergence is critically affected by incubation temperature and by other, as yet unidentified, factors that together cause the specific time of emergence and the degree of overlap to vary, leading to poor separation and inappropriate sex ratios in the production cages. The second is that the separation only occurs at the time of emergence, leaving no time for special, separate handling of the males, as for example for irradiation or shipping to a release site.

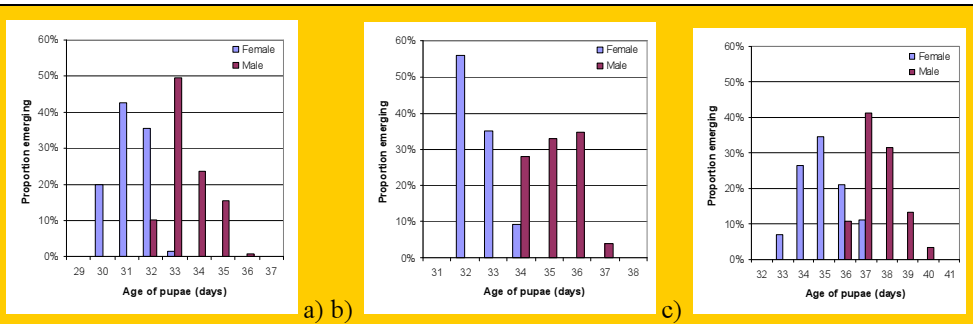


Figure 7. Natural emergence pattern of three species of tsetse, a) *Glossina pallidipes*, b) *G. fuscipes* and c) *G. brevipalpis* after incubation at 24 °C.

The discovery that near infrared spectroscopy can be used to distinguish the sex of tsetse pupae some days before emergence opens the possibility of automatic sexing and special handling of male pupae (**Annual Report 2004**). Work has continued on this system over the past two years.

During the initial work on the single kernel near infrared spectrometer system sorting efficiencies of 90-95% were achieved for pupae at five to four days before first female emergence, where each pupa was scored and emerged individually. Based on this initial calibration a number of automated sorting runs were made, but sorting efficiencies were lower in these runs.

When a pupa is scanned a "sort value" is derived from the spectrum. The calibration is arranged such that values near 1 represent females whilst values near 2 represent males. The pooled results from several hundred pupae show a clear bimodal distribution of the sort values (**Figure 8a)**. This distribution can be approximated by two Gaussian distributions of equal area but different means and spreads (red = female; green = male; blue = sum). The mean of the male curve lies close to 2, but the female curve mean is 1.35, and the spread is much wider than for males (s.d. = 0.27 for females, 0.17 for males).

Comparison of the readings from two individual runs shows that the distribution of sort values varies quite widely between runs (**Figure 8b**). The source of this variability between runs could come from a number of factors, such as variation in the

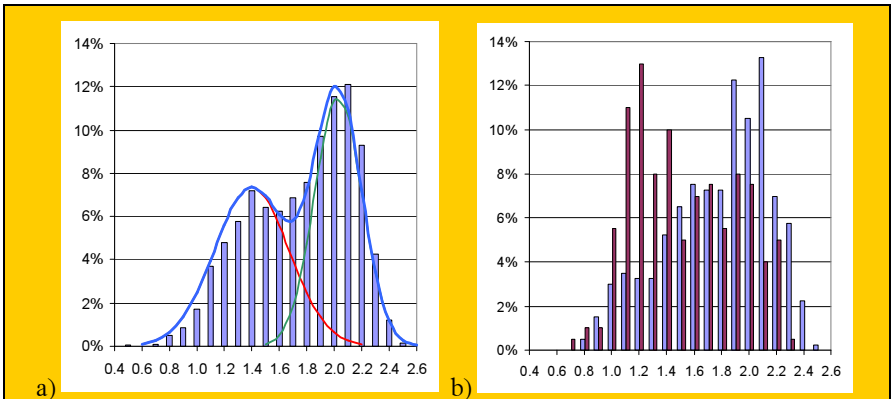


Figure 8 a) Overall distribution of sort values from the SKNIR for *G. pallidipes* pupae. b) Sort values from two individual runs.

machine, exact age of the pupae, errors in selecting the age of pupae or dirt on the surface of the pupae interfering with the signal. To try to control these factors the time of scanning was controlled so that scanning occurred at the same time each day, the SKNIR machine was switched on and allowed to warm up for at least 30 minutes before use, and extra care was taken with the labeling and selection of pupae for scanning to prevent errors. Additionally we took two samples of pupae, and compared the results of scanning the pupae before and after washing. The distribution of dirt on the surface was not expected to be uniform, so ten separate readings were taken from each pupa, and the mean and range calculated. Washing reduces the variability slightly and increases the absolute sort values, but does not do much to improve separation. Most notably though the variability in the readings from one pupa, even after washing off surface dirt, is still quite large.

Following this small trial we took repeated readings from thirteen unwashed batches of 15 pupae to measure the variability of successive readings from one pupa. Between each reading the pupa was repositioned in the machine so that the readings were taken from random locations around the middle of the pupa. **Figure 9** shows the mean and range of ten readings from each pupa (some groups contain less than 15 points because some pupae failed to emerge and were not sexed). The notional threshold value is shown by a blue dashed line. At this threshold 90% of female mean sort values lie below the line and 90% of male mean sort values lie above. Within each group the means are ordered in sequence of reading. It can be clearly seen that in group two there is a marked upward trend in the readings which may be due to instrument drift. The dividing point between males and females also differs between groups; e.g. in groups 1 and 12 a higher threshold would be required to get optimal separation, and in groups 6 and 11 a lower threshold. When the range of the readings from each pupa is also considered the magnitude of the overlap between the sexes due to the variation is apparent. The optimum threshold value becomes 1.4 based on the largest reading for each female and the smallest reading for each male, but in only 65% of females and 64% of males are all of the readings on the correct side of the threshold, leaving almost one third of the pupa that will sometimes be misclassified.

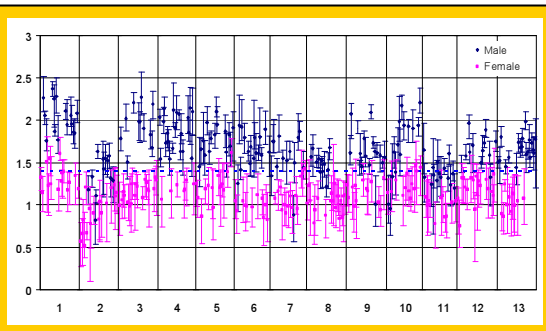


Figure 9. Mean and range of multiple scans from individual pupae.

Finally, tsetse pupae vary in size, and there is a (weak) correlation between size and sex. We therefore tested to see if the SKNIR sort values could be improved by separating the pupae by size. No trend was visible in the means, and the differences are not significant ($P > 0.4$ for males and females.)

So far we have not been able to identify the source of the variability from individual pupae. Size is not significant, and although surface dirt has some affect, it does not explain most of the variation. Another possibility is that the variation represents information about the developing adult fly; the infrared signal that forms the spectrum comes not only from surface reflection but also from layers below the surface and it is possible that the variation comes from the developing adult structures within the pupa. To test this we plan to position pupae within the spectrometer on a jig that will allow us to accurately rotate the pupa to take readings at known positions around the pupa.

1.4 Salivary Gland Hypertrophy Virus (SGHV)

As reported earlier some tsetse species carry a virus (**Figure 10**) that, in a certain proportion of individuals leads to salivary gland hypertrophy (SGH) and these individuals also show reproductive abnormalities. This virus is present in natural populations at a low level (0.5-5%), based on salivary gland dissection and in a colony of *G. pallidipes* maintained in the Unit, the frequency of SGH ranges from 4-10%. However, PCR analysis has confirmed that virus prevalence is almost 100%. The virus was also detected in samples of *G. pallidipes* from the colony maintained at the Kaliti facility in Ethiopia. Due to the negative impact of the virus on colony productivity under certain stressful conditions it is important to understand more about the virus with the goal to develop a management strategy for the virus. The most effective way to begin this study is to understand more about the virus in terms of its taxonomy and this can be done by obtaining nucleotide sequence information. Beside the sequence analysis the work aimed to gather information about the biology of the virus and its relation to the hypertrophy symptoms and sterility. Work on the effect of some antiviral drugs to suppress viral replication was also initiated.

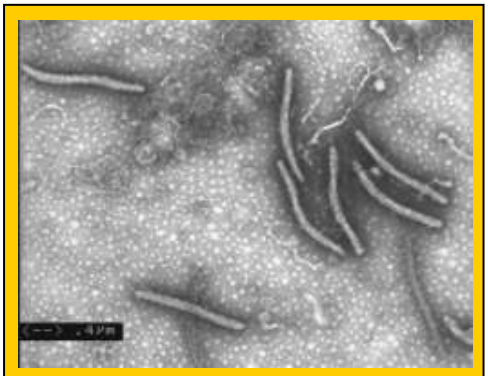


Figure 10. Purified SGHV under EM

1.4.1 Salivary gland hypertrophy virus (SGHV) genome sequence

As reported in the 2005 report, the sequencing of the SGHV DNA was started by generating an EcoRI DNA library; from 800 recombinant clones, 408 were sequenced. At a later date, 3µg of viral DNA was sent to 454 Life Science Company for sequencing the total viral genome. The sequence results from 454 Company produced 402 contigs. The sequence data obtained from 454 and from the sequenced clones and the sequence results from 108 sequences from PCR on viral DNA were assembled and analysed using different software, such as PHRAP, NTI Vector 10.0, DNASTAR, Lasergene, Seqman and GeneQuest. The assembly of these sequences produced one large contig of 189995 nt. The work is on going to determine whether the DNA as linear or circular. The length of the DNA was partially confirmed using long template PCR (**Figure 11**).

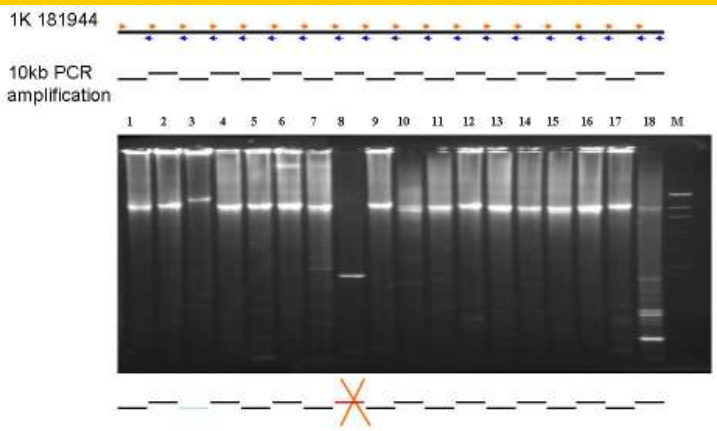


Figure 11. Checking the genome sequence with selected primers from the obtained sequence data with long template PCR kit.

The analysis of the resultant sequence by ORF finder software resulted in 263 ORFs of which 160 were annotated. The homology between the identified ORFs and the protein sequences in data banks was analysed using the Blastp programme and the GenBank virus database. The results indicate that there are some ORFs with homologies to virus genes such as: a) 25 putative homologues to baculo-granulovirus genes (three «pif-like», E66-ODV genes), b) 46 putative homologues to EPVs (AmEPV and MsEPV), c) seven putative homologues to viral A-type inclusion protein, d) two putative homologues to whispovirus, e) two putative homologues to chilo iridescent virus, f) DNA

polymerase, DNA helicase, thymidylate synthase DNA-directed RNA pol. Other ORFs showed homologies with non viral genes such as a) three putative homologues to *Wigglesworthia glossinidia* (endosymbiont) genes, and b) two putative chitinases (one from *Glossina morsitans*).

1.4.2 Virus classification

After the accumulation of the sequence data and to discus the results obtained on the viral genome and to have an overview of the possible classification of SGHV, a meeting was held in Vienna from 18-20 December 2006 with the title "Genome Characterisation of the Tsetse Salivary Gland Hypertrophy Virus". The participants for this meeting were Drion Boucias University of Florida USA, Max Bergoin Universite Montpellier, France, Johannes Jehle Agricultural Service Centre Palatinate, Germany, Just Vlak, Wageningen University, The Netherlands and John Burand University of Massachusetts, USA. During the meeting the sequence data were discussed and the repeat regions found in the sequence analysed. Also a phylogenetic analysis of the DNA polymerase was carried out which indicated that the predicted amino acid sequence of the DNA polymerase of SGHV was aligned with selected DNA POL of other large dsDNA viruses using Clustal W in Bioedit. Then a phylogenetic analysis was performed using MEGA3.1 software.

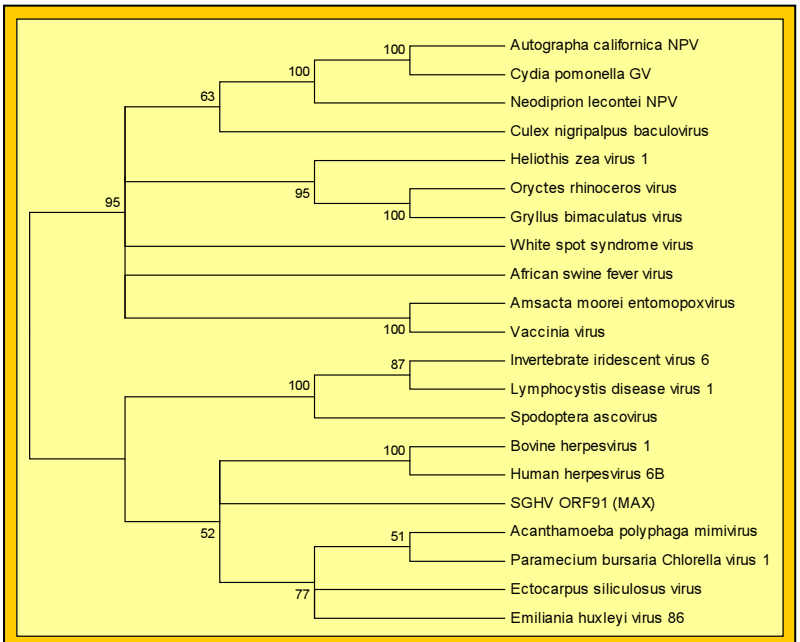


Figure 12. Phylogenetic tree of the SGH virus DNA POL

The generated tree shows that SGHV DNA polymerase does not branch with Baculoviruses and Nudiviruses but with Iridoviruses, Herpesviruses and Phycodnaviruses. The bootstrap values for this position is very high (95%). Therefore can be excluded that the SGHV DNA polymerase is is phylogenetically closely related to Baculoviruses or Nudiviruses. SGHV might represent a new virus family. Interestingly SGHV DNA polymerase clusters with other dsDNA viruses having a linear genome **(Figure 12)**.

1.4.3 Towards a better understandings of the SGHV distribution in the fly colony

During two years of research on the biology of the salivary gland hypertrophy virus (SGHV), several experiments were carried out with the major objectives to understand the impact of the virus infection on the colony performance and production and the mode of virus transmission. During detection of the virus by observing the development of salivary gland hypertrophy symptoms after dissection, we repeatedly found an average of three and 5% infected flies in females and males, respectively. The detection of virus in asymptomatic flies by PCR reveals that the virus is widely distributed in the colony ranging between 80-100%, with the exception of young, non-fed flies.

The observation of salivary gland hypertrophy in non-fed flies provides evidence for the vertical transmission of the virus from mother to larva. The observation of virus particles in the milk gland tissue may indicate that the virus could be transmitted to the larvae during feeding. The detection of the virus in the blood after feeding the flies shows that the virus is released into blood by infected flies during feeding; this indicates the possibility of horizontal transmission of the virus from infected to uninfected fly through the blood meal.

The absence of virus-free flies blocked progress towards determining the mode of transmission and assessing the biological impact of the virus infection. To overcome this problem the establishment of virus free colony was attempted. Some antiviral drugs were tested to suppress virus infection in the flies.

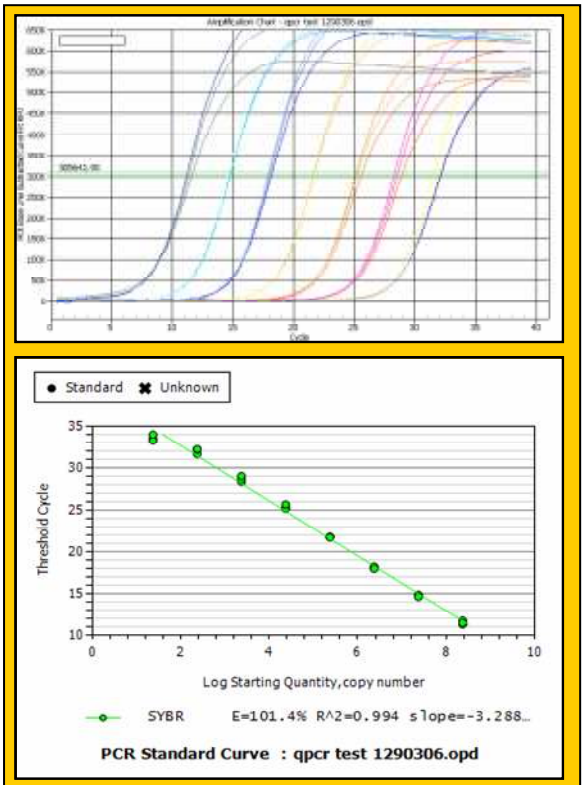


Figure 13. Optimization of QPCR with the SGHV DNA and the selected primers

1.4.4 QPCR analysis following anti-viral drug treatment

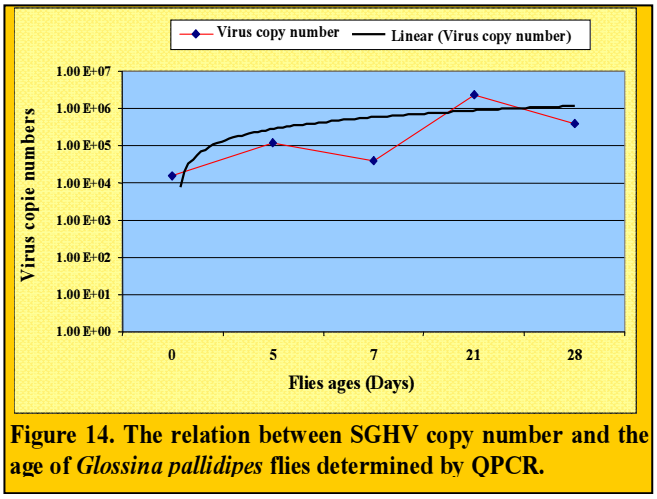


Figure 14. The relation between SGHV copy number and the age of *Glossina pallidipes* flies determined by QPCR.

After the establishment and optimization of a PCR test to detect SGHV in tsetse flies, the prevalence of the virus infection was found to be high (90-100%), mostly as asymptomatic infections. To determine the relationship between the virus infection load and the development of SGH, we needed to estimate the virus infection load in the symptomatic and asymptomatic infected flies. The Quantitative PCR (QPCR) represents an effective way to estimate the relative load of the virus infection by calculating the relative virus copy number. Another objective for establishing a QPCR was to assess the impact of the treatment with antiviral drugs on the prevalence of the virus infection. Two sets of primers were selected for the QPCR and the test was performed with Biorad instrument and instruction using the viral DNA as a standard (**Figure 14**). The virus copy number was estimated based on an estimated viral genome size of 185kbp, with the equation that 1µg DNA=4.7E+9 copies. Using the QPCR test the virus infection load was determined in flies

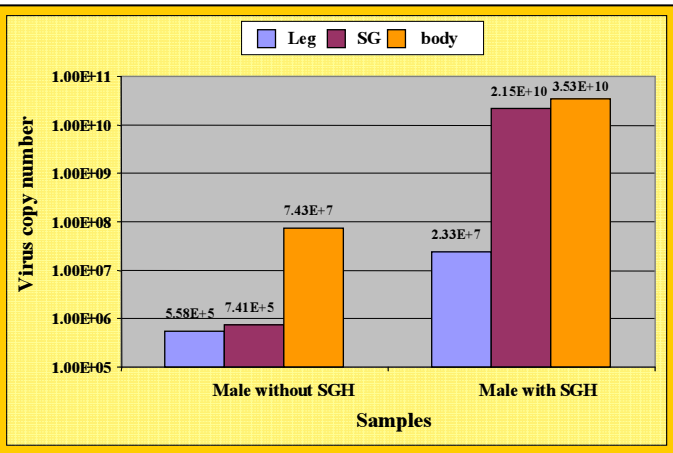


Figure 15. Comparison of virus copy number in males without SGH and those with SGH

of different ages (**Figure 14**) and in symptomatic and asymptomatic infected flies (**Figure 15**). While the tests indicated a slight increase in the virus copy number with increasing age of the flies, there is a significant increase in the virus copy number between the symptomatic and asymptomatic infected flies whether we use the salivary gland, one excised leg or the total body of the fly as a source of material from which to extract DNA.

1.4.4.1 Symptomatic and asymptomatic infection

Since we observed in the colony more asymptomatic infections than symptomatic, it is important to understand the factors that control the virus in this state and also to understand when and why this situation develops to the symptomatic state. Using the QPCR the virus copy number was estimated in flies with SGH to be around 2 x 10^{10} virus genome copies whereas flies without SGH have around 1 x 10^{5} virus particles. This result indicates the high load of virus particles associated with the SHG symptom which strongly indicates involvement of the virus in the development of SGH.

Based on this information; and taking into account that the number of virus particles acquired by larvae varied from one larva to another depending on the virus level in the mother; each larvae produces a fly with a certain virus copy number and will need a certain time for virus multiplication to accumulate the virus until reaching the virus copy number needed to develop salivary gland hypertrophy (**Figure 16**). In this matter; it is important to determine 1) the efficacy of the virus transmission from the mother to the larvae and 2) the rate of virus multiplication in adult flies taking into account the possible genetic variability of the individual flies. Determination these two factors may greatly help in the prediction of the development of the SGH symptom and sterility in the colony.

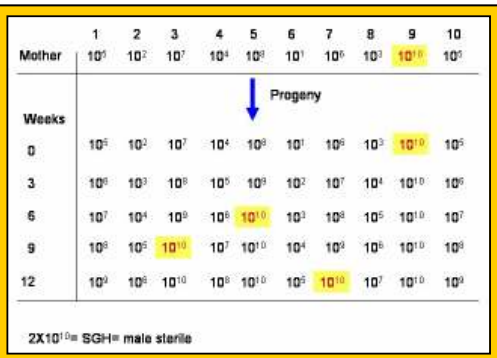


Figure 16. Incomplete model for the dynamic distribution of SGHV in tsetse colony.

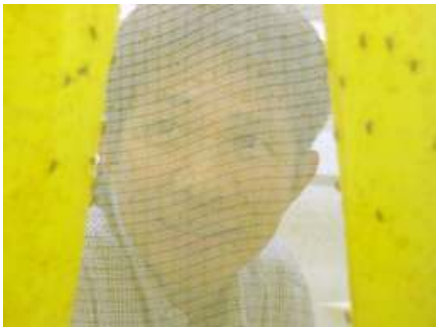
1.4.4.2 Virus infection and sterility

The previous reports on this virus indicate that males with salivary gland hypertrophy are often completely sterile and females with salivary gland hypertrophy are partially sterile. In our laboratory work and by dissection of the male after separation three days after mating we observed that, 63% of the males with salivary gland hypertrophy did not produce any progeny when mated with non infected females (PCR negative on leg tested before mating). For males which showed SGH three days after mating and produce progeny, the QPCR test showed that they had a significantly lower virus copy number (3 log lower) than the other males which have hypertrophied salivary glands. This result indicates that either the development of SGH occurred after mating or the virus invasion of the male accessory reproductive gland occurred progressively after infection of all the cells of the salivary glands. In this case damage to the male accessory reproductive glands and sterility will come after development of hyperplasia.

2. Fruit Fly Rearing and Quality Control

The first genetic sexing strains in Mediterranean fruit fly were developed using a white pupae *mutant whereby females emerged from white pupae and males from brown pupae and the current strains using the* tsl *mutation still incorporate the* white pupae *mutant to help with QC evaluation and strain monitoring. In* Bactrocera cucurbitae *and* Bactrocera dorsalis white pupae *sexing strains have been developed by colleagues in Hawaii and these are undergoing evaluation in the Unit. The* Bactrocera cucurbitae *strain appears to carry a complex chromosomal rearrangement and would not be suitable for mass rearing.*

Mating compatibility field cage tests are now essential components of quality control protocols for fruit flies to be used in SIT programmes. Many insect pests of economic importance have very wide geographic distributions raising concerns that not all the populations may be compatible with each other. This would mean that one strain mass reared for release could not be used in all situations. In 2006 mating compatibility tests were carried out with the olive fly Bactrocera oleae *and the South American fruit fly* Anastrepha fraterculus. *For the olive fly it was shown that mass-reared and wild populations from Greece were compatible with each other but they were less compatible with wild flies from Israel. The results on* Anastrepha fraterculus *confirmed previous studies that there exist major mating incompatibilities between some populations.*



In some species of fruit flies it has been shown that treatment of males with juvenile hormone analogues can accelerate sexual maturation. This is important as some species have very long maturation times requiring that sterile males have to be retained in the facility for some time before they can be released. In 2006 activities were started on Bactrocera cucurbitae *to assess the effect of methoprene treatment on male sexual maturation. For this species, males also need to be provided with protein to attain sexual maturity and it is not yet clear how the methoprene treatment interacts with the protein feeding. Experiments will be continued in 2007 to assess the effects of methoprene and protein on younger males.*

A major investment was made in the fruit fly facility in 2006 in terms of staff safety with the installation of a hydrochloric acid dispenser for larval diet preparation. This was accomplished through very good collaboration with the laboratory support group who also provided all the funds necessary for the installation.

2.1 Rearing GSS of the Oriental Fruit Fly (*Bactrocera dorsalis*) and Melon Fly (*Bactrocera cucurbitae*)

The Department of Entomology of the University of Hawaii and USDA/ARS Pacific Basin Area Research Centre have succeeded in isolating potential genetic sexing strains based on *white pupae* (*wp*) mutations for *Bactrocera dorsalis* and *Bactrocera cucurbitae*. In a collaborative project with Dr. D. McInnis, these strains have now been transferred to Seibersdorf for further studies on rearing protocols, quality control, cytology, stability and competitiveness. During after 12 generations colonies of both strains have been adapted to the Seibersdorf standard wheat bran Mediterranean fruit fly larval diet and production and quality control profiles during mass rearing are ongoing. A series of small scale rearing experiments were conducted to understand the biology of both strains and to assess their biological potential. Both strains are based on sex linked translocations and use the autosomal recessive *wp* mutation as a selectable marker.

Twenty experimental replicates of each strain were set up to assess egg viability, pupal and adult production. Eggs were collected over 5h periods from mature females between the third and sixth day of oviposition. Eggs were incubated for 24h and then counted onto moist black filter paper with 1000 eggs per replicate per stain. The filter paper was transferred to a Petri dish containing 200g of carrot diet. The number of eggs hatched after two days was recorded. Nine days after egg transfer pupae were collected and divided in two groups according to pupal colour (white or brown) and the number and sex of adults emerging was recorded. The results for the Oriental fruit fly (**Figure 17**) show a typical production profile of a sexing strain involving only one autosome (that carries the selectable marker) and the male determining chromosome. Due to the generation of balanced and unbalanced gametes when a single translocation is present, the expected strain viability is 50%. In this strain egg hatch and pupae production were reduced to 68±6% and 58±9% respectively and the overall adult production was, as expected, 50±4%.

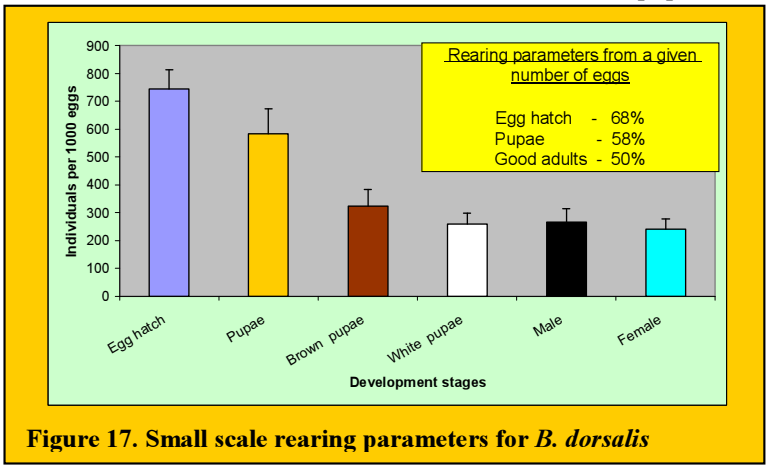


Figure 17. Small scale rearing parameters for *B. dorsalis*

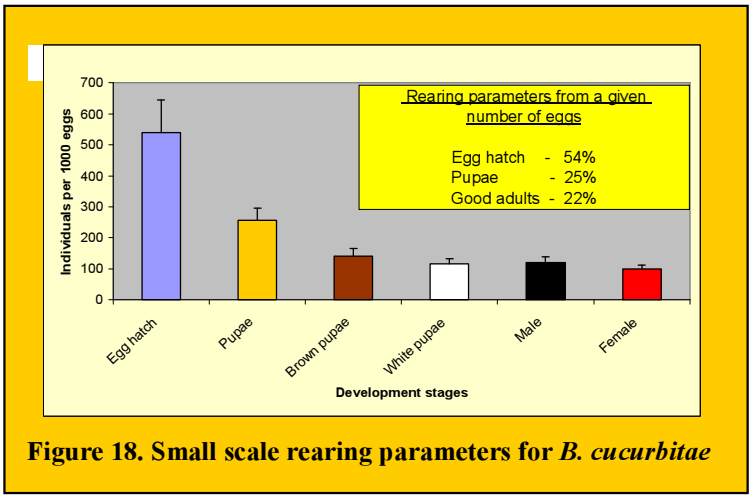


Figure 18. Small scale rearing parameters for *B. cucurbitae*

However the production parameters for the melon fly strain (**Figure 18**) were different, egg hatch and pupae production were more drastically reduced to 54±9% and 25±4% respectively and the adult production fell to 22±4%. These results indicate that a multiple translocation maybe involved which needs to be confirmed through cytological analysis.

After 12 generations both strains are genetically stable but show a low frequency of recombinant individuals. The percentage of males emerging from white pupae and/or females emerging from brown pupae was variable between generations but less that 0.20% and there was no accumulation of recombinants through the generations. Although the melon fly strain is stable, its quality control and production profile are lower than for other sexing strains. This will require the isolation of better translocations in order to generate more productive strains.

2.2 Effect of Methoprene and Protein on Bactrocera cucurbitae males

Operational SIT programmes for the melon fly are faced with the problem that sterile males only reach sexual maturity 10 days after adult emergence and it is known from *Anastrepha* species that a juvenile hormone analogue can speed up sexual development in males. To assess if the same acceleration can be induced in the melon fly, experiments have been carried out by a cost free intern Mr. Ihsan Ul Haq from Pakistan, who is registered for a PhD in the Department of Natural Resources & Applied Life Sciences, BOKU, Vienna. All the experiments were carried out with the *white pupae* genetic sexing strain.

2.2.1 Determination of peak mating time in field cages

Eight replicates were run and in each field cage twenty 12-15 days old males fed on yeast hydrolysate and sugar (1:3 ratio) were released at 14:20 hours, ten minutes later 20 females of the same age were released. Light intensity (Lux) was measured every 30 minutes and activity of males was recorded continuously until male calling ceased. The results showed that in all cases males started to call at 16:00 hours becoming active during the early dusk period. All activities such as calling, encountering and mating were recorded during the period 16:00 hours to 16:30 hours when light intensity started to decrease from 50lux. At 17:00 hours during late dusk (1-37 lux), all males stopped their activities. No calling and no mating were observed at this light intensity level **(Figure 19)**.

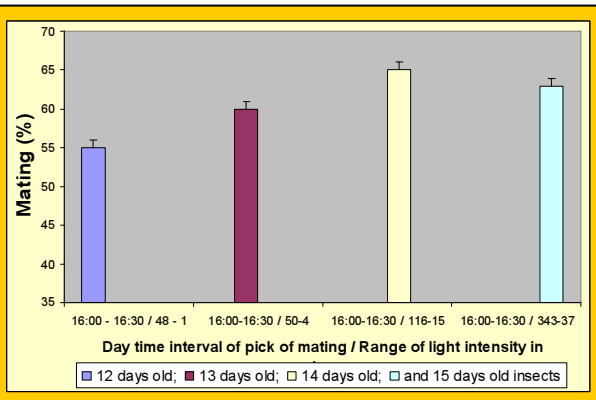


Figure 19. Time and light intensity for the peak of mating of melon flies of different ages

2.2.2 Mating success under laboratory conditions

The experiment consisted of four treatments. 1) males treated with juvenile hormone analogue methoprene (M) and fed protein (M+/P+), 2) males fed only protein (M-/P+), 3) males treated with juvenile hormone analogue methoprene and fed sugar (M+/P-) and 4) males fed only sugar (M-/P-). As with *Anastrepha fraterculus*, 1µl of methoprene solution (5µgr methoprene /1µl acetone) was applied topically to 3 hour old males. The protein source was Hydrolysate Enzymatic Protein ICN®) and mixed with sugar (in 1:3 ratio). Experiments were conducted under controlled laboratory conditions in small plastic cages (12 x 20 x 12 cm) with five males and five females of the same age. The results showed that methoprene and protein had a significant effect on mating success of *B. cucurbitae*. The treatment with methoprene and protein (M+/P+) gave many more matings than the treatment with protein alone (M-/P+) which in turn had more matings than the other two treatments. Treatment with methoprene but without protein showed no improvement over males fed only on sugar, however methoprene accelerated male sexual maturity and males started mating

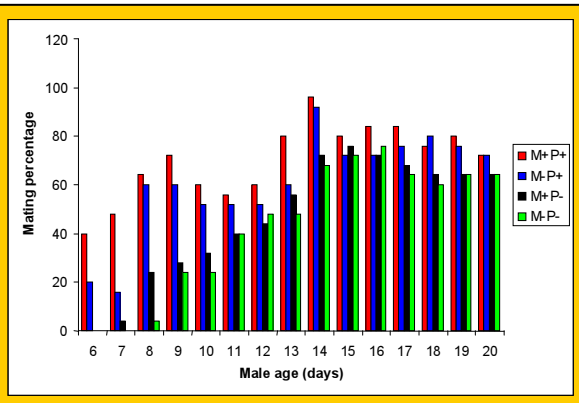


Figure 20. Percent of mating for males of different ages treated with methoprene feed with or without protein supplement

one day earlier than males not treated with methoprene in both protein and sugar fed groups. Males treated with methoprene and fed on protein started mating one day earlier than males fed on protein but without methoprene treatment, two days earlier than males fed on sugar with methoprene treatment and three days earlier than males fed only on sugar **(Figure 20)**.

2.2.3 Mating success under field cage conditions

The same treatments were used as those described above but the mating success was evaluated in field cages. Twenty males of each treatment identified by painting the thorax with a small spot of organic water based paint were released together into the field cage; twenty minutes later 20 mature females were released. The number of couples and mating duration were recorded for males of different ages, 13, 14, 15 and 16 days old. **Figure 21** shows that independently of age, males have better mating success when fed with protein supplement. There was no significant increase in mating success for males fed protein and treated with methoprene compared to males without methoprene.

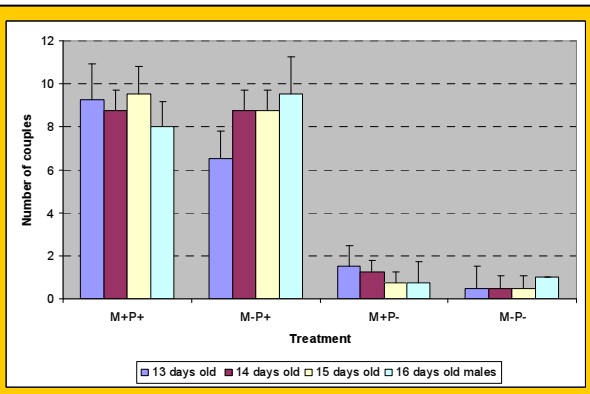


Figure 21. Average number of mating of methoprene treated mature males feed with or without protein supplement.

A second similar experiment was carried out comparing eight versus 14 day old males; the 14 day old males were not treated and fed only sugar. There were three treatments for the eight day old males, M+/P+, M-/P+ and M+/P-. The results **(Figure 22)** are similar to those obtained with mature males and suggest that protein supplement alone induces an acceleration of sexual maturity and increases sexual performance of male melon flies. As it is still not clear if methoprene accelerates male sexual maturity in melon fly, another series of experiments will be carried out using even younger males i.e. males four, five, six and seven days old.

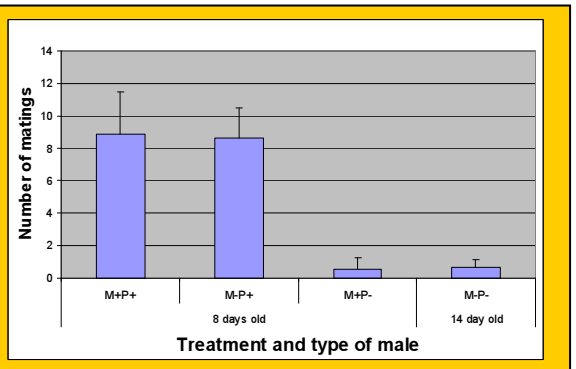


Figure 22. Number of matings of methoprone treated 8 days old males feed with or without protein supplement

2.3 Effect of Methoprene and Protein on *Anastrepha fraterculus* males

The South American fruit fly *Anastrepha fraterculus* is one of the major fruit pests in South America and as with *Bactrocera* species there is a long period of male sexual maturation. Earlier work (**Annual Report 2005**) carried out in Seibersdorf by Dr. Peter Teal from USDA-ARS, Gainesville, Florida, US has shown that topical applications of methoprene on newly emerged flies significantly accelerated sexual maturation of male *A. fraterculus*. There was a reduction in the mean time of maturation from seven to four days as evidenced by pheromone production; calling, and mating performance under laboratory conditions. In June-July 2006 Mr. Diego Segura, consultant from INTA Castelar, Buenos Aires, Argentina, carried out mating competitiveness tests in field cages using males treated with methoprene. The first objective was to compare the sexual competitiveness between treated males and untreated males and a second objective was to assess if methoprene treatment reduced the sexual isolation previously found for populations of *A. fraterculus* from Argentina and Peru.

2.3.1 Mating competitiveness

Twenty five treated, seven day old males, were released together with 25 untreated ten day old males, and 25 mature 14 day old females; as a control, a cage was set up with untreated seven day old males. These tests were carried out using flies from a laboratory population of *A. fraterculus* originating in Argentina. Methoprene treatment was carried out as described above and treated or untreated males were marked with a dot of water-based paint. The time at which copulation was first detected (latency), the duration of the copula and the Male Relative Performance Index (MRPI), were noted. For MRPI differences, significance was evaluated using a Chi-square test of goodness of fit. Differences between mean MRPI for seven day old treated and untreated males were evaluated by means of a one-way ANOVA (or Kruskal-Wallis

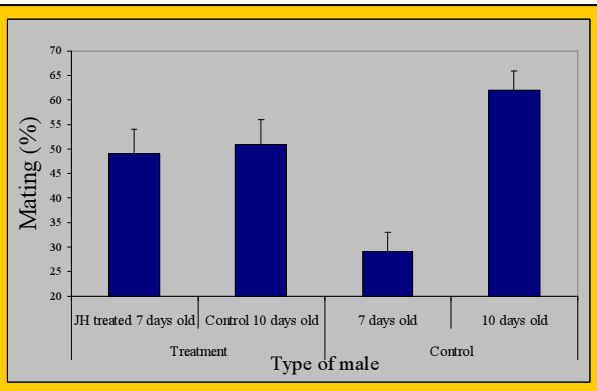


Figure 23. Mean percentage of mating obtained by males treated with methoprene

Figure 23 shows the mean percentage of mating achieved by each type of male. Methoprene treated males achieved a similar percentage of mating than untreated males that were three days older. When untreated seven day old males were compared with ten day old males, they achieved only 30% of the matings. The mean MRPI (7 d-old vs. 10 d-old) was -0.025 for treated males and -0.441 for untreated males. For the treated flies the MRPI proved to be significantly different from zero ($\underline{X}^2 = 24.83$, $P < 0.001$), while for untreated seven day old males the MRPI was not significantly different from zero ($\underline{X}^2 = 0.01$, $P > 0.05$). A Kruskal-Wallis test showed that the MRPIs differed statistically between treated and untreated seven day old males ($P = 0.0013$). These data show that hormonal treatment accelerates maturation in *A. fraterculus* males and produces competitive seven day old males. The performance of these males did not differ from that shown by mature untreated ten day old males.

2.3.2 Sexual isolation

These experiments were carried out using flies from Argentina and Peru. Twenty five treated, seven day old males of one of the two populations were released together with 25 ten day old untreated males of the other population, plus 25, 14 day-old females of each population. A control test was conducted using only 10 day old untreated males from both populations plus the females. The procedure and variables calculated were the same as those described above. The Index of Sexual Isolation (ISI) was calculated to evaluate de degree of assortative mating. The significance of the ISI values was evaluated using a Chi-square test of homogeneity, and compared between treated and control tests by means of a one-way ANOVA (or Kruskal-Wallis test).

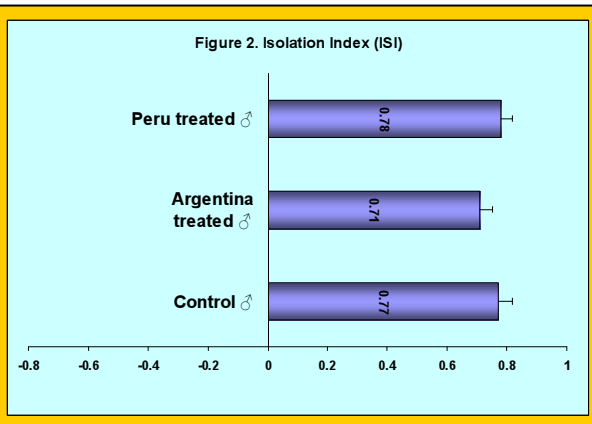


Figure 24. Isolation index for Peru and Argentina flies treated with methoprene

Figure 24 shows the isolation index (ISI) found in the control experiment (0.77±0.05), in the cages which included Argentina treated males (0.71±0.04) and for the cages with Peru treated males (0.78±0.04). The index was significantly different from zero in all cases. No differences were detected between treatments (ANOVA: $F = 0.67$, $P = 0.53$).

The mean percentage of matings achieved by each type of female and male are shown in **Figures 25 and 26**, respectively. The Male Relative Performance Index (MRPI) and Female Relative Performance Index (FRPI) were not significantly different from zero in any replicate, and no differences were found among treatments (ANOVA for MRPI: $F = 2.72$, $P = 0.10$; ANOVA for MRPI: $F = 2.39$, $P = 0.12$). After three years of laboratory rearing of the two populations at Seibersdorf, the degree of sexual isolation between them has not changed. The ISI found in the control treatment is almost identical to that reported earlier (**Annual Report 2005**). Thus, it seems that laboratory conditions did not to affect the mating behavior of the flies, at least concerning reproductive isolation. Also, this result indicates that no contamination between strains took place during the past three years.

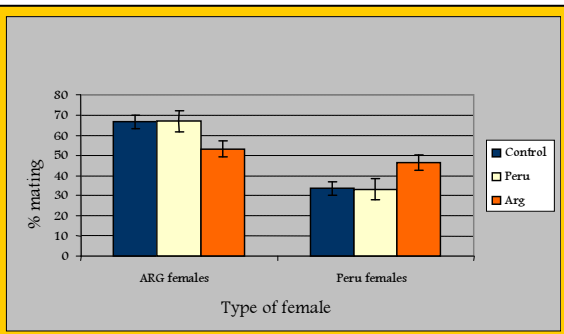


Figure 25. Percentage matings achieved by females with treated and untreated males.

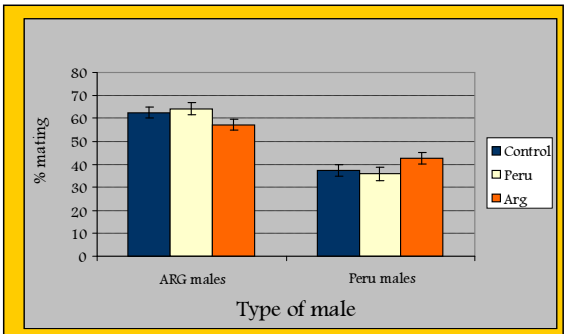


Figure 26. Percentage matings achieved by males with treated and untreated females.

2.4 Reproductive Isolation between Anastrepha fraterculus from Peru and Argentina

The data shown above (and in Vera *et al* 2006) indicated a high degree of sexual isolation between populations of *A. fraterculus* from Peru and Argentina. In addition, other reports in the literature have suggested that *A. fraterculus* consists of a complex of cryptic species. To further understand the basis of this degree of reproductive isolation between these two populations, a new study was undertaken to assess the presence of postzygotic isolation between these two populations. Postzygotic isolation is expected when two populations evolve separately and genetic differences produce deleterious effects in the hybrids principally in the heterogametic sex (Haldane's rule).

A cross was set up between the two populations using 200 Peru males and 200 Argentina females. After mating, eggs were collected from a silicone oviposition device. A known number of eggs were put then on a black moist filter paper and transferred onto artificial larval diet and the following parameters measured, egg hatch, number of pupae recovered, number of emerged adults and adult sex ratio (data not shown). There was a severe reduction in egg hatch (28±2%) compared to the parental Argentina strain (75±5%). In addition, sex ratio was significantly different from 1:1 (ca three females: one male, Chi square = 7.89, P = 0.05). The F1 offspring were inbred and in generation F3 a new assessment of viability was conducted by analyzing three replicates of 1000 eggs. Results of this second experiment are shown in **Figure 27**. As expected the hybrid population still showed low viability and high sex distortion in favour of females.

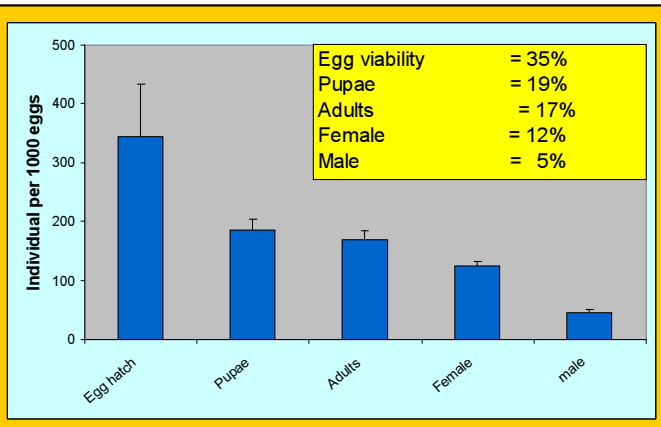


Figure 27. Viability of the progeny from crosses between Peru and Argentina flies

A cytological analysis of larval salivary gland polytene chromosomes from the hybrid population has been carried out by a consultant from University of Patras, Greece, Dr. Antigone Zacharapoulou. **Figure 28** show clearly extensive asynapsis between chromosomes indicating significant genetic differences between the populations. This difference could be one of the reasons that explain the high degree of lethality in embryonic and larval stages. Further work will be carried out to understand more about the genetic differences between these populations.

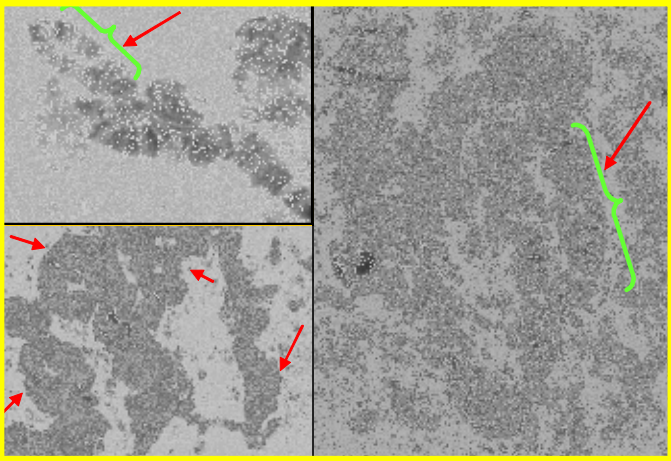


Figure 28. Polytene chromosomes of *A. fraterculus* hybrid population. Arrows show extensive asynapsis

2.5 Protein Sources for *Ceratitis capitata* Adult Diets

Trials were conducted by Mr. Luciano Arnold a fellow from the South African Mediterranean fruit fly SIT project to evaluate cheaper alternative sources of protein for adult diet. The main objective of this work is to reduce the production cost of male only sterile Mediterranean fruit fly pupae by avoiding the utilization of traditional expensive products. The specific objective of these trials was to evaluate four types of yeast hydrolyzate as source of protein for adult food.

The type of hydrolizate yeast used in this experiment is described in **Table 3**. Each experiment was setup by loading small mass rearing egging cages with approximately 31,000 females per generation. Each cage was provided with adult food (3:1 sugar:yeast hydrolyzate). Egg production, egg viability and pupal production during four consecutive generations were used as comparison parameters. However, data presented in this report are exclusively from the last evaluation step, generation F4.

Table 3. Type of hydrolvzate yeast evaluated and their relative cost in Austria.

Type of yeast	Cost/kg
AF–Animal Feed Abstract*	1
AY 150**	1.9
YE 100**	4.07
YE 300**	4.52
Control***	19.47

*Borregaard® Switzerland
**Locally available in South Africa
***ICN® Yeast enzymatic hydrolyzated

The main difference between types of yeast hydrolyzate is the percentage of protein. Adults fed with low protein hydrolyzate (AF) produced the lowest volume of eggs per cage (**Figure 29**). The effect over the generations is still on analysis but it seems that at least adults fed on lower protein concentration maintain lower production of volume of eggs between generations. Flies fed on protein YE-100 had better egg production after generation F2. In generation F4 egg production was lower but not significantly different from the control (**Figure 29**). Egg viability and pupal production from flies fed low protein were significantly higher. High protein concentration yeasts produced normal egg viability.

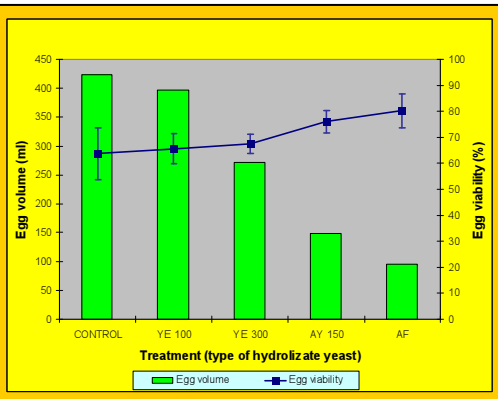


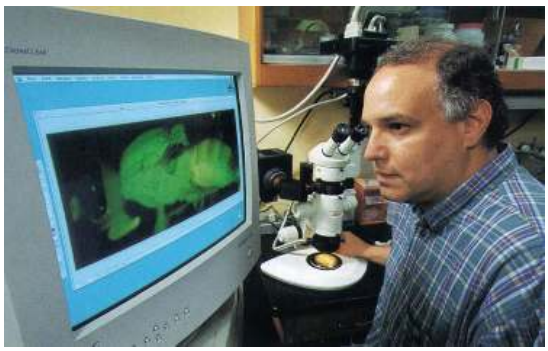
Figure 29. Egg production and egg viability from VIENNA 8 flies fed different protein diets.

Results from this evaluation show that the utilization of at least one of the above mentioned substitutes will result in a substantial saving in cost and will once again reduce substantially the cost of sterile pupae SIT technology and make it more affordable for current operational programs or for potential new projects. Affordability of the technology will enhance the expansion of the SIT in member states.

3. Fruit Fly Genetic Sexing

In 2006 a series of detailed evaluations was completed on Mediterranean fruit fly Ceratitis capitata *genetic sexing lines which carry the dominant visible marker Sergeant. The mutation has three white stripes on the abdomen as opposed to two in a normal fly. This mutation may be useful as a marker to identify released sterile males as work done in the Unit in 2004 showed that it had no significant effect on the mass-rearing or field cage competitiveness of genetic sexing strains carrying the mutation. The use of the marker enabled a detailed analysis to be made of recombination in the different strains it was demonstrated that as well as being a potential marker for released males, the mutation can also be used to improve the efficiency of the Filter Rearing System (FRS). The FRS is used to maintain the integrity of genetic sexing strains during large-scale mass rearing.*

The assessment of transgenesis to develop improved strains for eventual use in insect control programmes remains a high priority in the sub-programme and also in many other laboratories. As mentioned earlier there are developments on the regulatory side that will provide a framework for the deployment of these strains in control programmes. Activities in the Unit focus on the introduction of a visible marker into the current genetic sexing strain and the development of new genetic sexing strains based on transgenesis. Genetic sexing strains marked with a fluorescent protein have now been produced and are undergoing evaluation under mass rearing conditions and in field cages. A new strain in which the testes are labeled with fluorescent protein has also been developed.



mtDNA is a very useful molecule for population genetic analysis and has been used to try to understand the distribution of different genetic variants on the Island of Madeira where there is an ongoing SIT suppression programme. There has been a change in the different mtDNA variants over time as new variants have been recorded that were not present earlier. This implies that flies from other regions are colonizing the Island. Work carried out in the Unit and on Madeira in the past has demonstrated that the wild population on Madeira is much less compatible with released males form the genetic sexing than other field populations.

In line with our work on transgenic fruit flies, and together with other molecular biological activities in the laboratory, we have taken the initiative to register our work with the relevant Austrian Authorities through the setting up a Biosafety Committee.

3.1 Mediterranean Fruit Fly Sexing Strains Carrying the Mutation *Sergeant*

3.1.1. Strain CC59

This strain was developed by C. Caceres in Guatemala by irradiating males heterozygous for the dominant, homozygous lethal mutation *Sergeant* (*Sr*^{2}). The translocation breakpoint was mapped by A. Zacharopoulou (University of Patras) using polytene chromosomes isolated from male trichogen cells. **Figure 30** shows that the breakpoint is located at 58B, equivalent to 75C on the salivary gland polytene map (**Figure 31**). In **Figure 30,** one of the translocation fragments (Y-A) is displaced by the spreading procedure. This fragment contains the short arm, the centromere and a part of the long arm from the Y chromosome and from chromosome 5, the fragment spanning from the breakpoint to the tip of the right arm. The second translocation fragment (A-Y) is still aligned to the free chromosome 5 and consists of the rest of chromosome 5 (including the autosomal centromere) and the long arm of the Y chromosome. The breakpoint in CC59 is, based on cytology, identical to the one detected in translocation T(Y;5)3-129 (= VIENNA 7).

Figure 30. Polytene chromosomes from CC59 isolated from male trichogen cells.

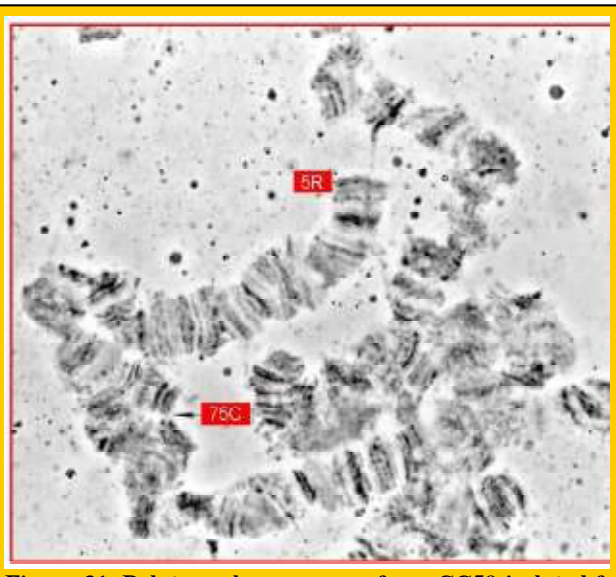


Figure 31. Polytene chromosomes from CC59 isolated from larval salivary glands showing an adj-1 individual with a triplication between the breakpoint and 5R.

Translocation strains are maintained by crossing each generation 50 females from a homozygous *white* (*w*) *white pupae* (*wp*) strain with 50 males carrying the translocation and any exceptional flies are removed. To determine the segregation pattern of the CC59 translocation the rearing level of the strain was increased. For 23 generations 34ml of unselected pupae were used to set up subsequent generations while a parallel sample of 40ml of pupae (equivalent to ca 2000 flies) was analysed. In total 19 generations were analysed and 38014 flies were screened.

Figure 32 shows that two major classes of exceptional flies are detected in addition to the normal w^+ wp^+ Sr^2 males and the *w wp* Sr^{2+} females. The first class of exceptional flies, w^+ *wp* Sr^{2+} females, represents type-1 recombination products where the exchange has taken place in the chromosomal region between *w* and the translocation breakpoint. It is assumed that the *white* mutation causes blindness and consequently the recombinant females have a clear selective advantage over the normal *w wp* females. Therefore, w^+ females accumulate steadily over generations in the colony

while consequently the frequency of *w* females declines.

The second class, *w* wp^+ Sr^2 males, is the result of adjacent-1 segregation during male meiosis. Such males contain two free chromosome 5 (*w wp* Sr^{2+}) and in addition the autosomal fragment spanning the region between the breakpoint and the right tip (**Figure 31**). In agreement with the position of the translocation breakpoint this fragment contains the wp^+ and Sr^2 alleles. The fact that these exceptional flies are males indicates that the translocation breakpoint on the Y chromosome is located distal of the Maleness factor, i.e. the Y centromere and the Maleness factor are on the same translocation fragment. Very typical for adjacent-1 flies, carrying a certain region of the genome in triplicate, is that many of them only half emerge or if they emerge completely a large fraction is crippled. In CC59, 82% of the *w* wp^+ Sr^{2+} males fall into this category. In contrast, translocation T(Y;5)3-129 which has the same breakpoint on chromosome 5 but where the Y-chromosomal breakpoint is located between the centromere and the Maleness factor, the adjacent-1 offspring are female and die as larvae. However, for translocation T(Y;5)2-22 (= VIENNA 6) where the triplicated area is slightly longer (breakpoint at 57D) a slightly higher frequency of half emerged/crippled flies is produced (92.7%). On average T(Y;5)2-22 produces 8.4% adjacent-1 offspring with a triplication. In case of CC59 the percentage of adjacent-1 males is difficult to determine because this frequency declines the longer the strain is reared (**Figure 32**) due to increasing number of w^+ *wp* Sr^{2+} chromosomes in the colony. In later generations the marker that is required to detect adjacent-1 males is lost and these males can no longer be distinguished from normal males. This can also be seen by the increasing number of w^+ wp^+ Sr^2 males per generation. If one uses only the values for the first 13 generations, than 13.8% of all flies are from adjacent-1 segregation.

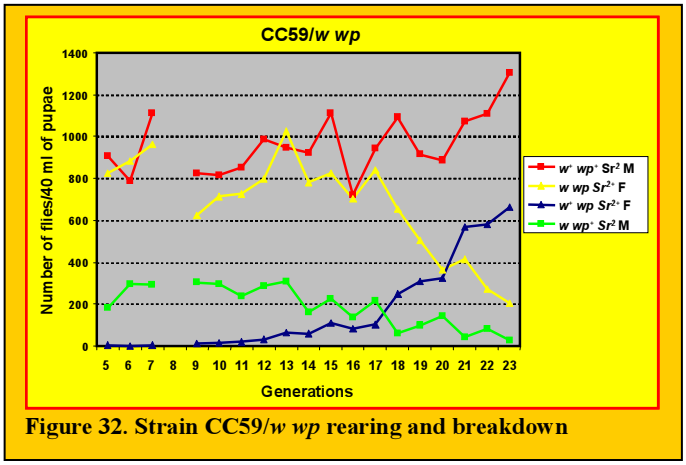


Figure 32. Strain CC59/*w wp* rearing and breakdown

In addition to the exceptional flies shown in **Figure 32**, three additional types are detected at very much lower frequencies. Among the 38014 flies screened two flies were detected that could represent type-1 recombination in the chromosomal region between *wp* and Sr^2, i.e. one male with w^+ wp^+ Sr^{2+} and one female with *w wp* Sr^2 phenotype. From the number of these reciprocal recombinants it can be calculated that the male recombination frequency in this interval is 0.0058% which is in agreement with the results obtained with other translocations (**Table 4**). As a third class seven males (= 0.0184%) with a *w* wp^+ Sr^{2+} phenotype are observed, probably due to a double recombination event, one between *w* and the breakpoint and one between *wp* and Sr^2.

Table 4: Recombinants produced by 1 million flies

	Gen's (counted)	Flies screened	Interval B-*wp*	Interval *wp*-Sr^2	Total recomb's
T(Y;5)101/*wp tsl*	18[a]	41164	1107	NA	
T(Y;5)101/D53	79 (76)	195786	143	NA	
T(Y;5)101-Sr^2/D53	55 (47)	119360	92	17	109
T(Y;5)3-129	147 (140)	283790	(106)[b]	NA	
CC59/*wp tsl*	60 (50)	93764	22	53	75
GS-9/*wp tsl*	51 (46)	101314	30	49	79
GS-9/D53	51 (46)	104873	57	124	181

[a]: only the first 18 generations can be used; then recombinants start to accumulate
[b]: based on doubling the male recombination frequency
B: Y-autosome translocation breakpoint

However, that would increase the *wp-Sr*^{2} recombination frequency to 0.0242%, a value that was not observed elsewhere. An alternative explanation for this type of male could be that they belong to the adjacent-1 offspring (*w wp Sr*$^{2+}$). Such males contain *Sr*^{2} together with two wild type alleles. The observed number of exceptional females with *wp*$^+$ phenotype cannot be used to calculate the recombination frequency because *wp*$^+$ females are also generated as a consequence of adjacent-1 segregation. However, as recombination should produce equal numbers of recombinant males and females one can estimate that the male recombination frequency is 0.0106% compared to 0.0022% in case of CC59. No recombinants were detected for the chromosomal interval between the breakpoint and *wp*. The breakpoint of CC59 is very close and more data have to be collected to be able to determine such low recombination frequencies more precisely (see next paragraph).

To construct a *tsl*-based sexing strain with translocation CC59, males where backcrossed twice with homozygous *wp tsl* females. The resulting strain was reared for 60 generations at our standard rearing level and only very few recombinants were detected (**Table 5**). This is particularly true for the chromosomal interval between the translocation breakpoint and *wp*. Here only a single recombinant, a *wp Sr*$^{2+}$ male, was detected which corresponds to a recombination frequency of 0.0011%. Although the strain T(Y;5)3-129 has its breakpoint at the same cytological position, recombination is higher; i.e. 0.0053%. For the *wp-Sr*^{2} interval 5 recombinants were detected. This is equivalent to a male recombination frequency of 0.0053%. As a comparison the female recombination frequency in this interval is roughly 100 times higher (0.48%).

Table 5. Comparison of productivity and stability of two GSS with different translocation

	T(Y;5)3-129/*wp tsl*	**CC-59/*wp tsl***
Generations (counted)	147 (140)	60 (54)
Adults counted	283790	93764
Males/40ml	1133	1010 (= -11%)
% males crippled or deformed	3.6	9.7
Females/40 ml	894	726 (= -19%)
% females crippled or deformed	5.1	6.8
Exceptional flies	*wp* males: 15 (0.0053%) *wp*$^+$ females: 46 (0.0163%)	*wp Sr*$^{2+}$ males: 1 (0.011%) *wp*$^+$ *Sr*$^{2+}$ males: 4 (0.0043%) *wp Sr*^{2} females: 1 (0.0011%)

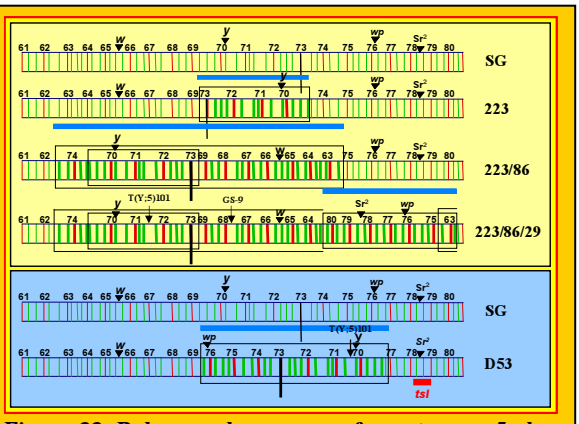


Figure 33. Balancer chromosome for autosome 5 also showing the inversion D53. SG salivary gland map.

The productivity of CC59 is relatively low when compared to T(Y;5)3-129 and even lower when compared to T(Y;5)101 (= VIENNA 8). CC59 produces 11% fewer males than T(Y;5)3-129 and 22% fewer males than T(Y;5)101. In addition, of the males produced ca 14% are adjacent-1 males, i.e. males of lower quality. This is also reflected in the relatively high frequency of half emerged/crippled males. Also the female production in CC59 is reduced, i.e. CC59 produces 19% less than T(Y;5)3-129 and 27% less than T(Y;5)101.

3.1.2 Strain GS-9

Strain GS-9 is based on a balancer chromosome for autosome 5, i.e. a chromosome with three overlapping inversions. This balancer was induced by A. Zacharopoulou and is based on the pericentric inversion #223 with its breakpoints at 69B and 73A on the salivary gland polytene map (**Figure 33** and **Annual Report 1999**). Inversion #223 was irradiated and the offspring was screened cytologically for newly induced inversions. In one family (#86), a second pericentric inversion was detected that completely includes #223, i.e. the breakpoints are at 62B and 74C. This double inversion chromosome was irradiated and a third inversion was induced (#29; 64C to 80B). The resulting balancer chromosome exists in several versions with respect to the markers it carries. However, all versions carry the mutation Sr^2 and therefore the balancer is lethal in a homozygous condition. Such chromosomes are very useful genetic tools because in a heterozygous condition single and all uneven numbered recombination events occurring within the inverted regions lead to unbalanced and therefore lethal offspring (while double and even numbered recombination events do not cause lethality in the offspring). This characteristic would also be very useful to increase the stability of sexing strains. The homozygous lethality of the balancer means that it has to be linked to the Y chromosome.

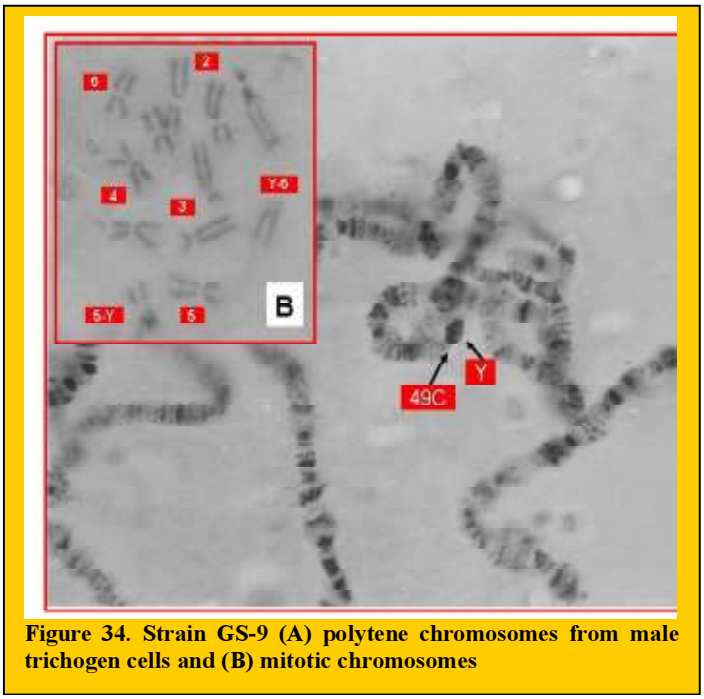


Figure 34. Strain GS-9 (A) polytene chromosomes from male trichogen cells and (B) mitotic chromosomes

To induce a Y-autosome translocation on the balancer, males were irradiated either with 40 or 50 Gy. The irradiated males were mass mated with *w wp* females. Single F1 males were crossed with *w wp* females and out of a total of 100 single male crosses one showed pseudo-linkage between the markers and sex (= GS-9). The breakpoint of the translocation was mapped by A. Zacharopoulou on polytene chromosomes isolated from male trichogen cells (**Figure 34**). The breakpoint is located at 49C (equivalent to 68A on salivary gland chromosomes).

GS-9 males were backcrossed twice with homozygous *y* (*yellow body*) *wp* females to identify adjacent-1 offspring. For this, two mutations are required; one for each translocated autosome fragment. The mutation *y* is located on the autosomal translocation fragment with the autosomal centromere (A-Y), while *wp* is located on the translocation chromosome with the Y chromosomal centromere (Y-A). The latter would be present in adjacent-1 offspring, i.e. the expected phenotype for adjacent-1 offspring should be *y* wp^+ Sr^2. In total ca 10000 flies were screened and no such flies were detected. The likely reason for this is that the triplicated region with this translocation is very long and adjacent-1 individuals die before reaching the adult stage.

With the translocation GS-9 two different sexing strains were constructed; in one strain the translocation was combined with the *wp tsl* chromosome and the second one with the D53 inversion chromosome (*wp tsl*). These strains were reared at the standard level for 51 generations of which 46 generations were screened for recombinants. Due to the complex structure of the balancer chromosome it is very difficult to assign recombination frequencies in the intervals between the three

markers used, i.e. the translocation breakpoint, *wp* and Sr^2. In principle the physical order of these markers on the balancer chromosome is: breakpoint-Sr^2-*wp* and all three are on the same chromosome arm. Based on this order an exchange in GS-9/*wp tsl* between the breakpoint and Sr^2 should lead to Sr^2 wp^+ females (none detected) and Sr^{2+} *wp* males (three detected). One would expect a second class of recombinants, i.e. Sr^2 *wp* males and Sr^{2+} wp^+ females due to recombination events between Sr^2 and *wp* (non detected). Instead, five recombinants were found where only Sr^2 was exchanged **(Table 6)**. In GS-9/D53 the situation is even more complicated. However, following the same logic as above the following results were obtained: recombination between breakpoint and Sr^2: four detected, recombination between Sr^2 and *wp*: two detected, exchange of only Sr^2: 11 detected. In general it can be concluded that the presence of the balancer does not reduce the recombination frequency to zero. In fact, if at least a double cross over is needed to produce these recombinants then the frequency is rather high. In case of GS-9/*wp tsl* the recombination frequency is very similar as in other strains. Combining D53 with GS-9 even increases the number of recombinants detected. Probably the best way to compare strains with *wp* and Sr^2 is to look at the total number of recombinants observed **(Table 5)**. Here GS-9/*wp tsl* (79 recombinants/million flies) is very similar to CC59/*wp tsl* (75 per million flies) despite the fact that this strain does not include an inversion. Adding D53 to GS-9 produces 181 recombinants per million flies. This is more than observed with T(Y;5)101/D53 (143 per million) or T(Y;5)101-Sr^2/D53 (92 per million).

Table 6. Comparison of productivity and stability of GS-9 combined either with *wp tsl* or with D53

	GS-9/*wp tsl*	GS-9/D53
Generations counted	51 (46)	51 (46)
Adults counted	101314	104873
Males/40ml	1195	1201
% males crippled or deformed	9.1	7.8
Females/40 ml	1007	1079
% females crippled or deformed	5.8	5.3
Exceptional flies	*wp* Sr^{2+} males: 3 (0.003%) wp^+ Sr^{2+} males: 5 (0.0049%)	*wp* Sr^{2+} males: 3 (0.0029%) wp^+ Sr^{2+} males: 11 (0.0105%) wp^+ Sr^2 females: 1 (0.001%) wp^+ Sr^{2+} females: 2 (0.0019%)

Overall GS-9 is very stable and no accumulation of recombinants was observed during 60 generations of rearing at the standard level. The productivity of both GS-9 strains is very similar. Compared to T(Y;5)101 the male production is slightly lower while female production is the same.

3.1.3 Strain T(Y;5)101-Sr2

This strain has been reared for 55 generations with D53 at the standard rearing level. In total 119360 flies were screened in 47 generations **(Table 7)**. The recombination frequency between the translocation breakpoint and *wp* is 0.0092%, i.e. the presence of the inversion reduces the recombination frequency in this region by ca 90% compared to T(Y;5)101/*wp tsl* **(Table 5)**. Furthermore, without the inversion the strain started to break down in generation 19, i.e. recombinants started to accumulate rapidly. This was not observed here nor with strain T(Y;5)101/D53 although in this strain the recombination frequency between the breakpoint and *wp* is even slightly higher (0.0143%). In T(Y;5)101-Sr^2 the recombination frequency between *wp* and Sr^2 is 0.0017%. This is the lowest frequency measured in any strain. This is possibly due to a "neighbourhood effect" of D53, i.e. although the inversion only partially covers this region, its presence exerts a recombination reducing effect on chromosomal regions in the near vicinity **(Annual Report 2005)**. Reducing the recombination in the *wp*-Sr^2 interval is important because the

tsl is closely linked to Sr^2. In a Filter Rearing System (FRS) recombinants are removed in the clean stream based on the *wp* marker. The presence of the *tsl* cannot be determined easily, i.e. recombination events between *wp* and *tsl* lead to a loss of the *tsl* but remains unnoticed in the FRS cleaning procedure. A loss of the *tsl* becomes only visible by an increasing level of *wp* females that are not killed by the temperature treatment. Therefore, including Sr^2 as additional marker would allow more accurate and more reliable cleaning in the FRS. If this is combined with the improved procedure for collecting the pupae to produce the next generation in the clean stream (wp^+ males from early pupal collection and *wp* females from late ones, **(Annual Report 2005)**) the overall accuracy of the FRS could be improved significantly.

Table 7. Comparison of productivity and stability of T(Y;5)101 with or without Sr^2

	T(Y;5)101/D53	T(Y;5)101-Sr^2/D53
Generations counted	79 (76)	55 (47)
Adults counted	195786	119360
Males/40ml	1426	1412
% males crippled or deformed	5.8	6.6
Females/40 ml	1051	1127
% females crippled or deformed	7.4	8.7
Exceptional flies	*wp* males: 6 (0.0031%) wp^+ females: 22 (0.0112%)	*wp* Sr^{2+} males: 7 (0.0059%) wp^+ Sr^{2+} males: 2 (0.0017%) wp^+ Sr^2 females: 4 (0.0034%)

3.2 New Transgenic Medfly Lines

In collaboration with A. Handler (USDA, Gainesville, USA) two constructs were injected into three fruit fly species (**Table 8**). The vector #369 carries three inverted repeats; two left (L) and one right (R) in the order L_1-L_2-R. Two fluorescent marker genes are inserted, EGFP (green fluorescence) between L_1 and L_2 and DsRed (red fluorescence) between L_2 and R. Both genes are under the control of polyubiquitin promoters. Integration of the entire construct is therefore manifested by the presence of green and red fluorescence. The screening is complicated because the fluorescence is present in the same part(s) of the fly and because the two colours overlap, i.e. the red colour is easily distinguishable while the EGFP fluorescence appears more as orange than as green. Successful integration was obtained previously in the wild type strain EgII from *C. capitata* and in this experiment in *A. fraterculus*.

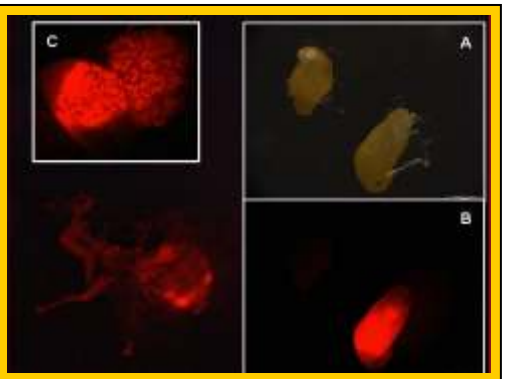


Figure 35. DsRed controlled by the ß2 tubulin promoter: transgenic male carrying the #369 construct. Insert A: two isolated testes viewed without fluorescent light; non-transgenic control: upper left; transgenic: lower right. Insert B: the same testes with fluorescent light. Insert C: Testes with fluorescing granular structures

The construction of the vector #369 allows stabilizing the transgene at its current insertion site by removing the region between L2 and R. That leaves only a partial, non-functional mobile element with only one inverted repeat behind. For this transgenic flies carrying #369 are re-injecting; this time only with transposase, i.e. with the helper plasmid. Flies where the L2-R region was removed become visible because they have lost the red fluorescence. This was demonstrated successfully in *C. capitata.* Several lines were generated where the DsRed was removed following a re-injection with transposase. Further analysis will be required to characterize these strains at the molecular level.

The vector #389 also carries EGFP and DsRed but here the markers are not part of a "suicide vector" system as in the one described above. The two fluorescent protein genes are under the control of two

Table 8. Results of embryo injections in three fruit fly species

Species	Strain	Construct	G0				G1 fluorescent adults	
			Larvae	Pupae	Males	Females	Males	Females
C. capitata	EgII	#389	117	85	45	29	7	5
C. capitata	D53	#389	57	20	5	6	0	0
C. capitata	D53	#369	124	72	28	22	0	0
A. fraterculus		#369	250	194	62	47	2	5
B. cucurbitae		#369	5	1	1	0	0	0

different promoters. The EGFP is controlled by the polyubiquitin promoter and provides an easily screenable body marker to detect successful transformation. The DsRed is under the control of the *C. capitata* ß2 tubulin promoter. In several species it has been shown that this combination allows incorporation of the fluorescent protein into sperm. Labeled sperm would be very interesting for the practical application of transgenic insects in SIT programmes. It would allow the determination of whether a trapped female was mated by a wild or a released male. **Table 8** shows that 12 lines were generated in the *C. capitata* wild type strain EgII. They are maintained as heterozygotes by outcrossing each generation with EgII wild type flies. In **Figure 35** the expression pattern of the ß2 tubulin-controlled DsRed marker is shown. The testes exhibit very high levels of fluorescence (**Figure 35B**). In some cases granular structures with fluorescence are observed (**Figure 35C**). We have a minimum of three independent lines as the transgenic G1 flies were detected in three independent cages with G0 crosses. However, probably there are more independent lines if one considers the different expression patterns of the fluorescent markers (in particular EGFP). So far we were unable to detect fluorescent sperm in any of these lines using a microscope with a 400x magnification.

3.3 mtDNA Haplotype Distribution on the Madeira North Coast

The mitochondrial DNA (mtDNA) haplotype of various wild medfly samples was determined using the method described in **Annual Reports 2000** and **2001**. In brief, the presence or absence of a restriction site in a particular segment of the mtDNA is determined for four different enzymes after PCR amplification of the respective region. The results are shown with a four letter code where each position corresponds to one of the four enzymes (order: EcoRV, XbaI, MnlI and HaeIII) and "A" refers to presence and "B' to absence of the site. For the analyses DNA is isolated from individual flies.

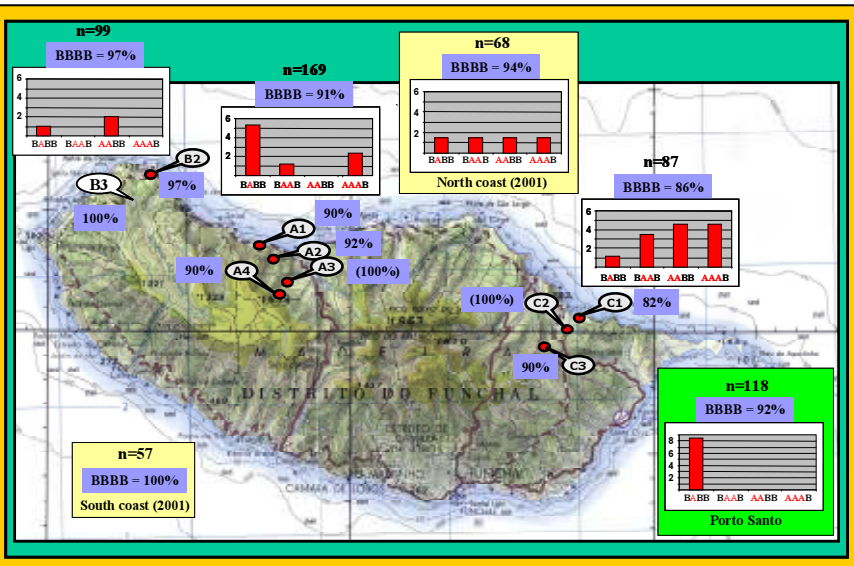


Figure 36. Distribution of mtDNA haplotypes on Madeira and Porto Santo.

that Madeira flies carry exclusively a BBBB haplotype. However, in 2001 samples from the north coast and from the neighbouring island Porto Santo showed that a certain percentage of flies carried different haplotypes (see **Annual Report 2004**). This survey was extended to samples from three different locations from the north coast (A to C). From each location flies were sampled from different altitudes.

For example in the region A flies were collected in 0-200 m (A1), 200-300 m (A2), 300-600 m (A3) and above 600 m (A4). **Figure 36** shows a summary of the distribution of the four non-BBBB haplotypes detected in the areas A to C and it also shows the percentage of remaining BBBB haplotypes for each sampling area and altitude.

Comparison between the three different sampling areas: There seems to be a clear trend of decreasing diversity from area C in the east to area B in the west. This decrease is expressed by the lower number of different non-BBBB haplotypes, i.e. there are four in region C, three in region A and two in area B. Usually such conclusions are difficult to draw because of the effects of small sample sizes. However here the smallest sample (87 in C) shows the highest diversity and the reduction in diversity in area B cannot be explained by a smaller sample size (n = 169). In parallel, the percentage of remaining BBBB haplotypes increases from east to west from 86% in C, 91% in A and 97% in B. There is no clear trend in the distribution of each individual non-BBBB haplotype. For example, the haplotype AAAB is one the most prominent types in C, is absent in A and is again the most prominent one in B.

It is difficult to compare data from the different sampling altitudes in each area because the sample sizes are very different and in some cases rather small. However the diversity of different haplotypes and the percentage of non-BBBB haplotypes tend to be lower at higher altitude.

The present data from the north coast are very similar with that from Madeira and Porto Santo in 2001 but it is not known from which region the older samples originated from. It is also not known whether today the south coast still shows exclusively BBBB haplotypes. The Porto Santo sample shows a single non-BBBB haplotype and, therefore, differs significantly from the Madeira north coast. It could be argued that a single invading female with a BABB haplotype would have been sufficient to cause this change.

4. Mosquito R and D

A first effort was undertaken in 2006 to develop a genetic sexing strain based on classical genetics. The approach taken was to try to link an insecticide resistant gene to the male determining chromosome using a translocation so that female larvae would be killed by the application of a discriminating dose of insecticide whereas males would survive. This approach has been successful for other mosquitoes. Three potential sexing lines were obtained but none with the required discrimination between females and males. The work will be continued and expanded in 2007.

Another approach to genetic sexing has been successful although whether it can be applied on a larger scale has still to be assessed. The approach is transgenic and involves the expression of a fluorescent protein in the male testes; larvae could then be passed through a scanning device and sorted according to fluorescence. Several lines with this characteristic have been isolated and one of them has been successfully made homozygous.

The mosquito group has been pioneering the use of stable isotopes as markers either for sperm or as markers to identify released insects once they are trapped in the field. The aquatic environment of the larval stage provides an efficient way to incorporate the stable isotope into the mosquito. The results showed that this technique can indeed be used to identify labeled sperm in female spermathecae and can probably be used to assess competitiveness in field cage environments. The use of stable isotopes has now been expanded to Mediterranean fruit flies where it may be possible to use differences in the diet of mass reared flies, as compared to flies reared on fruit in the wild, to differentiate released flies from wild flies

The mosquito group has had major staffing changes in 2006 which has resulted in some disruption of activities.

4.1 Classical Genetic Sexing Systems Based on a Conditional Lethal

A first attempt to produce a genetic sexing system for *Anopheles arabiensis* using a classical approach has been completed with only moderate success. The conditionally lethal allele confers resistance to the insecticide dieldrin. Mosquitoes heterozygous for this allele show a level of resistance intermediate between the two homozygotes and can be easily identified using a discriminating dose of the insecticide. The initial objective of the experiment is to induce a chromosomal translocation involving the Y chromosome and chromosome 2, where the breakpoint on the autosome is as close as possible to the dieldrin resistance locus. The intended outcome is a stable inbreeding strain, where males are heterozygous and will survive the discriminating does of insecticide, whereas females are homozygous susceptible and therefore do not survive. The approach is standard, homozygous resistant males were irradiated with 60 Gy and crossed to homozygous susceptible females. The F1 males were then backcrossed to susceptible females. Eggs were collected separately from individual females and checked for semi-sterility. The semi-sterile lines were maintained and checked for a distortion in the ratio of males to females surviving the discriminating dose of dieldrin. To date three lines have been obtained that show such a distortion, but none that would provide the basis of a satisfactory genetic sexing system. This outcome was not entirely unexpected as the induction of a breakpoint at a suitable location is a matter of chance and therefore the experiment will now be repeated until successful.

4.2 Novel Genetic Sexing Based on Transgenic Sex Specific Phenotypes

A transgenic sexing strain of *An. arabiensis* has been created based on testes specific expression of enhanced green fluorescent protein (EGFP). Previously, a group at Imperial College London produced a similar transgenic sexing strain for *Anopheles gambiae* using a germline transformation vector derived from the TTAA specific transposon *piggyback* in a construct that carries EGFP under the control of the beta2-tubulin promoter. This construct is designed to express DsRed in the eyes and central nervous system of both sexes and EGFP in the male gonads. The same construct was used to produce the first ever successful germline transformation for *An. arabiensis* (**Figure 37**).

Figure 37. Fourth instar larvae showing expression of DsRed in the eyes and nervous system (top) and GFP in testes (bottom)

In initial experiments, expression of DsRed and EGFP was observed in larvae hatched from injected eggs, but it took several attempts before we were successful in establishing lines in which inheritance of both traits was observed. In total six positive larvae were selected from the first generation after two separate injection sessions. These were outcrossed to wild type females and six separate lines established. Similarities in the EGFP phenotype amongst the lines descended from the first four positive larvae suggest that these may have been from the progeny of a single female, but until molecular characterisation is complete all four lines are being maintained. The remaining two lines show marked differences in EGFP expression, so it appears that at least three transformation events were achieved. In an attempt to make the lines homozygous for the transgenes, individuals that showed no DsRed expression were removed at each generation. Despite this selection, in five of the six lines, the frequency of the wild type phenotype was always considerably higher than expected, suggesting lethality amongst transgene homozygotes. The sixth line, which shows noticeably lower levels of expression, is now considered to be homozygous.

4.3 Mating Competitiveness of Sterile Males under Laboratory Conditions

In the previous annual report we reported the findings of two pilot experiments on competitiveness of males irradiated at 70 Gy, and we stressed the importance of performing these experiments in larger cages. Larger cages were purchased and tested in a room newly equipped with temperature and humidity control for this purpose. However, the conditions in the room were sub optimal resulting in large and unpredicted mortality due to drafts in the room, and subsequently varying degree of mating success. Therefore, these experiments were discontinued and will be continued in a different room where conditions are expected to be better in 2007. In the meantime, small cage experiments in the insectary were performed, and preliminary results indicated a decent competitiveness of irradiated males. However these results will be confirmed in the larger cages in 2007.

4.4 Identification of Re-captured Sterile Males and Sterile Male Inseminated Females

4.4.1 Stable isotope labelling of semen

The potential use of stable isotopes to study mosquito mating was investigated by tracing the fate of labelled semen into spermathecae. Labelled-^{13}C glucose was incorporated in the diet of the malaria mosquito *An. arabiensis*. Treatments included labeling of either the larval water or adult sugar water, or a combination of both. After mating, "spiked" spermathecae were analysed for isotope ratios using mass-spectrometry. Results demonstrated that spermathecae positive for semen could successfully be distinguished from empty ones or controls (i.e. filled with unlabeled semen) using the raw δ ^{13}C values (**Figure 38**). The labeling during larval development and combined labeling of larvae and adults resulted in detectable values; adult labeling alone proved not to be sufficient. In addition, we found that the label persisted in spermathecae for up to 7 days after mating, and the unlabelled sugar feeding of adult males labeled in the larval stage did not result in a detectable turnover of the semen label. There were no detrimental effects of the addition of labeled glucose on larval development and survival, adult size, male longevity, and mating performance. We conclude that this technique can be used to study competitiveness of mosquitoes in laboratory and semi-field settings.

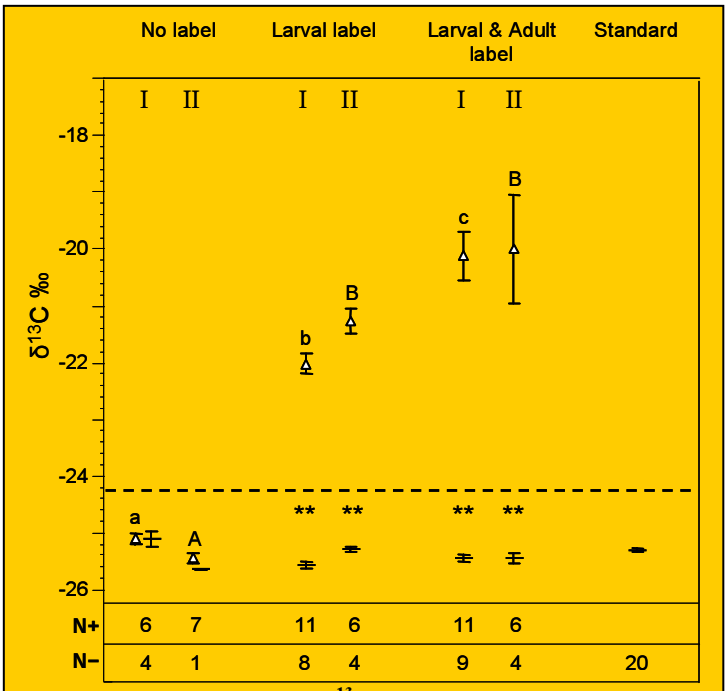


Figure 38. Mean (± s.e.m.) δ^{13}C‰ values of inseminated (open triangles) and uninseminated (bold line) spermathecae. Males were either labelled as larvae or as larvae and adult, and a control with no label was included. Dissection treatments are I: dissected the day after mating; or II: dissected 3 days after mating. N is the number of samples analysed for inseminated (+) and uninseminated (-) spermathecae. Dotted line indicates the threshold value of 3 SD above mean δ^{13}C‰ of virgin females. Standard samples are included. Means without letters in common are significantly different at p< 0.05 for I (lower case), or II (upper case). ** indicates significant difference between positive and negative spermathecae at p< 0.01.

The same principle was tested with another stable isotope; ^{15}N. Labelled-^{15}N glycine was added to the larval diet, and the transfer of ^{15}N labeled semen determined by mass spectrometry. However, the amount of nitrogen in the spermathecae was insufficient to accurately measure samples and this research was discontinued.

4.4.2 PCR identification based on dieldrin resistance using legs and spermathecae

The standard published PCR based protocol for the identification of homozygous or heterozygous dieldrin resistant *An. gambiae* and *An. arabiensis* requires DNA extraction. We have established that a simple variation of the method using legs taken from live individual mosquitoes, which are spun down directly into the PCR reaction mixture, can be developed for a high throughput screening method suitable for use in the field. A further variation can be used to identify susceptible females inseminated by dieldrin resistant males. This involves dissection of spermathecae into a small volume of lysis buffer containing proteinase-K. After a short incubation a few microlitres of the lysate is added directly to the PCR reaction. No further DNA extraction is necessary. The protocol is still being improved to establish optimum timing and volumes, but again should be suitable for use in a field laboratory.

4.5 Technology Development for Mass Rearing Mosquitoes

A number of activities are currently in progress to develop procedures and equipment for mass rearing of mosquitoes. The goal is a modular system with a fixed daily output of eggs sufficient to produce 100 000 sterile males that is as fully automated as possible, with minimal mosquito handling and built in checks on production quantity and quality. To this end various stages in the rearing process are being examined in detail and a large mass production cage has been built and evaluated.

In general, optimisation of rearing conditions involving modifications in water depth, food quantity and larval density have reduced rearing time from hatch to adult by two days. Work on artificial blood feeding was reported in the previous newsletter and studies are still in progress to compare the use of bovine and human blood. This includes an assessment of the long term impact of exclusively using one, or other, of the two blood sources.

Investigations are in progress on the scope to use larval and pupal cold tolerance to manipulate development time, to aid transportation and as part of a pupal harvesting strategy. Early results indicate that both larvae and pupae will recover from exposure to a rapid drop in temperature, to short exposures (<1 hour) to 0ºC or less and long exposures (<5 days) to 4ºC, but all markedly reduce adult emergence. Exposures to temperatures in the region of 12-14ºC appeared to have little impact on overall survival, but were low enough to aid separation of larvae and pupae, or to significantly delay development.

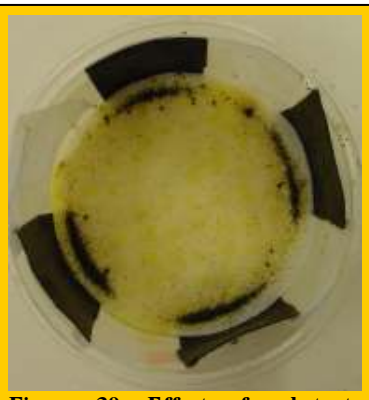


Figure 39. Effect of substrate colour on oviposition

Past observations on *An. arabiensis* oviposition behaviour indicated a stark preference for a black rather than a white substrate. This was investigated further to determine if this could be exploited for mass rearing. In preliminary experiments, two oviposition pots were introduced in each cage, one lined with black paper and the other with white; the materials were of the same undulated texture and were fitted inside the walls of the pots. To ensure that only mosquitoes in a prepared physiological state for oviposition were used for these experiments, four day old inseminated females that had not taken a previous blood meal were selected and then only

those that probed an offered human arm placed were transferred to the experimental cages. The black lining was clearly preferred (**Figure 39**). The experiment was then repeated with oviposition pots divided into sections of black and white and again the results showed that the females preferred to deposit their eggs next to the black sections. In an alternative design the black and white sections were placed on the bottom of an oviposition tray that had very low walls, but egg laying on this appeared random. We are now examining the use of a black strip held vertically above a white substrate to direct females to lay their eggs in long rows, which would aid counting and handling for mass production.

As part of efforts to develop a mass production system for malaria mosquitoes, an adult holding cage has been designed based on improvements on an initial mass production cage jointly developed with the Malaria Repository (MR4, CDC, Atlanta, GA) (**Annual Report 2005**). The cage is a self-contained unit that allows for stocking, feeding, brood maintenance, egg collection and cleaning without having to open or interfere with life activities inside the cage. The intention is to develop a modular unit with specific capacity that can be multiplied to give the required production output.

Unlike its predecessor, which has a footprint of 1 x 1 m and a height of 0.8 m with sloped sides, the second generation cage (**Figure 40**) is a rectangular box (1 x 0.8 x 0.8 m), which is more robust and allows for stacking. It incorporates all the features required for pupal introduction, sugar and blood feeding, as well as egg collection (**Figure 41**) as described in the first generation prototype (**Annual Report 2005**). Other important modifications include: the provision of sleeves on each side and at the top for easy access into the cage during sampling, cleaning and observations; the resting sites are also made of bunches of tubes made from black construction material, unlike in the first prototype where they are made of strips of similar material positioned in the form of louvers across the cage.

Figure 40. Adult rearing cage

Figure 41. Access ports to adult rearing cage

Currently the cage is being evaluated for ease of use and production capacity at the Seibersdorf Laboratory. Initial results indicate that adult mosquitoes survive well in the cage and exhibit proper mating behaviour, which are essential for mass production. Density dependence of mating in the cage has been observed and is being studied further to determine the optimum densities for production.

In order to ensure that the cage when fully developed can be used to rear different types of mosquitoes under different conditions, it is being multiplied in the Mechanical Workshop of the Seibersdorf Laboratory, for parallel evaluation in Sudan, under semi-arid conditions for *An. arabiensis*; Ghana, under tropical conditions for *An. gambiae* and French Polynesia (Tahiti), under tropical conditions for *Aedes polynesiensis*.

5. Appendices

5.1 Publications

BARNES, B.N., **A. TARGOVSKA** and **G. FRANZ** (2006). Origin of a Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), outbreak determined by DNA analysis. African Entomology 14: 205-209.

BOURTZIS, K. and **A.S. ROBINSON** (2006)**.** Insect pest control using *Wolbachia* and/or radiation. In "Insect Symbiosis Vol. 2". Eds K. Bourtzis and T.A. Miller, CRC Press, Boca Raton, Florida, USA. pp 225-246.

BRAGA SOBRINHO, R., **C. CACERES, A. ISLAM, V. WORNOAYPORN** and W. ENKERLIN (2006). Diets based on soybean protein for Mediterranean fruit fly. Pesq. Agrop. Brasileira 4: 705-708.

CHANG, C.L., R.I. VARGAS **C. CACERES,** E.B. JANG and I.K. CHO (2006). Development and assessment of liquid larval diet for *Bactrocera dorsalis* (Diptera: Tephritidae). Annals of the Entomological Society of America 99: 1191-1198.

FRANZ, G. (2006) Transgenic arthropods and the sterile insect technique. In: Status and risk assessment of the use of transgenic arthropods in plant protection. IAEA-TECDOC-1483, 37-44.

HELINSKI, M.E.H., A.G. PARKER and **B.G.J. KNOLS** (2006). Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis*. Malaria Journal 5: 41.

HELINSKI, M.E.H., B. EL-SAYED and **B.G.J. KNOLS** (2006). The Sterile Insect Technique: can established technology beat malaria? Entomologische Berichten 66: 13-20.

KNOLS, B.G.J., H. BOSSIN, G. FRANZ, A.S. ROBINSON and R. MUKABANA (2006). GM sterile mosquitoes - a cautionary note. Nature Biotechnology 24:1067-1068.

MUTIKA, G.N. and **A.G. PARKER** (2006). Induced sterility of *Glossina pallidipes* Austen males after irradiation in a nitrogen atmosphere. Entomological Science 9: 47-53.

VAN DEN BOSSCHE, P., K. AKODA, B. DJAGMAH, T. MARCOTTY, R. DE DEKEN, C. KUBI, **A. PARKER** and J. VAN DEN ABBEELE (2006). The effect of a single treatment of tsetse flies with isometamidium chloride on the fly's subsequent susceptibility to trypanosome infections. Journal of Medical Entomology 43: 564-567.

VERA, M.T., **C. CÁCERES, V. WORNOAYPORN, A. ISLAM, A.S. ROBINSON**, M.H. DE LA VEGA, J. HENDRICHS and J.P. CAYOL (2006). Mating incompatibility among populations of the South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae). Annals of the Entomological Society of America 99: 387-397.

In press and published 2007

ABD-ALLA, A., H. BOSSIN, F. COUSSERANS, **A. PARKER,** M. BERGOIN and **A.S. ROBINSON** (2007). Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. Journal of Virological Methods 139: 143-149.

BENEDICT, M.Q. and **A.S. ROBINSON** (2007). Impact of technological improvements on traditional control strategies. (In Press "Transgenesis and Management of Vector-Borne Disease" Ed S. Aksoy).

BRICEÑO, R.D., W.G. EBERHARD and **A.S. ROBINSON** (2007). Copulation behaviour of *Glossina pallidipes* (Diptera: Muscidae) outside and inside the female, with a discussion on genitalic evolution. (In press Bulletin of Entomological Research).

CÁCERES, C., D.O. MCINNIS, T.E. SHELLY, E.B. JANG, **A.S. ROBINSON** and J. HENDRICHS (2007). Quality management systems for fruit fly (Diptera: Tephritidae) sterile insect technique. Florida Entomologist 90: 1-9.

CÁCERES, C., E. RAMÍREZ, **V. WORNOAYPORN, S. ISLAM** and **S. AHMAD** (2007). Protocol for storage and long-distance shipment of Mediterranean fruit fly (Diptera: Tephritidae) eggs. I. Effect of temperature, embryo age, and storage time on survival and quality. Florida Entomologist 90: 103-109.

GARIOU-PAPALEXIOU, A., G. YANNOPOULOS, **A.S. ROBINSON** and **A. ZACHAROPOULOU** (2007). Polytene chromosome maps in four species of tsetse flies *Glossina austeni, G. pallidipes, G. morsitans morsitans* and *G. m. submorsitans* (Diptera: Glossinidae): a comparative analysis. Genetica 129: 243-251.

HELINSKI M.E.H., **R. HOOD-NOWOTNY**, L. MAYR and **B.G.J. KNOLS** (2007). Stable isotope-mass spectrometric determination of semen transfer in malaria mosquitoes. Journal of Experimental Biology 210: 1266-1274.

KNOLS, B.G.J., H.C. BOSSIN, W.R. MUKABANA and **A.S. ROBINSON** (2007). Transgenic mosquitoes and the fight against malaria: managing technology push in a turbulent GMO world. (In press American Journal of Tropical Medicine and Hygiene).

MAMÁN, E., and **C. CÁCERES** (2007). A protocol for storage and long-distance shipment of Mediterranean fruit fly (Diptera: Tephritidae) eggs. II. Assessment of the optimal temperature and substrate for male-only production. Florida Entomologist 90: 110-114.

NESTEL, D., E. NEMNY-LAVY, **S ISLAM, V. WORNOAYPORN** and **C. CÁCERES** (2007). Effects of pre-irradiation conditioning of medfly pupae (Diptera: Tephritidae): Hypoxia and quality of sterile males. Florida Entomologist 90: 80-87.

PARKER, A.G. and M. KISHOR (2007). Sterile Insect Technique: A model for dose optimization for improved sterile insect quality. Florida Entomologist 90: 88-95.

ROBINSON, A.S., **B.G.J. KNOLS,** M.Q. BENEDICT, A. BOUSSAHA, G. VOIGT, P. ANDREO, Y. TOURE and J HENDRICHS (2007). Development of the sterile insect technique for African malaria vectors. I. Conceptual framework and rationale. (submitted to The Malaria Journal).

5.2 Travel

Staff Member	Destination	Period	Purpose of Travel
Abd Alla, A.	Wu Han, China	27 Aug-1 Sept	Presentation at the IX Int. Colloquim on Invert. Path. and Microbial Control
Bossin, H.	Washington, Atlanta, Gainesville, USA	7-17 Nov	Presentation at meeting "Microbial Biopesticides and Transgenic Insects", Wash. D.C. Presentation at 55th ASTMH conf. Atlanta, GA. Pres. at USDA-CMAVE, Gainesville, FL,
	London, UK	3-4 Aug	To discuss the possibility of automated identification and efficient sorting of fluorescent transgenic male individuals from a mixed population of male and female larvae. Imperial College, London, U.K.
	Paris, France	11 Dec	To discuss with French authorities possible partnerships for Mosquito SIT on Reunion.
Caceres, C.	Bahia, Brazil	10-15 Sep	Attend 27th International Symposium on Fruit Flies of Economic Importance, and present a poster
	Crete, Greece	13-17 Nov	Visit Olive fly facility at the University of Crete to review and collect technical and scientific information on mass rearing and QC procedures, and discuss with local staff topics for future research collaboration.
	Bahia, Brazil	5-9 Sep	2nd RCM on Development of mass rearing for new world (Anastrepha) and Asian (Bactrocera) fruit fly pests in SIT. Scientific Secretary.
Franz, G.	Bangkok, Thailand	2-6 Nov	2nd RCM on Molecular technologies to improve the effectiveness of the sterile insect technique. Scientific Secretary
	Tapachula, Mexico	28-29 Nov	To supervise technical project activities (Tapachula, Chiapas (MEX)
Helinski, M.	Belfast, Northern Ireland	13-18 Aug	Attend the 5th International Conference on Applications of Stable Isotope Techniques to Ecological Studies
	Thessaloniki, Greece	10-14 April	Attend and present a poster at the SOVE meeting in Serres.
Kabore, I.	Dundee, Scotland	8-10 May	Training in the use of the UV blood decontamination equipment
Malcolm, C.A.	Paris, France	11 Dec	To discuss with French authorities possible partnerships for Mosquito SIT on Reunion.

Parker, A.	Bratislava, Slovakia	6 Sep	To consult with the contract holder regarding the production and shipping of *Glossina pallidipes* and *G. fuscipes* to Ethiopia.
	Addis Ababa	16-20 Oct	Follow up technical review mission to assist the c/p with rearing in module 1&2 at the Kaliti facility including assistance with resolving any problems with running or equipment of the modules.
	Addis Ababa	27 Feb-3 March	Further follow up technical review mission to ensure that modules 1 and 2 of Kaliti mass rearing facility is ready to receive pupae shipments from Seibersdorf and that equipment is correctly installed and functional;
	Bratislava, Slovakia	28 March	To accompany the counterparts from Ethiopia on a technical visit to the tsetse rearing facility at the Institute of Zoology, Slovak Academy of Sciences.
Robinson, A.S.	Izmir, Turkey	17-22 Sept	Attend the European Congress of Entomology and give an invited presentation.
	Raleigh, NC, USA	6-8 June	Deliver the keynote address and participate in workshop sessions at APHIS/ARS Fruit Fly Research & Technology Development Workshop,
	Tapachula, Chiapas, Mexico	4-5 Sept	Review and conduct the final evaluation of the *Anastrepha ludens* genetic sexing project.
	Tuxtla Gtz, Mexico	7-8 Sept	Invited presentation at an International Screwworm Seminar
	Canberra, Australia	11-15 Dec	4th RCM on Enabling technologies for the expansion of the SIT for Old and New World Screwworm. Scientific Secretary
	Gainesville, Florida, USA	15-18 May	Lecture at Interregional Training Course on the Use of the Sterile Insect and Related Techniques for the Integrated Area-wide Management of Insect Pests
	Montpelier, France	1-3 Feb	To lecture and demonstrate at the Leverhulme Trust Tsetse Research Network,
	Penticton, BC, Canada	13-17 March	Invited participant in a NAPPO meeting regarding the development of an IPPC standard for transgenic insects
	Arlington, VA, USA	23-27 Oct	Invited member of EPA Scientific Advisory Panel
Thailayil, J.	London, UK	3-4 Aug	To exchange practical experience on microinjection techniques of mosquito embryos. Discuss aspects of transgenic strain establishment and characterization, and transgene stabilization.

5.3 Fellows

	Name	Fellowship Code	Month/Days	From	To
1	RADONJIC, Ms. S.	SCG/04011	3/00	2005-10-01	2006-03-31
2	EL-KHOLY, Ms. E.M.S.	EGY/01042P	3/00	2005-10-03	2006-04-02
3	KIGODA, Ms. K.J.	URT/04013	3/00	2005-10-06	2006-04-05
4	KEMBOI, Mr. S.K.	KEN/04011	4/00	2005-11-01	2006-04-30
5	GERBU, Mr. M.H.	ETH/004012	4/00	2006-02-19	2006-06-19
5	GERBU, Mr. M.H.	ETH/004012	1/00	2006-06-20	2006-07-19
6	KAPANGE, Mr. A.	URT/04010	6/00	2006-03-31	2006-09-30
7	MUSIE, Mr. K.	ETH/06004	8/00	2006-04-05	2007-04-04
8	BRAVO SANZANA, Mr J.A.	CHI/06010	1/00	2006-06-01	2006-06-30
9	VAN HANSEN, Ms. E.	SAF/06015	3/00	2006-06-01	2006-08-31
10	FORTUIN, Mr. S.	SAF/06014	3/00	2006-06-01	2006-08-31
11	M'SAAD, Ms. M.G.	TUN/06017	1/00	2006-06-12	2006-07-11
12	ARNOLDS, Mr. L.E.	SAF/06013	5/20	2006-07-03	2006-12-22
13	KYELA, Mr. M.S.	URT/06026	3/00	2006-09-25	2007-03-24
14	OSAE, Mr. M.	GHA/06011	2/15	2006-10-15	2007-04-15
15	GARCIA MARTINEZ, Mr. V.	MEX/06005	1/00	2006-11-01	2006-11-31
			52/05		
Scientific Visitors					
1	DUGASSE, Ms. D.	SEY/06003V	0/05	2006-12-04	2006-12-08
			0/05		

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