# CONSULTANTS' GROUP MEETING

*on*

# TSETSE GENETICS IN RELATION TO TSETSE/TRYPANOSOMIASIS CONTROL/ERADICATION

*12 - 16 October 1992*

*Vienna, Austria*

## CONTENTS

## INTRODUCTION

The FAO and IAEA have long recognized the need for methods for insect and pest control based upon approaches other than simply the widespread use of insecticides. Through the past several years the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has expended a considerable amount of effort in the development of a SIT programme applicable to tsetse. Such programmes have proved to be a highly successful component of the integrated control of tsetse flies. However, the pilot programmes undertaken to date have been applied to areas of limited size and future integrated control programmes for tsetse must cover much larger regions. The Consultants' Group (see Annex 1) was cognisant of the continued need for improvements in the cost effectiveness in the mass production of tsetse, particularly for SIT programmes. The Consultants' Group recognized also that FAO/IAEA plays an important leadership role in the development of new technologies for the control of insect pest populations, and in the transfer of such technologies to assist in the improvement of agricultural production, particularly in developing countries.

In addition to research on the development of methods for insect control (emphasizing application of the Sterile Insect Technique), the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has established and implemented five "Co-ordinated Research Programmes" on tsetse and has, from time to time, convened groups of consultants to discuss and make recommendations on specific subjects. At least two such meetings (in July 1975 and November 1987) focused on genetic methods of insect control. In section IV B (2) of the 1987 report, it was noted that "information currently available on tsetse genetics is still too incomplete to postulate ways of making use of genetic methods other than SIT to facilitate control and/or eradication of the tsetse fly and/or trypanosomes transmitted by the fly." The recent, rapid developments in molecular biology have stimulated interest in the application of genetic techniques to the problem of tsetse and trypanosomiasis control in Africa, and a Consultants' Group was convened in Vienna, from 12 to 16 October 1992 and charged with the following responsibilities:

a) Discuss, review and advise on how genetic R&D may be useful for and in managing the tsetse/trypanosomiasis problem.

b) Identify broad areas of research and development that are most likely to be helpful in supporting current and future activities against tsetse in Africa.

c) Recommend specific research thrusts to be addressed by a Co-ordinated Research Programme on tsetse genetics planned for 1993-98.

d) Write a report that summarizes the above discussions and that provides a set of conclusions and implementable recommendations.

The Consultants' Group reviewed the current information on tsetse genetics and relevant aspects of molecular biology (Annex 2) and identified several areas of research which the Consultants' Group felt would contribute to a solution to the tsetse/trypanosomiasis problem in Africa. The Group felt that the research recommended would support existing SIT programmes whilst also providing direction for the incorporation of newly developed genetic and molecular technologies into future programmes for tsetse control employing techniques based upon ionizing radiation.

## SPECIFIC AREAS FOR FUTURE RESEARCH

### 1 Mapping of genomes

This research will provide knowledge or systems aimed at improving the efficiency of the SIT, including genetics of colonization, hybrid sterility, improved monitoring methods, genetic sexing and other genetic manipulations. Given the present state of knowledge of tsetse genetics we advise that work be continued on *Glossina m. morsitans* while some of the longer term research projects be initiated on a riverine species (*G. p. palpalis*, *G. p. gambiensis* or *G. tachinoides*).

***Recommendations***

1.1 Undertake inbreeding to isolate visible autosomal recessive mutations (see 1.2) and to establish recombinant inbred (RI) strains to be used as standards for molecular genetic maps. If technically feasible, both colonization and inbreeding will begin with field-collected flies in order to maximize genetic variability. (We suggest studying *G. p. gambiensis* at the Bobo Dioulasso laboratory and *G. m. morsitans* at the University of Alberta, Howard University, and other appropriate laboratories.)

1.2 Mutagenesis (using both irradiation and chemicals such as EMS) and Mendelian breeding programmes should be established to isolate and map dominant and recessive genetic mutations. We suggest the use of vacuum treatment to apply chemical mutagens to tsetse (protocol available from G. Foster, see Annex 1). (We suggest using *G. m. morsitans* at the University of Alberta and other appropriate laboratories.)

1.3 Development of molecular methods of detecting genetic variation, and preparation of maps. (We suggest studies on *G. m. morsitans* at the University of Alberta, the University of Saskatchewan, Howard University, and other appropriate laboratories.)

1.4 Development of standardized photographic polytene chromosome maps and correlation with molecular and conventional genetic maps. (We suggest engaging an established cytogeneticist followed by a training course to diseminate the techniques developed.)

## 2 Development of molecular monitoring tools for SIT and other control operations

This research would aim to improve molecular methods of recognizing existing and as yet undetected taxa, and to identify genetic differences between geographically separated populations within taxa. We stress at this point that these techniques are currently available but will need to be adapted for application to tsetse. These methods could be used to type target and nearby populations before and during control programmes. In the event of loss of population control, these tools and information could be used to determine the origin of the problem (e.g. immigration *vs* rearing or release procedures). The goal should be to develop these tools for use in laboratories in Africa. (The Group suggests techniques be developed at the University of Saskatchewan and the International Centre for Insect Physiology and Ecology [ICIPE] in collaboration with the Institute of Molecular Biology and Biotechnology [IMBB].)

***Recommendations***

2.1 Molecular techniques be utilized immediately to elucidate the phylogenetic relationships between *Glossina* species.

2.2 Immediate use be made of DNA fingerprinting probes to monitor changes occurring during laboratory colonization and for determining the genetic variability in mass reared populations for SIT control.

## 3 The application of transgenic technology to tsetse fly

Transgenic technology may provide the opportunity to manipulate directly the genome of tsetse flies so as to create strains with desirable characteristics for existing SIT programmes as well as for novel future control strategies. The anticipated advantages of this approach include (a) the ability to carry out genome manipulations without the concurrent genome disruption normally associated with a genetic cross, and (b) an increased ability to manipulate genes across species barriers. Initially there is a need to develop the technology necessary to carry out direct genetic manipulations of the tsetse genome. For example, nothing has been published on the presence of transposable genetic elements in tsetse. Such mobile elements could be exploited to form the core of a DNA vector system for use in tsetse. In addition, the possibility of utilizing Rickettsia-like organisms (RLOs) as transformation vectors should be further investigated. As this technology becomes available it should be incorporated into SIT programmes to generate novel strains - for example for genetic sexing. (We suggest that techniques be developed at ICIPE, and at the Universities of Liverpool, Saskatchewan and Yale.)

Concurrent with the above studies, research should be orientated towards the molecular analysis of genes and genetic loci which may have potential for future manipulations in relation to tsetse control or the ability of these flies to transmit trypanosomiasis. (We suggest studies be done at the Universities of Bristol and Yale.)

In parallel, research is necessary to investigate the methodologies which would provide the means of driving desirable gene constructs through tsetse populations by the use of cytoplasmic incompatibility (e.g. utilizing *Wolbachia*). If this is achieved, transgenic technology could allow desirable gene constructs to be introduced into target strains and species of tsetse fly and hence through target populations without the need for mass rearing and release. (We suggest such studies be carried out at Yale.)

***Recommendations***

3.1 Immediate efforts should be directed towards developing the methodologies necessary to undertake direct genetic manipulation of the tsetse genome. This should include an investigation of methods to introduce DNA into the tsetse germline and into tsetse symbionts, and an investigation of potential DNA vectors (such as the identification and molecular characterization of putative transposable elements in the tsetse genome and RLO constructs) which may be employed in the tsetse system. Such vectors will necessarily have incorporated in them selectable markers and the necessary stage and tissue specific promoters to control expression of desirable genes. Hence, further research should be directed towards the molecular cloning and analysis of phenotypic markers and promoters for incorporation into a tsetse DNA transformation vector.

3.2 Research should be directed to the molecular characterization of genes which have potential for future manipulation within tsetse flies and their symbionts. The genes of particular interest include tsetse genomic loci involved in trypanosome susceptibility and refractory mechanisms, insecticide resistance genes, behavioural genes and foreign genes producing trypanocidal products.

## 4 Development of genetic sexing methods

This research would be aimed at improving the separation of males and females at the puparial stage during mass rearing, with minimal deleterious effects on colony fertility. Among the major potential benefits of success would be: reduction of labour in mass rearing, elimination of damage to flies caused by current sex-sorting procedures, doubling of the numbers of males available for release, and the use of excess females for other purposes (e.g. sterile virgin female ecological monitoring [SVFEM], colony maintenance or expansion). (We suggest studies on *G. p. gambiensis* at the Bobo laboratories and *G. p. palpalis*, and possibly *G. tachinoides*, at Seibersdorf.)

***Recommendations***

4.1 Use traditional genetic and cytogenetic methods to construct highly fertile and stable genetic sexing strains. This will depend upon progress in Recommendations 1.1 and 1.4 above.

4.2 Use molecular methods either as an alternative to, or in conjunction with, traditional methods, if mutations or methods become available. This depends upon progress in Recommendations 1.3, 1.4, 3.1 and 3.2 above.

## 5 Integration of ecological models of tsetse populations and genetic control models as a decision and research tool

These models would enable assessment of individual or combined elements of integrated control programmes including genetic and non-genetic approaches. Such models have many uses, including identification of research priorities, design and evaluation of modifications to SIT (e.g. reducing radiation dose to increase male competitiveness), alternative genetic options, analysis of trial data, and evaluation of the consequences of genetic responses of populations to control measures (e.g. development of pesticide resistance, changes in symbiont/parasite/host relationships, behavioural changes). (The Zimbabwe Department of Veterinary Science is suggested as a source of such expertise.)

***Recommendations***

5.1 Inclusion of a specialist in tsetse population dynamics in the proposed CRP (see Annex 3).

5.2 Development of a model with regular updating as appropriate to allow inclusion of new data/concepts.

## 6 Investigate the possibility of using males from one taxon as agents against several closely related taxa

The ability of certain tsetse species to participate in inter-taxon mating, under both laboratory and field conditions, raises the possibilities that a) release of sterile males of one species could be used to control more than one species, and b) genetic mutations developed in one species could be transferred to other species.

***Recommendations***

6.1 Investigation of the inter-taxon mating propensities in *G. p. palpalis*, *G. p. gambiensis* and *G. f. fuscipes*, using available methods. (We suggest that these studies be carried out at Seibersdorf.)

6.2 Investigate, under laboratory conditions, the effectiveness of sterile males from one taxon in sterilizing females from other taxa.

6.3 Investigate the effectiveness of this system under field conditions.

## 7 Research and development of improved storage methods for tsetse

Research on the stockpiling of flies for release would benefit rearing for SIT when tactics or other factors dictate interrupted release programmes. Stockpiling would decrease the costs of production by reducing the need to maintain a large colony. Also, success in this area could lead to improvement of long-distance transport and release methods. (We suggest that Seibersdorf is an appropriate laboratory for these studies.)

*Recommendations*

7.1 Research on the acclimation of wandering stage larvae, or of puparia, to improve puparial survival in cold conditions.

7.2 At various temperatures, determine optimal stages during puparial development for sub-ambient temperature storage.

7.3 Selection of lines with more cold-tolerance and/or prolonged puparial development times (without loss of fitness).

7.4 If necessary, a search should be initiated for cold-tolerant genotypes in natural populations and the incorporation of these genes into SIT colonies.

## 8 Research on RLO/tsetse relationships

Research in this area would provide a better understanding of the susceptibility of tsetse flies to trypanosome infection, and could lead to methods to increase refractoriness. (Yale and Bristol are suggested as appropriate locations for such work.)

*Recommendations*

8.1 Investigate causes of the increases in RLO frequencies in colonies and methods of minimizing this increase.

8.2 Investigate genetic interrelationships between tsetse species & RLOs.

8.3 Research on methods for introducing into tsetse flies modified RLO constructs which have desirable properties such as antitrypanosome activity, or which promote refractoriness.

## RESEARCH PRIORITIES

*High Priority Research areas*

It is recommended that:

- in support of the existing SIT programmes, research be initiated for the development of a genetic sexing system. Progress in this area will be facilitated by the next two recommendations.

- mapping the tsetse genome be expanded using classical and molecular approaches. As a specific objective, it is recommended that priority be given to identifying and engaging a cytogeneticist to develop cytogenetic techniques for use in the tsetse system. Subsequently, this expertise should be communicated to a larger number of individuals *via* a focused training course.

- molecular approaches be immediately utilized to elucidate the phylogenetic relationships between *Glossina* species. In addition, use should be made of molecular techniques such as DNA fingerprinting to monitor changes in the tsetse genome occurring during laboratory colonization and in mass reared populations during SIT programmes.

***Medium priority research areas***

It is recommended that:

- the application of transgenic technology, in relation to tsetse control, be fully explored. This should include the development of the technology for the incorporation of foreign DNA into the tsetse genome.

- molecular techniques should be fully explored to extend our understanding of the involvement of RLOs in vectorial capacity.

***Other areas for research***

It is recommended that:

- the proposed CRP (see Annex 3) include expertise in the area of modelling and that integrative control models, as a decision and research tool, be developed.

- an investigation be initiated into the possibility of using males from one of the following taxa, *G. p. palpalis*, *G. p. gambiensis* and *G. f. fuscipes*, as agents for the control of all three taxa in a SIT programme.

- research be undertaken into the development of methods for the storage of tsetse puparia.

## CONCLUDING REMARKS

The recommendations outlined above are envisaged as an integrated approach to the use of nuclear and of genetic techniques for current and future tsetse control. It should be emphasized that an important component of the proposed research is the training of staff, from contract holder institutions, in the relevant techniques through the Agency's Fellowship Programme. The research is also envisaged as increasing the medium- to long-term cost effectiveness of tsetse SIT programmes.

The research recommended requires the diverse expertise and facilities which currently exist in various institutions. To facilitate co-ordination of the research recommended in this report, the Consultants' Group suggests that the Joint FAO/IAEA Division establish a Co-ordinated Research Programme (see Annex 3).

Annex 1

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Annex 2

**Working Papers**

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| Dr. G. Foster: | "GENETIC APPROACHES TO MANAGING INSECT PEST POPULATIONS WITH REFLECTIONS ON THE TSETSE/TRYPANOSOMIASIS PROBLEM" |
| Dr. R. Gooding: | "RECOMMENDED GENETIC STUDIES ON TSETSE SPECIES IN RELATION TO THE CONTROL OF ANIMAL TRYPANOSOMIASIS" |
| Dr. A. Blanchetot: | "RESEARCH ON THE MOLECULAR BIOLOGY OF TSETSE: OPPORTUNITIES AND RELEVANT APPROACHES" |
| Dr. J.M. Crampton: | "GENETIC ENGINEERING OF INSECCTS AND APPLICATIONS IN BASIC AND APPLIED ENTOMOLOGY" |
| Dr. I. Maudlin: | "INHERITANCE OF REFRACTORINESS TO TRYPANOSOME INFECTION IN TSETSE" |
| Dr. A. Robinson: | "DEVELOPMENT AND USE OF DNA PROBES FOR MAPPING INSECT GENOMES" |
| Dr. R.A. Leopold: | "APPROACHES TO THE STOCKPILING OF INSECTS USING LOW TEMPERATURE STORAGE TECHNIQUES" |

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| Dr. S. Aksoy: | "GENETIC TRANSFORMATION AND EXPRESSION OF FOREIGN GENES IN THE NATURALLY OCCURRING SYMBIONTS OF TSETSE FLIES" |

Annex 3

**Proposed**
**Co-Ordinated Research Programme**

**TITLE:** Development and Application of Genetic Methodology for Tsetse and Trypanosomiasis Control or Eradication.

**OBJECTIVES:**

1 To develop genetic and molecular approaches to be used to aid in separation of male and female tsetse flies.

2 To conduct a genetic and molecular analysis of the tsetse genome.

3 To determine the genetic factors involved in vector competence for the transmission of trypanosomes.

4 To develop recombinant/transgenic tsetse.