1. CRP Number and Title

Genetics Application to Improve the Sterile Insect Technique for Tsetse Control/Eradication including Population Genetics

2. Section/Division: IPC/NAFA

3. Project Officer: Udo Feldmann and Alan Robinson

4. Period Covered: 1997-2003

5. List of Participants

Name (CSI)	Institute/Country
Mr. Elsen, Pierre	IMT/BEL
Mr. Solano, Philippe	Pierre Richet/IVC
Mr. Solano, Philippe	CIRDES/BKF
Mr. Kinyua, Johnson	KETRI/KEN
Mr. Aksoy, Serap	Yale Univ./ USA
Mr. Krafsur, Elliot	Iowa State Univ./USA
Mr. Perera, O.P.	USDA/USA
Mr. Gooding, Ronald	Alberta Univ/ CAN
Mr. Yannopoulos, George	Patras Univ./GRE
Ms. Torti, Cristina	Pavia Univ/ITA

Research Co-ordination Meetings

Date	Location
10-14 February 1997	Addis Ababa, Ethiopia
03-07 October 1999	Mombassa, Kenya
19-23 March 2001	Rome, Italy
23-27 June 2003	Edmonton, Canada

6. Objectives of CRP

a) *Overall:* To improve the planning, implementation and evaluation of tsetse SIT programmes by the development of appropriate genetic tools.

b) Specific:

- To determine the isolation status of tsetse populations by the use of genetic analysis.
- To develop strains of tsetse that are refractory to infection by trypanosomes.
- To initiate a physical and genetic map for tsetse.

7. Outputs

RESEARCH:

Knowledge of tsetse fly genetics has increased by orders of magnitude in the past five years. Here we identify the principle developments.

Many new genetic markers have been made available

We have developed a suite of molecular genetic markers that include allozyme, mitochondrial, and microsatellite loci. These markers have been used for basic and applied studies of tsetse genetics, including linkage maps, studies of interspecific hybridization, and population genetics. These are discussed in more detail below.

Genetic analysis, linkage maps and cytogenetic maps have been established

Polytene chromosome maps have been developed in *morsitans* group tsetse and *G. austeni*. These maps provide for the first time in tsetse powerful tools for a wide range of cytogenetic analyses including the precise localization of cloned genes, mapping insertions in transgenic flies, population genetics, and evolutionary relationships. Moreover, polytene chromosome analysis may assist in the design of stable genetic sexing systems, as has been accomplished in the medfly.

Tsetse fly symbionts and parasites have been identified and characterized at the molecular level

Three organisms, *Wolbachia, Sodalis*, and *Wigglesworthia* have important effects on tsetse. These organisms offer opportunity for genetically engineering tsetse flies with desirable properties. *Wolbachia* may serve to drive advantageous genes into natural populations.

cDNA libraries have been developed and tsetse-trypanosome interactions studied at the molecular level

Genes coding for anti-parasitic substances have been identified. The usefulness of anti-parasitic substances is that natural tsetse symbionts can, in principle, be engineered to express substances that would prevent the successful development of trypanosomes in tsetse flies. Two applications of the technology are apparent: release of sterile males that are refractory to trypanosome infection, and development of refractory release strains that could replace natural vector strains of the same species.

Population genetics and gene flow measures have been established in some economically and medically important tsetse flies

The breeding structure of some *palpalis* group and *morsitans* group populations have been characterized in terms of gene flow. All genetic evidence indicates surprisingly restricted gene flow among natural populations. The relevance of low gene flow to the

SIT is that the return of tsetse flies to regions where they have been eliminated is likely to be a slow process.

The genetics of speciation and studies on the genetics of sex determination have been initiated

Heritable factors on the X chromosome have been found that help explain speciation in the *morsitans* group, and other X chromosome factors account for biased sex ratios (causing excess numbers of females) in *G. m. submorsitans*. Such biased sex ratios in breeding stocks used for SIT are adverse. In principle, however, research may allow the development of breeding stocks in which male-biased sex ratios occur.

Hybridization studies have confirmed the major cause of hybrid male sterility is incompatibility of sex chromosomes from different taxa

Such work indicates that hybridization asymmetry [i.e., markedly different success in reciprocal crosses] is due to chromosomal rather than maternally inherited factors such as *Wolbachia*. The results emphasize the need for experimental work to establish whether *Wolbachia*-based asymmetry occurs within individual tsetse taxa (i.e. species or subspecies).

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ADDITIONAL RESEARCH OUTPUTS

- *Monoclonal antibodies have been developed against major tsetse gut proteins.* This provides an additional basis for understanding tsetse-trypanosome interactions and provides putative targets for disrupting these interactions. Flies bred for release could, in principle, be designed with multiple mechanisms that confer resistance to trypanosome infection, thereby reducing greatly the chances of finding a natural trypanosome population that can infect 'resistant' tsetse flies

- *Studies have begun of the ecological physiology of wild tsetse flies and genetic adaptation.* In particular, cross correlations with breeding structure, resistance to desiccation, and temperature tolerances are under investigation

- *EST libraries have been developed and are of use in tsetse genomics.* These will enable many interactions between the tsetse and its symbionts to be studied in great detail at the molecular level. They can also be used as probes for hybridization to polytene chromosomes to help develop a physical map for tsetse.

OTHERS

- Enhanced collaboration among and between African, European, and North American laboratories

- Capacity building in East and west Africa

- Fellowship support

- Focused international attention on the contribution of genetics to efficacious area-wide tsetse/trypanosomosis control

- Recruitment of new workers into the tsetse fly community

8. Effectiveness of the CRP

a) In reaching specific objectives

(i) In reaching Specific Objective 1: *To determine the isolation status of tsetse populations by the use of genetic analysis.*

Studies on *morsitans* group and *palpalis* group tsetse flies show unanticipated levels of genetic differentiation. Genetic drift is the likely operating mechanism, but adaptation cannot be ruled out. Current and planned research is investigating the likelihood of local adaptations in maintaining the high levels of genetic differentiation.

ii) In reaching Specific Objective 2: *To develop strains of tsetse that are refractory to infection by trypanosomes.*
Much basic research has been accomplished and promising results have been obtained in engineering the secondary symbiont *Sodalis* to express anti-trypanosomal substances thus making released flies inherently refractory. An additional number of antibiotic candidate substances have been identified. We must plan for field evaluation of these promising developments.

iii) In reaching Specific Objective 3: *To initiate a physical and genetic map for tsetse.*
State of the art cytogenetic [i.e. physical] maps have been constructed for *G. m. submorsitans, G. m. morsitans, G. pallidipes*, and *G. austeni*. Genetic maps have been developed for *G. m. submorsitans, G. m. morsitans*, and *G. p. palpalis*. New biochemical and molecular markers have been incorporated into these linkage maps and additional work is anticipated.

b) In contributing towards Overall (i.e. Agency project) Objective: *To improve the planning, implementation and evaluation of tsetse SIT programmes by the development of appropriate genetic tools.*
Genetic diversities of colonized tsetse indicate conservation of genetic variation, testifying to the careful husbandry required to maintain thriving tsetse cultures. The question of genetic compatibility of released, sterile mass reared flies and their wild conspecifics has not been investigated directly, but genetic studies of natural populations show high levels of population structure. Although there is no *a priori* reason why genetically differentiated populations should predict a degree of mating incompatibility between released and wild flies, the possibility should be investigated thoroughly.
Cryptic species may occur within the *palpalis* group. Evidence for this comes from population genetics of natural *G. p. gambiensis* populations and from breeding experiments on *G. p. palpalis* cultures that originated from different regions.
The development of new microsatellite genetic markers for *morsitans* and *palpalis* group tsetse allow, for the first time, the unambiguous genotyping of natural populations, in addition to monitoring genetic drift [i.e., random changes] in cultured breeding stocks.
Techniques necessary to engineer refractory tsetse flies have been developed via genetic engineering of the secondary symbiont, *Sodalis glossinidius*.

c) Factors, if any, which adversely affected the effectiveness of the CRP

i) Appropriateness of the CRP
The CRP is deemed to be highly appropriate.

ii) Formulation of the CRP
No adverse factors were identified. The CRP effectively facilitated coordinated research.

iii) Management problems during implementation of the CRP

a. Core facilities to support collection and dissemination of field samples were not successfully established.

b. Timing of CRP meetings was sometimes awkward for participants in universities.

c. Above all, no provision for project continuity exists but there is a compelling need for long term collaborative research.

iv) Intellectual, financial and other inputs from participants

a. Participants had great difficulties in obtaining support to perform needed research. The objectives of this CRP did not usually correspond with the priorities of granting agencies to which CRP participants apply. Nevertheless, after many years of writing proposals, two CRP members have succeeded in obtaining U.S. NIH support.

b. There is a continuing loss of workers and, therefore, a significantly reduced number of tsetse experts. For example, three members of this CRP have retired. Another essential worker has had to change careers and now teaches secondary school biology.

9. Impact of the CRP

Three major impacts can be identified: (a) The development of genetic tools for studying tsetse flies; (b) the development of tsetse as a model system for studies on insect immunity, and (c) the finding that tsetse populations show an unexpectedly high degree of genetic differentiation. All impacts are noteworthy from a purely scientific point of view, and each has profound consequences for the successful application of the SIT.

10. Relevance of the CRP

The CRP succeeded in focusing attention of investigators on the practicalities of instituting an operation SIT programme. The CRP fortuitously corresponded with the PAATEC initiative.

11. Recommended future action by the Agency

Training

The establishment of reciprocal fellowships between African and European/North American institutions could greatly facilitate interest in the tsetse/trypanosomiasis problem in addition to furthering institutional capacities and scientific progress.

We also recommend establishing an annual or biennial training course. The target groups would include technologists and managers. The syllabus would cover both theory and practice:

A. Field and laboratory practice

- Geographic positioning systems, geographic information systems, polymerase chain reaction, agarose and acrylamide electrophoresis, and DNA sequencing.
- Essential equipment necessary to accomplish the foregoing.
- Exercises in genetic statistics by using current software

B. Elementary theory and principle applications

- Genetic analysis theory:
- Genetic analysis in practice: trypanosome genetics and tsetse genetics
- Representative sampling and analytic procedures
- Tsetse pathogens and symbionts: biology and applications
- Theory of the SIT and chief genetic enhancements
- Ecological physiology: tsetse temperature and desiccation tolerances
- Physical basis of survival in hot, terrestrial environments

Capacity building: regional centres and core facilities

Two or three regional centers are required in Africa. Study of trypanosome transmission by tsetse is a huge constraint to furthering knowledge of trypanosomosis epidemiology. Clearly, maintaining tsetse cultures is impossible for most research laboratories. Field sampling both vector species and the trypanosomes they transmit is expensive, difficult, and time consuming when attempted by individuals from European and North American laboratories. Large scale molecular surveys of trypanosome and tsetse populations are desperately needed and, in principle, would benefit greatly from economies of scale afforded by commercial technology companies who use delicate, advanced instrumentation unaffordable to the vast majority of laboratories. Moreover, the maintenance requirements of such technology are extreme and very costly. We suggest that African laboratories (e.g., KETRI, CIRDES) maintain tsetse cultures and trypanosome stocks, sample tsetse and trypanosomes and accomplish essential ecological research. High tech applications would depend on extramural support with the collaboration of investigators from 'cutting edge' laboratories. Centrally located biological stocks would greatly advance research by allowing laboratories to rely on their particular strengths and advantages while reducing costs and sharing effort and opportunity over a larger array of investigators.

Scientific opportunities

Tsetse flies, their symbionts and parasites are among the most interesting and unusual organisms known. We have learned a great deal about them in the past five years, and achieved a much deeper understanding of tsetse biology and vector-parasite relationships.

Newly available recombinant DNA technologies allow the investigation of yet deeper questions relevant to the control and eradication of African trypanosomosis. Rapidly advancing developments in fundamental science support a search for new application methodologies. Thus control methods, which were until recently only hypothetical, are becoming increasingly practical to develop and evaluate in the field. But tsetse flies and their associated microflora are inaccessible to the vast majority of scientists. This is why both European/North American and African institutions need to be involved, working much closer together than before.
In particular, we now can produce expressed sequence tags, search for single nucleotide polymorphisms and build bacterial artificial chromosomal libraries of tsetse genomes and we can 'engineer' tsetse symbionts to express antiparasitic substances. Physical mapping of genes can be done expeditiously where advanced laboratories have easy access to thriving tsetse fly cultures. Sequencing large regions of the tsetse genome is a distinct possibility. All of the foregoing have important applications for functional genomics, proteomics, and exhaustive bioinformatic analyses.

Communication

Tsetse and African trypanosome workers are few and geographically scattered. There is no venue for periodic meetings, and little support for international travel. The research environment is far richer for mosquito-borne pathogens and the current epidemics of sleeping sickness get incredibly little attention from health or agricultural scientists. Formal linkages need to be established and supported. These could include a well maintained web page and access to a tsetse-trypanosome database. There is PAAT and TTIQ, but these are poorly maintained and no longer current, mostly displaying organizational logos and advertising their relevance. Perhaps an international agency could support financially a website maintained by an interested scientist.
Earlier RCMs, sponsored by the IAEA, have been markedly successful in bringing research workers together, catalyzing needed research, and encouraging technology transfer. But more is needed – 5 year research and outreach projects are, quite simply, too short.

Movement of biological material

Shipping biological materials, such as DNA, ethanol-preserved tsetse flies and cryopreserved materials, is becoming extremely difficult. The application of laws to protect wildlife and endangered species has delayed movement of tsetse material. Laws governing genetically modified organisms may prohibit field trials of transgenic, sterile tsetse flies. If present trends continue, it will soon be quite impossible to carry out research that requires any form of transport of biological specimens.
We therefore recommend the agency keep scientific collaborators and applicators appraised of rules and laws regarding GM organisms. Scientists must be aware of current rules and regulations: can IAEA help in this? Can IAEA provide good offices in encouraging member states to lessen the red tape that increasingly interferes with needed medical and agricultural research?

A new CRP is required

For all the foregoing reasons, a new CRP would greatly encourage and support progress in the control and eradication of African trypanosomosis. It would do so by providing research and outreach focus on obtainable goals, disseminating fundamental scientific knowledge, new scientific applications, and, above all, encourage technology transfer from 'rich' countries to sub-Saharan Africa.