CRP Final Evaluation Report

1. Title:
Improveing SIT for Tsetse Flies through Research on their Symbionts and Pathogens

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NAFA

3. Project Officer:
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5. Participating Countries:

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<th>Country</th>
<th>Institute</th>
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<td>AUS</td>
<td>Laboratories of Genome Dynamics</td>
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<td>BEL</td>
<td>Prince Leopold Institute of Tropical Medicine</td>
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<td>BKF</td>
<td>Ministère des ressources animales</td>
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<td>CAN</td>
<td>Laboratory for Molecular Virology</td>
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<td>CMR</td>
<td>Université de Yaounde I</td>
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<td>CPR</td>
<td>Wuhan Institute of Virology</td>
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<td>FRA</td>
<td>Institut de recherche pour le développement (IRD)</td>
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<td>FRA</td>
<td>Université Montpellier II</td>
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<td>FRA</td>
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<td>GFR</td>
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<td>Julius Kühn-Institut</td>
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<td>GHA</td>
<td>Biotechnology and Nuclear Agriculture Research Institute</td>
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<td>GRE</td>
<td>Department of Environmental and Natural Resources Management</td>
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<td>ITA</td>
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<td>KEN</td>
<td>Trypanosomiasis Research Institute</td>
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<td>International Centre of Insect Physiology and Ecology (ICIPE)</td>
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<td>URT</td>
<td>Tsetse and Trypanosomiasis Research Institute (TTRI)</td>
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6. CRP Overall Objective:
The overall objective of the CRP was to understand and exploit interactions between tsetse flies and their microbes to enhance the efficacy of tsetse SIT programs.

Specific Objectives of the CRP
- To clarify tsetse, symbiont and other microbe interactions
- To better understand and manage tsetse - virus interactions in laboratory populations
To manipulate microbial flora to express parasite refractoriness traits
To harness symbiont mediated natural mating incompatibilities
To improve tsetse suppression technologies
To disseminate knowledge among disease endemic country researchers to improve field application of SIT through better decision making and capacity building.

Contribution towards the Agency project:

The CRP was implemented in Project 2.1.4.3 (Strengthening expertise and capacities to integrate SIT in area-wide integrated pest management (AW-IPM) approaches against selected tsetse and screwworm populations) as part of Sub-programme 2.1 (Sustainable Control of Major Insect Pests). The results and information provided by this CRP were directed to contribute to improving SIT technology through a better understanding of the role that microbes play in the laboratory and field biology of tsetse.

7. Specific Research Objective(s):

1. Decipher host-symbiont interactions to understand tsetse’s nutritional ecology and improve mass rearing procedures,
2. Investigate tsetse pathogen interactions to improve tsetse control,
3. Study the population dynamics of tsetse microbial flora,
4. To understand and manage tsetse virus interactions in laboratory populations
   a. identify DNA database for virus populations
   b. determine transmission mode of tsetse SGHV
   c. maintain SGH symptom free colonies
5. Develop parasite refractory lines,
6. Identify trypanosome inhibitory products,
7. Incorporate parasite resistance traits into SIT lines
8. Cytoplasmic incompatibility-mediated gene drive system
9. Harness cytoplasmic incompatibility for SIT application
10. Disseminate discoveries to endemic countries and interested parties and relevant databases
11. Publish results in scientific journals

8. Activities:

Activity 1. Hold Consultants meeting. This activity has been completed.

Activity 2. Form a network of researchers to address the points listed above. The CRP has involved researchers from USA, Canada, France, Italy, Kenya, Greece, Belgium, Uganda, Tanzania, China, Germany, Nederland, Ghana, Austria, Cameroon, Slovakia, Burkina Faso, and South Africa: This activity has been completed.

Activity 3. Inform PAAT and PATTEC as a means to disseminate discoveries and to support strategies to tsetse and trypanosomosis interventions including related genomics activities. This activity has been partially completed.
Activity 4. Issue a Technical Contract to enable dissemination of tsetse fly laboratory material to CRP members. This activity has been completed.

Activity 5. Issue a Technical Contract for collection/analysis and dissemination of field samples to CRP members. This activity has been completed.

Activity 6. Organize the 1st RCM in Vienna 2007 and planned for workshop to standardize protocols for population sampling, DNA extraction and identification of microbial flora. This activity has been completed.

Activity 7. Organize the 2nd RCM together with the 1st workshop in Bobo Dioulasso (Burkina Faso, 2009) to analyze progress in delivering the research outputs and to plan the next phase of the project. This activity has been completed.

Activity 8. Organize the 3rd RCM with the second workshop on genotyping in Nairobi (2010) to analyze progress in delivering the research outputs and to plan the next phase of the project. This activity has been completed.

Activity 9. Organize the 4th RCM in Vienna (2012) to analyze progress in delivering the research outputs and to review the final publication to disseminate tsetse, virus and symbiont genomics information for tsetse control and set a future agenda. This activity has been completed.

Activity 10. Publish the results of the CRP. Sixteen mini-reviews and seven research articles.

9. Expected CRP Output:

1. Decipher host-symbiont interactions to better understand tsetse’s nutritional ecology and improve mass rearing procedures
2. Investigate tsetse pathogen interactions to improve tsetse control
3. Study the population dynamics of tsetse microbial flora
4. To better understand and manage tsetse virus interactions in laboratory populations
   a. DNA database for virus populations identified (accomplished)
   b. Transmission mode of tsetse SGHV determined (accomplished)
   c. SGH symptom free colonies maintained (partially accomplished)
5. Develop parasite refractory lines
6. Identify trypanosome inhibitory products
7. Incorporate parasite resistance traits into SIT lines
8. Cytoplasmic incompatibility-mediated gene drive system
9. Harness cytoplasmic incompatibility for SIT application
10. Disseminate discoveries to endemic countries and interested parties and relevant databases
11. Publish results in scientific journals

10. Factors, if any, which adversely affected the effectiveness of the CRP:

1. Inability to efficiently transmit Sodalis between generations has slowed progress on development of paratransgenic lines.
2. Inefficient secretion of recProteins in Sodalis has hampered the utility of paratransgenic application.
3. Low density Wolbachia infections in individuals of natural tsetse populations complicated true prevalence information.
4. Lack of knowledge on species specific host-symbiont interactions limited scope
5. Long life cycle of the tsetse fly has limited the study of viral replication and suppression strategies.
6. Lack of suitable cell culture system that supports virus replication.

11. Impact of the CRP:

1. Decipher host-symbiont interactions to understand tsetse’s nutritional ecology and improve mass rearing procedures:
   i. Developed the methodology to generate fertile tsetse lines without symbionts through dietary supplementation,
   ii. Obtained Knowledge on the functional role of Wigglesworthia in tsetse symbiosis.
2. Investigate tsetse pathogen interactions to improve tsetse control:
   see 4 below.
3. Study the population dynamics of tsetse microbial flora:
   i. Discovered varying Sodalis genotypes in natural populations,
   ii. Observed correlation of Sodalis occurrence with parasite infection in natural populations.
4. To understand and manage tsetse virus interactions in laboratory populations
   a. DNA database for virus populations identified:
      i. identified and characterized the causal agent (SGHV) of salivary gland hypertrophy,
      ii. sequenced, annotated, and published genomes of three SGHVs (two strains of GpSGHV and one strain of MdSGHV),
      iii. classified a novel virus family (Hytrosaviridae) with two genera (Glossinavirus and Muscavirus), accepted by the ICTV; proposed that hytroviruses are related phylogenetically with baculoviruses and nudiviruses,
      iv. genomic sequence provided a framework to:
         - developed specific and universal PCR primers to detect virus in field and laboratory tsetse colonies,
         - identified genes that are stable, essential, and suitable candidates for the development of antiviral therapies,
         - identified antiviral dsRNAs for RNAi suppression of viral replication,
         - evaluated the level of GpSGHV genetic diversity in field and laboratory tsetse fly populations,
         - determined the SGHV proteomes and identified targets for the development of antibodies to suppress viral infection.
   b. Transmission mode of tsetse SGHV determined:
      i. recognized that in field populations the primary mode of transmission is vertical whereas in colonies horizontal transmission also occurs and that infected flies secrete the virus during membrane feeding,
      ii. utilized PCR-based detection tools and showed that tsetse colonies with low prevalence of SGH symptoms (approx. 5%) harbour high levels (80-100%) of asymptomatic infections,
      iii. determined (by qPCR) that asymptomatic flies (i.e., infected flies that do not show SGH symptoms) contain variable levels of GpSGHV,
      iv. discovered that in colonies, membrane feeding facilitates horizontal virus transmission resulting in infected F1 progeny.
c. SGH symptom free colonies maintained:
i- discovered that the tsetse fly virus exists in both an asymptomatic and symptomatic state whereas the house fly virus expresses only symptomatic infections,
ii- associated symptomatic GpSGHV infection with reduced fertility of both sexes,
iii- surveyed field populations throughout Africa to determine the prevalence of both symptomatic (by dissection) and asymptomatic (by PCR) infections in flies; prevalence determined by PCR varied widely (0-90%) among and within different Glossina spp,
iv- designed and tested different strategies to manage virus levels in tsetse colonies: clean feeding (i.e., fresh blood membranes are used for each feeding), RNAi, polyclonal antibodies, oligopeptides, and antiviral drugs; each of these strategies reduced virus loads to varying degrees,
v- found that integrating several strategies (such as clean feeding and antiviral drugs) eliminated symptomatic infections and significantly reduced the levels of asymptomatic infections in tsetse colonies.

5. Develop parasite refractory lines:
Because of the inability to efficiently transmit Sodalis between generations, nevertheless, we have:
i- identified tsetse endosymbiont transmission routes, and
ii- development of symbiont-based expression system.

6. Identify trypanosome inhibitory products:
produced anti-trypanosom nanobodies.

7. Incorporate parasite resistance traits into SIT lines:
could not be achieved in the time frame of this CRP

8. Cytoplasmic incompatibility-mediated gene drive system:
i- discovered the presence of chromosomal insertions of Wolbachia symbionts in many tsetse species,
ii- discovered low titer Wolbachia infections in natural populations and colonies,
iii- determined tsetse Wolbachia genome sequences,
iv- discovered polyandry in natural tsetse populations.

9. Harness cytoplasmic incompatibility for SIT application:
i. discovered the functional role of Wolbachia in inducing high CI,
ii- A mathematical model was developed to use CI for paratransgenic application to derive desirable phenotypes.

10. Disseminate discoveries to endemic countries and interested parties and relevant databases:
i- we have built capacity in Tsetse & Trypanosome Research in participating DECs in Africa. This includes:
  - Training of 6 PhD students and 8 research fellows from DECs in collaborating laboratories,
  - Training of DEC researchers in collaborating laboratories through short-term visits,
  - Presentations of research findings by DEC scientists in international meetings,
  - Ability to recruit independent research funds to DEC scientists in collaboration with participating scientists and laboratories,
  - Promote development of genomics knowledge, including genome sequencing and functional studies in multiple tsetse species through participating in International Glossina Genome Initiative.

11. Publish results in scientific journals:
See research publication list.
12. Relevance of the CRP:

The results of the CRP are very relevant to ongoing technology transfer activities in support of technical cooperation projects in Sub-Saharan Africa. The technical information generated by this CRP will contribute to improving SIT technology through a better understanding of the role that microbes play in the laboratory and field biology of tsetse. The goal is to improve the quality of decision making related to field implementation of SIT projects. The emphasis on the field component of the CRP will ensure effective transfer of technology and effective capacity building.

13. Additional Information:

In addition to the extensive achievement of this CRP (see section 12), the CRP was an excellent opportunity to bring together researchers from different countries to work together on a technical topic of relevance to Agency development activities. Researchers from US, China, several countries in Europe, including France, Germany and the Netherlands, and from African countries worked on the tsetse virus and symbionts. During the CRP around 67 research articles, reviews and book chapters were published, in addition to publishing a special issue (with 20 articles including research article and mini-reviews) in the journal of Invertebrate Pathology with the CRP results and achievements.

The CRP was also a great opportunity for collaboration on technology transfer and for fostering South-North collaborations. Two workshops were held during the CRP in Bobo-dioulasso (Burkina Faso) and Nairobi (Kenya) with the aim of training and technology transfer. During the CRP four RCM were held (two in Africa and two in Vienna) enabling the participants to exchange laboratories visits. During the CRP several M.Sc. and Ph.D. student graduated within the framework of the CRP.

14. Recommendation:

1. Extend knowledge accumulated in *G. morsitans* to other tsetse species
2. Make available laboratory strains and natural biological material from different species to the research community
3. Study symbiont expression systems:
   - Research on symbiont secretion systems and transformation
   - Research on parasite resistance conferring molecules
4. Research paratransgenic applications:
   - Research on rec-symbiont transmission to host progeny
   - Research the ability of *Wolbachia* to drive paratransgenic lines
5. Harness *Wolbachia* mediated CI to improve SIT applications
6. Study the relationship between polyandry and *Wolbachia*, and its impact on SIT
7. Characterize host-symbiont-pathogen interactions:
   - Microbiome characterization through metagenomics
   - Relate different states of viral infection (symptomatic/asymptomatic) and Trypanosome infection to the microbiome and physiological status (e.g., age, nutrition, stress) of the fly.
   - Research the role of symbionts on host physiology including reproduction and behavior
8. Identify the factor(s) responsible for the switch from asymptomatic to symptomatic viral infection

10. Expand networking and strengthen linkages among all collaborators.

11. Disseminate knowledge through linking CRP meetings with international conferences

12. Organize workshop to disseminate information to CRP participants on genomic resources relevant for TNT research.

15. Remarks:

A follow-up CRP with a focus on “Enhancing vector refractoriness to trypanosome infection” has been approved for initiation in 2013.

16. Resulting Publications:

A. Research articles:


B. Mini Reviews:


1. **Articles published by the CRP participant during the CRP period (2007-2012)**
Book Chapters:

Reviews:

Research Articles:


