WORKING MATERIAL

ENHANCING VECTOR REFRACTORINESS TO TRYPANOSOME INFECTION

THIRD RESEARCH COORDINATION MEETING

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1. INTRODUCTION AND CURRENT STATUS

**Tsetse and African trypanosomosis.**

Tsetse flies (Diptera: Glossinidae) are the only cyclical vectors of African trypanosomes, which are the causative agents of human and animal African trypanosomiases (HAT and AAT, respectively). HAT is endemic to 36 countries in sub-Saharan Africa with about 70 million of the inhabitants at risk. In 2009, the number of new cases of HAT reported to WHO dropped below the symbolic number of 10,000 (WHO update Febr. 2016). However, given that the disease affects hard to reach rural populations, and that active surveillance in war-torn areas is non-existent, the disease prevalence numbers are undoubtedly a gross underestimation. The related disease AAT causes estimated losses to African agriculture of US$ 4.5 billion per year and has a profound effect on development of the continent.

The most economically important African trypanosomes are transmitted during the bite of the tsetse fly. The AAT-causing trypanosomatids *T. vivax*, *T. congolense* and *T. brucei brucei* are major pathogens of livestock. Humans are only infected by *T. b. rhodesiense* and *T. b. gambiense*.

Following the success of the SIT programme in Zanzibar and the PATTEC (The Pan African Tsetse and trypanosomiasis Eradication Campaign) initiative of the AU (African Union), i.e. reducing the fly population size, interest in the use of SIT for tsetse and trypanosomosis control is increasing. To date, the IAEA-supported SIT projects have been in areas without human sleeping sickness, but future projects could include areas of actual or potential human disease transmission. In such projects it would be ethically unacceptable to release flies capable of transmitting the parasite to humans. Therefore, methodologies to prevent parasite transmission by released sterile male flies must be developed. To achieve this goal, a complete understanding of the underlying mechanisms involved in vector competence is necessary. The current CRP proposal addresses this issue by proposing experiments that will decipher interactions between the tsetse fly, its symbiome, and pathogenic African trypanosomes.

**Tsetse-trypanosome interactions.**

The transmission cycle starts when a tsetse fly feeds on an infected vertebrate host. Distinct trypanosome species exhibit different developmental cycles in various tissues and organs of the tsetse fly vector. *T. vivax* has the simplest life cycle, with development occurring exclusively in tsetse’s mouthparts. So far, only limited information is available on factors that affect *T. vivax* development in the fly. For *T. congolense* and *T. brucei* initial establishment of infection occurs in the fly midgut, with subsequent maturation in the fly’s proboscis and salivary gland, respectively. During this journey, these parasites undergo several rounds of differentiation and proliferation, finally ending in the metacyclic form that is end-stage infective to the vertebrate host. The success rate of trypanosome colonization in the tsetse fly is low and often fails during initial establishment of the fly’s midgut. Tsetse present a chitinous peritrophic matrix (PM), on which assembly an array of proteins, that lines the midgut. The PM serves as a formidable front-line barrier that trypanosomes must circumvent in order to establish an infection in the tsetse midgut. The flies also mount a robust innate immune response, and experimental down regulation of the Imd pathway increases midgut infection prevalence. Similarly, RNAi silencing of tsetse’s immune-responsive glutamine/proline-rich (EP) protein promotes trypanosome establishment, and reactive oxygen species are also important determinants of resistance to infection. Following colonization of tsetse’s midgut, trypanosomes again differentiate and then migrate to the fly’s salivary glands (SGs). This process, which represents another significant bottleneck that mediates parasite transmission, is not fully understood. The cardia (the tissue that forms the junction between tsetse’s foregut and midgut), which is an immune-active tissue, produces nitric oxide synthase and contains increased
levels of nitric oxide, reactive oxygen intermediates and hydrogen peroxide (H₂O₂). From the cardia, only a few trypanosomes will succeed in migrating to, and colonizing tsetse’s SGs. The tsetse fly SG and proboscis function as biotopes to which parasites adhere, multiply within and undergo their final re-programming into infectious metacyclic forms. Information on the molecular composition of saliva is scant, and was derived mainly from *in silico* interpretation of a SG EST library and a limited ‘proteomics’ analysis. Recent progress through this CRP action has provided more information on the key players on the pathogen host interactions in tsetse’s midgut and SGs.

Now additional information is emerging regarding the underlying mechanisms that facilitate 1) trypanosome adaptation to different tsetse microenvironments, and 2) trypanosome circumvention of tsetse immune responses during their developmental journey through the fly.

**Insect Symbiosis.**

Symbiosis is ubiquitous in nature and has had significant consequences in promoting evolution and biodiversity. This is particularly true for insects that establish both endo and ectosymbioses. These symbiotic associations are just starting to be understood. Symbiotic microorganisms affect different aspects of their insect host’s physiology, including development, nutrition, reproduction, speciation, defense against natural enemies and host preference.

Currently, insect symbiotic associations can be divided into at least three categories. The first category includes mutualistic symbionts that provide their host with nutrients such as amino acids and vitamins. In return, the mutualist receives an nutrient-rich, protective niche in which to reside and thus can maintain a highly reduced gene inventory. The second category includes commensal symbionts that provide their hosts with the ability to survive heat stress and develop resistance to parasitic infections. Finally, parasitic symbionts manipulate the reproductive properties of their hosts, inducing phenomena such as parthenogenesis, feminization, male-killing and cytoplasmic incompatibility (CI).

**Tsetse symbiosis.**

All tsetse flies, examined to date, harbor an obligate symbiont of the genus *Wigglesworthia*. This relationship is ancient (50-80 million years), and likely serves to complement tsetse’s vertebrate blood-specific diet. In fact, *Wigglesworthia*’s highly reduced genome encodes several vitamin biosynthesis pathways, the products of which are absent from vertebrate blood.

Two populations of *Wigglesworthia* exist in tsetse. The first is found within bacteriocytes, which collectively comprise an organ called the ‘bacteriome’ that is located immediately adjacent to tsetse’s midgut. This population of *Wigglesworthia* presumably supplements metabolites absent from its host’s diet. Tsetse’s second population of *Wigglesworthia* is found extracellularly in the female milk gland. This milk-associated population of cells colonizes developing intrauterine larvae.

Recent experiments demonstrate that *Wigglesworthia* also has an immuno-modulatory role in tsetse. Adult tsetse that lack this symbiont (*Gmm<sup>Wgm</sup>*<sup>-</sup>) exhibit an immuno-compromised phenotype compared to their wild type counterparts (*Gmm<sup>WT</sup>*). When challenged with trypanosomes, gut infections are established in a large percentage of *Gmm<sup>Wgm</sup>* flies. Conversely, *Gmm<sup>WT</sup>* flies are highly resistant and can efficiently clear parasite infections. Interestingly, *Wigglesworthia* does not directly enhance immunity in wild type individuals, as elimination of this bacterium from adult flies via antibiotic tetracycline treatment does not result in an immune compromised phenotype. Instead, this obligate symbiont must be present during immature larval stages in order for tsetse’s immune system to develop and function properly during adulthood.
Tsetse’s second symbiont, *Sodalis*, is a commensal bacterium found in all lab-colonized tsetse lines and some natural populations. This polytropic bacterium is found intra- and extracellularly in tsetse’s gut, hemolymph, salivary glands and reproductive tract (*Sodalis* is also transmitted to intrauterine larvae via maternal milk secretions). Unlike *Wigglesworthia*, *Sodalis* from different tsetse species are closely related, thus indicating this bacterium’s recent association with its tsetse host. *Sodalis* exhibits genotypic traits similar to those found in several free-living microbes, and can be cultured outside of tsetse and is amenable to genetic manipulation. Furthermore, *Sodalis* co-inhabits tsetse’s gut along with pathogenic trypanosomes. Recent data suggest that tsetse’s midgut microbiota (*Sodalis* and *Wigglesworthia*) can modulate trypanosome development. These characteristics make *Sodalis* an ideal candidate for use in tsetse paratransgenic disease control strategies.

Tsetse’s third symbiont is the alpha-proteobacterium *Wolbachia*, making it the most successful symbiont on earth. This bacterium infects up to 40% of arthropods, including insects, terrestrial crustaceans, spiders, scorpions and springtails, as well as filarial nematodes. *Wolbachia*, which is strictly intracellular, is mainly transmitted maternally to progeny with the egg cytoplasm (although more and more reports emerge in literature showing that horizontal transfer events are more common than earlier anticipated). *Wolbachia* found in different arthropod species exhibit varying levels of co-evolution with their host. As such, this bacterium can present parasitic, commensal or mutualistic (obligatory) phenotypes. In order to enhance their own spread through host populations, *Wolbachia* cause a number of reproductive phenotypes in many arthropods, including CI, male killing, feminization and parthenogenesis. Furthermore, as part of their co-evolutionary physiological inter-relations at cellular and metabolic levels, *Wolbachia* can manipulate host fitness, fecundity, immunity and longevity, development and even sexual behaviour. As recently shown in hybrids between closely related *Drosophila* hosts, mutualistic *Wolbachia* can lose host-directed replication control, thus over-replicating so as to transform into pathogens. Such artificial hybrid-host systems can serve as sensitive biomonitors for studying symbiont dynamics in *vivo*.

As demonstrated recently, *Wolbachia* mediate more host-pathways than was earlier anticipated. For example, *Wolbachia* protects *D. melanogaster* from infections with RNA viruses, whereas similar studies on the *Wolbachia*-mediated protection against entomopathogenic fungi in *D. melanogaster* are still inconclusive. The analyses of *Wolbachia*-mediated protective effects against pathogenic microbes has recently been extended from the *Drosophila* host system to *Aedes aegypti* and *Anopheles stephensi* mosquitoes. In these cases, *Wolbachia* infection interferes with the establishment of viral infections, as well as infections with *Plasmodium* and filarial nematodes.

In addition, *Wolbachia*-infected *Ae. aegypti* have reduced lifespans, as expected from the phenotype in its original host. *Wolbachia*-infected *Ae. aegypti* are less likely to transmit viral diseases such as dengue (virus), which require specific incubation periods in their mosquito vectors before transmission to humans can occur. The application of this virulent *Wolbachia* is therefore a novel strategy for fighting vector-borne diseases, such as dengue, malaria and filariasis, by interfering with vector biology. As recently demonstrated, such artificially infected mosquitoes spread rapidly in the wild and provide dengue protection to their novel host. Because of these seminal discoveries, *Wolbachia* continues to attract an applied-research interest as a novel biocontrol agent for arthropod pests and vectors such as mosquito-transmitted malaria or even tsetse fly-transmitted trypanosomiasis. Many tsetse species and populations harbour closely related but distinct *Wolbachia* strains at different titres and with different infection prevalences. In addition, a number of tsetse species have *Wolbachia* sequences insertions in their chromosomes. In the laboratory-reared *G. m. morsitans*, crossing studies between *Wolbachia*-infected and -uninfected individuals have demonstrated the expression of strong CI. Surveying the potential capacity of natural *Wolbachia* infections to functionally interfere with trypanosome transmission is of pivotal importance.
**Symbiont-based tsetse control strategies**

CI-inducing *Wolbachia* strains can cause high levels of embryonic lethality. Thus, this symbiont can be applied for population suppression of insect pests and disease vectors in a way analogous to SIT (this process is referred to as incompatible insect technique, IIT). As recently shown, strong and promiscuous CI-inducing *Wolbachia* strains can be used as a novel, environment-friendly tool for the control of insect populations such as *Ceratitis capitata* (the Mediterranean fruit fly), *Bactrocera oleae* (the olive fly) and *Culex pipiens* and *A. aegypti* (mosquitoes). *Wolbachia* induced CI can also be used to augment the sterility caused by irradiation and enhance the efficacy of current SIT methods. Furthermore, *Wolbachia*-induced CI can be applied as a natural driver system for spreading transgenic insects and maternally transmitted modified symbionts (in paratransgenesis approaches, see below) through populations. Because transgenic organisms are likely to be less fit than their wild-type counterparts, transgenic traits must be actively driven into the population, in spite of fitness costs by means of population replacement. By a variety of mechanisms, *Wolbachia*-infected females have a reproductive advantage relative to uninfected females, allowing infection to spread rapidly through host populations to high frequency in spite of fitness costs. Hence, *Wolbachia* can be exploited to drive ‘valuable’ transgenes or modified symbionts into vector populations for disease control.

Tsetse’s symbiont population is also amenable to the use of paratransgenesis as an approach to reduce fly susceptibility to infection with trypanosomes. Expression of parasite resistance genes in *Sodalis*, followed by the bacterium’s introduction into laboratory reared sterile tsetse prior to release in the field, can now be harnessed for disease control. *Sodalis* paratransgenesis, coupled with SIT, would further decrease the risk of trypanosomiasis transmission to the human populations in areas designated for control.

**Viral pathogens**

An essential aspect of SIT is the ability to establish large colonies of tsetse lines. Several species of tsetse can be infected with the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV). Infection can cause salivary gland hypertrophy (SGH) and significantly reduce the fecundity of the infected flies. Although SGH syndrome prevalence is likely very low in wild tsetse populations (0.5-5%), in *G. pallidipes* mass rearing facilities, prevalence can reach up to 80% and cause colony collapse. For example, high prevalence of SGH led to the collapse of the *G. pallidipes* colony in Seibersdorf, Austria in 1978 and 2002. The first report of the SGH was described in wild populations of *G. pallidipes* in the 1970s, but the pathology was later observed in other tsetse fly species from several African countries. The causative agent of this syndrome was described initially as a rod-shaped, enveloped DNA virus averaging 70 by 640 nm in size. This virus was associated also with testicular degeneration and ovarian abnormalities, and its presence affected the development, survival, fertility and fecundity of naturally or experimentally infected flies. Mother-to-offspring transmission, either transovum or through infected milk glands, is thought to be the mode of virus transmission in natural tsetse populations. In colonized tsetse the main route of virus transmission is horizontal, this being facilitated by artificial silicon membranes that are used for large-scale fly feeding. SGH syndrome has been observed in two other dipterans, the narcissus bulb fly *Merodon equestris* and the housefly *Musca domestica*, but recently a SGHV look-alike was found in the parasitic wasp *Diachasmimorpha longicuadata*.

Although PCR detection of GpSGHV in old *G. pallidipes* shows widespread asymptomatic virus infection (up to 100%), only 5-10% of the infected individuals develop SGH symptoms. It is unclear why some infected flies show symptomatic infection whereas others remain asymptotically infected. Although a positive correlation was found between symptomatic SGH and increased virus copy number (which indicate an accumulation effect of the virus related to the SGH symptoms), other unknown factors related to the fly’s genetics and interaction with its
microbiota (symbionts) cannot be excluded. Preliminary data indicate a possible negative correlation between virus infection and *Wolbachia*. Therefore, it will be important to analyze the interaction between virus infection and tsetse’s other associated microorganisms (*Wolbachia*, *Sodalis*, *Wigglesworthia*, *Spiroplasma* and trypanosomes) in different tsetse species.

Currently, the genetic analysis of two genotypes of GpSGHV (Ethiopia and Uganda isolates) with differential pathologies has been completed. Out of the 174 open reading frames, at least 140 of these are transcriptionally active and 83 encode confirmed proteins based on SG analysis. The most divergent open readings frames were used to determine genetic diversity SGHV found within tsetse in sub-Saharan Africa.

**Dissection of tsetse-microbial associations towards vector and trypanosomiasis control.**

Tsetse’s bacterial symbionts are important during all fly life stages. They may also impact trypanosome vectorial competence and hence could enhance the efficacy of SIT programs. We seek to resolve four key questions related to tsetse’s microbial community:

- Can the elucidation of tsetse-trypanosome molecular interactions assist in the development of novel methods and approaches to reduce or prevent the transmission of trypanosomes by irradiated tsetse flies?
- Can tsetse symbionts be used to develop novel vector and disease control tools, complementary to the SIT?
- Can the characterization of tsetse’s symbiome and viral pathogens improve the efficacy of SIT?
- Are tsetse symbionts (including the gut microbiota), and the fly’s competence as a vector of trypanosomes, affected by radiation?

These questions are expanded in the following paragraphs.

**Tsetse-trypanosome molecular interactions**

The transmission of major medically and veterinary important trypanosome species (*T. brucei* ssp., *T. congolense* and *T. vivax*) relies on the specific biological relationship between the parasites and the blood feeding tsetse fly. Indeed, depending on the trypanosome species, the parasite has to go through an obligatory developmental cycle that varies from a short cycle in tsetse’s mouthparts (*T. vivax*) to a longer, more complex life cycle in the fly’s midgut and mouthparts (*T. congolense*) or midgut, mouthparts and SGs (*T. brucei* ssp). For both *T. congolense* and *T. brucei*, the molecular interplay at different developmental stages in the fly will determine if the parasite develops to its final infective stage. The elucidation of these interactions is essential to understand the determinants of tsetse vector competence for a given trypanosome species population and how they can be affected. High resistance (refractoriness) to trypanosome infection has been demonstrated both in laboratory lines as well as in natural populations. Understanding the genetic basis of tsetse’s resistance (vector competence) will help to develop tools to enhance fly refractoriness to trypanosome infection.

**Tsetse microbiota**

All laboratory colonized tsetse harbour three distinct, maternally-transmitted bacterial endosymbionts. Additionally, some colonized tsetse are also infected with *Spiroplasma* and SGHV. Field-captured flies are also colonized with a population of bacteria transiently acquired from their environment. In other vector arthropod systems, gut microbiota prevents establishment and/or transmission of pathogens. Thus, acquiring a better understanding of the symbionts and pathogens present in the tsetse vector, and the physiology that underlies their interactions, may allow us to manipulate tsetse flies so that they exhibit increased refractoriness to trypanosome infection.

**Symbiotic organisms and novel control tools**
IIT, which is based on the mechanism of *Wolbachia*-induced CI, has been successfully tested for control of the agricultural pests, *Ceratitis capitata* (the Mediterranean fruit fly) and *Bactrocera oleae* (the olive fly), under laboratory conditions. Also, *Wolbachia*-induced CI has been successfully used as a driving mechanism of desirable traits in mosquito *A. aegypti* populations under laboratory and field conditions in Australia and Brazil. Such approaches should be considered, alone and/or in conjunction with SIT, for the control of tsetse flies and trypanosomiasis.

At present, SIT has been proven effective in reducing or eradicating isolated tsetse populations. This method relies on the massive release of sterile male flies, which could result in a temporary increase in the number of potential trypanosome vectors in the release zones. As such, the use of a tsetse that present a trypanosome-resistant phenotype would render SIT a much less controversial component of integrated vector control strategies.

Tsetse’s commensal bacterium, *S. glossinidius*, is ideally suited for use in paratransgenesis because:
1) it resides in different tsetse tissues (midgut, haemolymph, salivary glands) and in close proximity to the pathogenic trypanosomes; 2) it can be cultured and genetically modified *in vitro* and made suitable to delivery effector molecules; 3) recombinant *Sodalis* can be re-introduced into female flies; 4) re-introduced recombinant *Sodalis* are maternally transmitted to larval offspring via milk secretions; and 5) due to large-scale gene erosion, *Sodalis* is metabolically dependent on its tsetse host niche, suggesting that this bacterial symbiont cannot survive outside of the fly. A key component of *Sodalis*-based tsetse paratransgenesis is the identification of anti-trypanosomal effector molecules that be used in the paratransgenic system. The tsetse-trypanosome interaction research proposed in this CRP will provide several potential candidates as anti-trypanosome effector molecules to be delivered by *Sodalis*.

### 4. Effects of radiation on symbionts and pathogens

Before their release into the field for SIT, male tsetse are irradiated to render them sterile. This radiation treatment may alter the bacterial community of the fly so as to create an imbalance between the different bacterial communities in the gut and other organs/tissues. Hence, this could have an impact on the tsetse’s physiology and trypanosome vector competence. Understanding the effects of radiation may enable us to design responses that address them in a manner that optimizes SIT efficacy. In addition, radiation may also result in the development and isolation of mutant strains of endogenous symbiotic bacteria leading to novel insect symbiotic associations that may affect tsetse vector competence. Moreover, in the context of the possible application of a paratransgenic approach to enhance refractoriness to trypanosome infection in released tsetse flies, evaluation of the impact of radiation on the transformed bacterial community is essential.

In conclusion, the elucidation of the molecular interactions between the host, it’s symbionts, and associated pathogens can have profound effects on the development and application of efficient control strategies for tsetse flies and trypanosomiasis. The current CRP aims to characterize and develop methods to harness the tsetse fly-trypanosomes-symbiont tripartite association in order to:
- (a) unravel the molecular interplay among tsetse flies, symbionts, and trypanosomes,
- (b) characterize tsetse microbiota under field and laboratory conditions as well as in the presence / absence of tsetse pathogens and trypanosomes,
- (c) develop novel, symbiont-based and SIT-compatible control tools for tsetse flies and trypanosomiasis,
- (d) determine the effects of radiation on tsetse symbionts and pathogens. We believe that this initiative will lead to better and more cost-effective SIT programmes aimed at reducing tsetse populations and trypanosomiasis. A better understanding of the vector-trypanosomes-symbionts tripartite association is essential to develop methodologies that will lead to the enhancement of refractoriness of the tsetse flies to trypanosome infection.
5. Genomics and transcriptomic research

The following genomics resources relevant to tsetse and its associated microbiota are publicly available:

- **VectorBase** (www.vectorbase.org). The Glossina Genome Cluster project has completed the whole genome sequencing of *G. pallidipes*, *G. austeni*, *G. brevipalpis*, *G. fuscipes fuscipes* and *G. palpalis gambiensis*. Tissue-specific transcriptomes are also available to assist with genome assembly and annotation.

- **NCBI** (http://www.ncbi.nlm.nih.gov/pubmed). The genetic analyses of the genomes of Ugandan and Ethiopian strains of GpSGHV and the MdSGHV are complete. This information is complemented with transcriptomic and proteomic data. Additionally, genomic resources relevant to tsetse’s symbiotic microbes are available at NCBI.

- **TriTrypDB** (http://tritrypdb.org/tritrypdb/). A database containing comprehensive trypanosome-related genomics data.

- **Genomes Online Database** (https://gold.jgi.doe.gov/). A World Wide Web resource for comprehensive access to information regarding genome and metagenome sequencing projects, and their associated metadata, around the world.
## 2. REVISED LOGICAL FRAME WORK

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<th>Important Assumptions</th>
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<td>Overall Objective:</td>
<td>N/A</td>
<td>N/A</td>
<td>African countries continue to suffer from tsetse fly-vecared human and animal African trypanosomoses (HAT and AAT, respectively). Prevention and treatment measures remain ineffective. Drug resistance remains a problem. The increasing demand for area-wide integrated vector management approaches to control vector-borne diseases, including where appropriate the SIT as non-polluting suppression/eradication component, and SIT will be expanded into areas of potential human disease transmission. Financial and human resources are available. Novel approaches will be accepted on ethical ground.</td>
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<td>Specific Objectives:</td>
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<td>1. To decipher the molecular interplay between vector-symbiont-trypanosomes</td>
<td>1. Mechanisms and molecules that cause parasite resistance identified in one model species.</td>
<td>1. Reports and publications.</td>
<td>1. Tools and experimental models for functional research are available.</td>
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<td>2. To characterize tsetse symbiome including gut microflora.</td>
<td>2. Diversity and composition of tsetse symbiome identified</td>
<td>2. Biological (field and laboratory) material and next generation sequencing and bioinformatics tools are available.</td>
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<td>Project Design Elements</td>
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<td>3. To determine the effect of radiation on vector symbiome</td>
<td>3. Effects of radiation on vector symbionts assessed.</td>
<td>3. Scientific reports and peer reviewed publications.</td>
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<td>4. To develop innovative symbiont-based strategies, in conjunction with SIT, to control African trypanosomosis.</td>
<td>4. Symbiont-based approaches, in conjunction with SIT, developed and validated.</td>
<td>4. Scientific reports and peer reviewed publications.</td>
<td>4. Symbionts can be manipulated as tools for the control of African trypanosomosis.</td>
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<td>1. Molecular cross-talking between vector-symbiont-trypanosomes characterized</td>
<td>1. Factors and mechanisms involved in molecular interplay determined.</td>
<td>1. Scientific reports and peer reviewed publications.</td>
<td>1. Tools and experimental models are adequate and valid.</td>
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<td>2. Vector symbionts characterized and impact on host physiology identified.</td>
<td>2. Vector’s symbiotic partners determined and impact identified.</td>
<td>2. Scientific reports and peer reviewed publications.</td>
<td>2. Available genomics and bioinformatics tools are optimal.</td>
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<td>3. The impact of radiation on vectors and symbionts.</td>
<td>3. Qualitative and quantitative changes on the experimental systems determined.</td>
<td>3. Scientific reports and peer reviewed publications.</td>
<td>3. Experimental tools are available.</td>
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<td><em>T. vivax</em> infections</td>
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<td>genomics and bioinformatics</td>
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<td>species and hybrid</td>
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<td>genomics and bioinformatics</td>
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<td>Project Design Elements</td>
<td>Verifiable Indicators</td>
<td>Means of Verification</td>
<td>Important Assumptions</td>
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<td>2c. Impact of viral pathology on the tsetse symbionts.</td>
<td>2c. Symbionts of SGHV symptomatic and asymptomatic tsetse hosts characterized.</td>
<td>2c. Reports and peer reviewed publications.</td>
<td>2c. Biological material, genomics and bioinformatics tools are available.</td>
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<tr>
<td>3a. The effect of radiation on tsetse vectors, their symbionts and pathogens determined.</td>
<td>3a. Impact of irradiation in mass-reared species determined.</td>
<td>3a. Reports and peer reviewed publications.</td>
<td>3a. Irradiation damages hosts, symbionts and pathogens in a dose and species-specific manner.</td>
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<td>3b. The mutagenic effect of radiation on paratransgenesis.</td>
<td>3b. Effect in at least 1 target species assessed.</td>
<td>3b. Reports and publications</td>
<td>3b. Paratransgenesis developed.</td>
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<tr>
<td>4a. <em>Wolbachia</em>-based population suppression and/or replacement strategies assessed.</td>
<td>4a. CI in target species characterized and <em>Wolbachia</em> impact on mating behaviour assessed.</td>
<td>4a. Reports and publications</td>
<td>4a. <em>Wolbachia</em> is amenable to characterization by classical genetic and molecular approaches.</td>
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<tr>
<td>4b. Develop parasite resistant paratransgenic tsetse.</td>
<td>4b. Anti-trypanosome molecules identified and expressed in paratransgenic tsetse.</td>
<td>4b. Reports and peer reviewed publications.</td>
<td>4b. Symbiont-based trypanosome control strategies can be developed against target vector species.</td>
</tr>
</tbody>
</table>

**ACTIVITIES:**

<table>
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<tr>
<th>Project Design Elements</th>
<th>Verifiable Indicators</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Announce project amongst established vector entomologists, trypanosome biologists, virologists and symbiologists</td>
<td>2. CRP announced, and research contracts and agreements submitted, evaluated and forwarded to IAEA committee.</td>
<td>2. Issued contracts and agreements.</td>
<td>2. Suitable proposals submitted and approved by IAEA committee.</td>
</tr>
<tr>
<td>3. Organize first RCM to plan, coordinate and review research activities</td>
<td>3. 1st RCM held.</td>
<td>3. Working material printed and distributed for 1st RCM.</td>
<td>3. Research activities started. Reports published and distributed following each RCM.</td>
</tr>
<tr>
<td>4. Carry out R&amp;D.</td>
<td>4. Research carried out by contract and agreement holders.</td>
<td>4. Reports and publications.</td>
<td>4. Renewal requests and continued funding of RCM’s and CRP.</td>
</tr>
<tr>
<td>5. Second RCM to analyse data and draft technical protocols as required</td>
<td>5. 2nd RCM held.</td>
<td>5. Working material printed and distributed for 2nd RCM; Research published in scientific literature and disseminated to member states and scientific community.</td>
<td>5. Research activities continue, progress satisfactory.</td>
</tr>
<tr>
<td>6. Continue R&amp;D.</td>
<td>6. Research carried out by contract and agreement holders.</td>
<td>6. Reports and publications.</td>
<td>6. Renewal requests and continued funding of RCM’s and CRP.</td>
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<td>Project Design Elements</td>
<td>Verifiable Indicators</td>
<td>Means of Verification</td>
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<td>7. Review the CRP after its third year.</td>
<td>7. Mid-CRP review carried out.</td>
<td>7. Report of mid-CRP review.</td>
<td>7. Mid-CRP review by Agency committee is positive.</td>
</tr>
<tr>
<td>8. Convene third RCM to evaluate and standardize protocols.</td>
<td>8. 3rd RCM held beginning of June 6-10, 2016 in Lyon, France.</td>
<td>8. Working material printed and distributed for 3rd RCM; Research published in scientific literature and disseminated to member states and scientific community.</td>
<td>8. Research activities continue, progress satisfactory.</td>
</tr>
<tr>
<td>9. Hold workshops on “Microbial bioinformatic methods and in silico methods and fluorescence microscopy.”</td>
<td>9. Workshop held May 30th to June 5, 2016 in Lyon, France. Harmonized procedures and trainees capable of implementing novel techniques</td>
<td>9. Workshop report.</td>
<td>9. There is need for training; techniques, equipment and instructors are available.</td>
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<tr>
<td>10. Continue R&amp;D.</td>
<td>10. Research carried out by contract and agreement holders.</td>
<td>10. Reports and publications.</td>
<td>10. Renewal requests and continued funding of RCM’s and CRP.</td>
</tr>
<tr>
<td>11. Hold final RCM to review data and reach consensus.</td>
<td>11. Final RCM held.</td>
<td>11. Final CRP report.</td>
<td>11. Research and dissemination activities concluded.</td>
</tr>
<tr>
<td>12. Evaluate the CRP and submit evaluation report.</td>
<td>12. CRP evaluation carried out.</td>
<td>12. CRP evaluation report.</td>
<td>12. CRP evaluation by Agency committee is positive.</td>
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<tr>
<td>Project Design Elements</td>
<td>Verifiable Indicators</td>
<td>Means of Verification</td>
<td>Important Assumptions</td>
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### 3. INDIVIDUAL WORK PLANS FOR THE NEXT 18 MONTHS

<table>
<thead>
<tr>
<th>Activities / PI laboratories</th>
<th>Drion Boucias</th>
<th>Inna Malele</th>
<th>Jean Manjanina</th>
<th>Gisèle Ouedraogo</th>
<th>Just Vlak</th>
<th>Florence Watmuri</th>
<th>Jan Abeele</th>
<th>Serap Aksu</th>
<th>Floberkine</th>
<th>Abdeslat Haddi</th>
<th>Anna Makrida</th>
<th>Peter Takac</th>
<th>Martin Kehlerpohl</th>
<th>Soerge Kelm</th>
<th>George Tsiamis</th>
<th>Solomon Mekonnen</th>
<th>Marc Ciosi</th>
<th>Wolfgang Miller</th>
<th>Ikbal Agagh Ince</th>
<th>Anne Geiger</th>
<th>Astani Tregre</th>
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<tbody>
<tr>
<td>Mechanism of trypanosome resistance in model tsetse identified</td>
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<td>Comparative genomics of different <em>Glossina</em> species and outgroups related to immunity</td>
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<td>Comparative transcriptomics of <em>Glossinia</em> in response to trypanosomes</td>
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<td>Male accessory gland (MAG) function in reproduction (behavior)</td>
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**National Institute for Controlling and Eradication of Tsetse and Trypanosomosis (NICETT)**
Solomon Mekonnen


With the exception of three well-characterized symbionts, the microbiota that resides within tsetse’s gut are poorly documented. Acquiring a better understanding of other microbes present in the tsetse vector, and the physiology that underlies their interactions, may allow us to manipulate tsetse flies so that they exhibit increased refractoriness to trypanosome infection. By utilizing different microbiological and molecular methods, our research will identify and characterize microbes associated with *G. pallidipes* and *G. fuscipes* and their impact on the performance of the flies in colony development in the Kality mass rearing center, Ethiopia. SGH also affected the *G. pallidipes* and *G. fuscipes* colonies. The infestation of SGH within the colony will be further controlled in order to enhance the fecundity of the colonies. The Kality insectary will serve as a source of *G. pallidipes* and *G. fuscipes*, and several *Glossina* spp. will also be collected from the field. All individuals will be screened to determine their symbiont profiles (including *Spiroplasma*) and SGHV infection status. Flies will kept at 23–25°C with 75%-80% RH and fed on defibrinated and irradiated bovine blood. All flies will be sterilized (once with 5% sodium hypochlorite and twice with 70% ethanol) prior to dissecting midguts. Microbiological and PCR techniques will be conducted at the National Animal Health Diagnostic and Investigation Center (NAHDIC), which is a National and East African referral laboratory and Seibersdorf Lab.

Planned for the next 18 months:
1. Collect samples from lab and wild
2. Symbiont and virus identification of wild and laboratory *G. pallidipes* and *G. fuscipes fuscipes* flies
3. Provide samples to partners for SGH and symbiont diversity studies
4. Identify the impact on the performances of the flies in colony development
5. Assess the impact of radiation on symbiont and SGH presence in the colonies
6. Recollect released flies and analyse for the presence of parasites and symbionts

**Gisele Ouedraogo**

**Ecole National de l’Elevage et de la Santé Animale (ENESA) Laboratoire Vétérinaire**


**Work plan**

For the coming 18 months our group will continue to collect tsetse flies from Ghana and Ivory Coast in an effort to evaluate virus, symbiont and trypanosome prevalence. We will:
1. Set up a small *G. medicorum* colony in the laboratory.
2. Propagate the virus from different *Glossina* species (*G. p. gambiensis, G. m. submorsitans, G. tachinoides* and *G. medicorum*) to assess the SGHV genetic diversity.
3. Study the genetic variability of virus, symbionts and trypanosomes in West Africa using sequencing and phylogenetic analysis. These analyses will be based on variability of *Wolbachia* variable number of tandem repeats (VNTRs), trypanosome internal transcribed spacer (ITS) and GpSGHV diverged ORFs and/or VNTRs.
4. Study of the ecological distribution of different strains of virus, symbionts and trypanosomes in West Africa. These analyses will be based on GPS localization and genetic diversity within samples.
Institute of Tropical Medicine, Belgium
Jan Van Den Abbeele, Linda De Vooght
Collaborators: S. Kelm, Seibersdorf laboratory, S. Aksoy, W. Miller, A. Malacrida, S. Mekonnen, P. Takac

The current Sodalis expression system will be continued to be used for the expression of anti-trypanosome components (Nbs and antimicrobial peptides) in the tsetse fly midgut and evaluated for its impact on T. brucei development in the tsetse fly. The selected anti-Tbb procyclic Nb19 that was found to enhance trypanosome midgut establishment will be further characterized by epitope mapping. The Sodalis expression system could also be used to express T. congolense trans-sialidase and its variants, candidate proteins to interfere with trypanosome development in the tsetse fly midgut. We can participate in screening the existing anti-T. brucei procyclic Nb-library for Nbs that bind to recombinant T. brucei trans-sialidases. Since an efficient mother-to-offspring transmission of the transformed Sodalis is a key issue to establish a sustainable paratransgenic tsetse fly colony, we will continue to optimize this transmission and study the biological mechanism that is driving this vertical transfer of Sodalis. In addition we will assist in the analysis of the paternal transmission of Sodalis to evaluate the role of Sodalis in the molecular machinery of G. morsitans morsitans fly semen.

Additionally, we are currently investigating which immunological pathways the tsetse fly uses to control the recently established commensal Sodalis, and how the Sodalis symbiont affects these pathways and subsequently trypanosome infection/transition. For this we have established a Sodalis-free G. morsitans morsitans colony.

Laboratory of Virology, Wageningen University, The Netherlands
Just M. Vlak
Collaborators: (i) H. Kariithi, I.A. Ince, Seibersdorf laboratory (ii) I. Malele, F. Wamwiri, O. Koekemoer, F. Njiokou, T. Astan

The major objectives are: (i) to understand the control mechanisms of overt (G. pallidipes) and covert (other Glossina spp) GpSGHV infection in symptomatic and asymptomatic tsetse flies, (ii) to investigate the host responses in salivary glands to GpSGHV and parasite infections (iii) to determine the extent of genetic diversity of SGHV in Glossina spp. over Africa.

In many Glossina spp. GpSGHV seems to be present in an asymptomatic state, i.e. the virus is there either as an episome in the host nucleus, or as a low-replicating virus e.g. in salivary gland cells. Salivary glands) of symptomatic and asymptomatic G. morsitans morsitans and G. pallidipes flies will be screened for GpSGHV expression (transcription) by (deep) RNA sequencing. The role of RNAi in the control of GpSGHV expression in asymptomatic flies (G. morsitans) versus symptomatic flies will be further investigated. Asymptomatic flies (both model species) will be treated with RNAi (Dicer-2, Ago-2) in the F0 and F1 to see if the RNAi response is blocked and induces GpSGHV replication. These experiments would indicate whether RNAi is involved in the asymptomatic state of GpSGHV in. Alternatively, if RNAi is not involved, cues may emerge from
the transcript analysis whether GpSGHV and host genes are involved in maintaining the virus in an asymptomatic state.

(ii) In contrast to symbiotic bacteria, GpSGHV and trypanosomes are infectious agents specifically targeting the salivary glands of tsetse flies. To control or eliminate both GpSGHV and the parasite from the fly, it is important to know the key molecular defense responses and to select targets for paratransgenesis. The predominant approach will be a proteomic analysis of GpSGHV-infected and non-infected flies in the presence or absence of parasites.

(iii) The diversity of GpSGHV in the field is important for evaluating the potential for GpSGHV evolution and its contribution to refractoriness for trypanosomes. The first task is to identify and characterize SGHVs from the various Glossina species around sub-Saharan Africa, initially using the sequence of the standard PCR product, if need be variable genes or repeat regions (VNTRs). To obtain a much better phylogenetic picture, more divergent genes (as opposed to the very conserved genes used previously) as determined recently by comparing the Uganda and Ethiopia strain of GpSGHV to detect genetic variation. Sequences from as many G. pallidipes throughout Africa will be screened for the presence of GpSGHV and the diversity determined. When the different Glossina spp. represent separate GpSGHV lineages, the analyses would reveal the overall genetic variation and possible evolution of GpSGHV in tsetse flies in Africa.

ICIPE, Kenya
Fathiya Khamis

Infection of G. fuscipes flies with the entomopathogenic fungus Metarhizium anisopliae reduces vector competence in acquiring and transmitting Trypanosoma, in addition to the multiplication of the parasite in the host. However, the underlying mechanisms are still unknown. The objectives are therefore to understand the tritrophic interactions between fungus/parasite/tsetse.

(i) Investigate the infection process of M. anisopliae in tsetse fly and the coexistence with Trypanosoma in hemocoel, midgut and proboscis.

(ii) Investigate the effects of fungal metabolites on GpSGHV infection in situ (salivary glands) and Trypanosoma in vitro. Entomopathogenic fungi must overcome and / or avoid host immune defenses during infection process by production of metabolites such as destruxins in the insect hemocoel. For instance, destruxins cause morphological and cytoskeletal changes in insect plasmatocytes in vitro, and this adversely affects insect cellular immune responses, such as encapsulation and phagocytosis. The crude destruxins from M. anisopliae were recently reported to inhibit the gene expression and replication of hepatitis B virus (HBV) both in vitro and in vivo. They may be therefore serve as potential antiviral agents. Metabolites from several isolates of M. anisopliae will be screened for their toxic activities against GpSHGV and parasite infection. In the case of GpSHGV, metabolites will be mixed with blood for flies to feed on. Different concentrations of fungal metabolites will be injected into the culture of Trypanosoma in artificial medium. Metabolites with toxic activities will be characterized using chemical and molecular techniques.

(iii) The infection of adult G. fuscipes by M. anisopliae has an effect on their hemocytes. Heddi’s Lab has expertise in insect hemocytes, which will be used to better understand their description and cytology.

(iv) Investigate whether infection by M. anisopliae has an effect on midgut and proboscis microbiome from G. fuscipes.

(v) Investigate the interaction of trypanosomal trans-sialidase with M. anisopliae.

(vi) Investigate the interaction between infection with M. anisopliae and GpSGHV.

(vii) Screen for anti-fungal products that delay fungal killing.
University of Glasgow, UK
Marc Ciosi
Collaborators: I. Malele, F. Wamwiri, Seibersdorf laboratory (potentially also G. Ouadreago and S. Kelm)

Progress over the last 18 months:
The data collection in the presence/absence of several trypanosome species and clades of *T. congolense* in sympatric populations of tsetse of multiple species was completed for tsetse populations in Nigeria and Kenya. The analysis of that data for the Nigerian populations was completed and the results have been recently published in Parasites and Vectors (Isaac, Ciosi et al. 2016 Parasites & Vectors, 9: 301). The data analysis of the data collected for the Kenyan populations is still underway.
The data collection corresponding to the blood meal identification was initiated but revealed a high proportion (>30%) of multiple blood meals that we could not identify by Sanger sequencing.
The data collection corresponding to the estimation of the prevalence of *Sodalis glossinidius* in tsetse populations in Kenya and Nigeria was completed. These data were analyzed for the Nigerian population but remain to be analyzed for the Kenyan populations.
The development of multiplex microsatellite PCRs to genotype *T. congolense* Savannah was completed. It is of little use in the field because the number of trypanosomes in field samples is too often too low to be able to amplify the microsatellites. However, it is very useful to characterize *T. congolense* Savannah once they have been expanded into a vertebrate host in the laboratory.

Work plan for the next 18 months:
- We will complete the data analysis of the prevalence of several trypanosome species in several tsetse species collected in several sites in Kenya.
- Regarding the blood meal analysis we will clone some of the PCR products associated with multiple infection and sequence some clones to try to obtain a better understand of which hosts are represented in these multiple blood meals.
- We will complete the data analysis of the correlations of trypanosome presence/absence with that of the symbiont *S. glossinidius*.
- We will complete our analysis of the trypanosome internal transcribed spacer (ITS1) sequences obtained over the last 18 months.

Vector& Vector Borne Diseases Research Institute (VVBDRI) – Tanga, Tanzania
Imna Malele

At VVBD the following will be conducted in relation to enhancing tsetse fly refactoriness to trypanosome infection within the next 18-month period.
1) We will continue to investigate on gut microbiota and trypanosomes interaction and their role in the epidemiology of trypanosomiasis in wild tsetse species. This time we will focus on sequencing the gut microbiota isolated from *G. pallidipes* field populations from a HAT area and *G. m. morsitans* from the coastal area a non-HAT area. The obtained gut microbiota from the two species will be compared with the gut microbiota that has been obtained from *G. pallidipes* trapped from a HAT-free areas. Results from *G. pallidipes* gut microbiota indicated a dominance of the obligate endosymbiont *Wigglesworthia glossinidia* and the commensal endosymbiont *Sodalis glossinidius* and a rich diversity of bacteria (*Proteobacteria* and *Firmicutes*).
2) We will continue with research on the interaction of SGHV with other gut microbiota interaction (pathology) from *Glossina pallidipes* and *G. m. morsitans* and from any other sympatric tsetse species found in the trapping area.

3) Lastly, we will investigate on the use of entomopathogenic fungi (*Metarhizium anisopliae* isolate ICPE 30) as a tool for tsetse and trypanosomes control at another nearby site where we can easily access even when there are prolonged rainfalls. Before, our study site on the use of entomopathogenic fungi was in Meatu district, but due to prolonged rainfalls were experienced for the past two years, it was difficult to assess the impact of the control tool. The generated information will also be published in per reviewed journals (about three papers will be published).

**Faculty of Sciences and Technics, Mali**

**Mme Astan Traore**

Collaborators: Entomology and Parasitology Laboratory, G. Ouédraogo, Seibersdorf lab, S. Kelm, G. Tsiamis.

The proposed research aims to decipher the relationships between tsetse and pathogen as a component of the sterile male release technique program. To date, sampling has been conducted in Mali where trypanosomes are prevalent. A total of 307 flies have been collected from 3 locations of Mali. The flies screened of *Glossina palpalis gambiensis* indicate an average prevalence of SGHV virus at 4.88% (15/307). The average prevalence of *Wolbachia* was 3.58% and the average prevalence of *Trypanosoma* spp was 4.23%.

We will extend tsetse collections to include others regions of Mali. Collect flies along streams, using biconical traps (Charlier - Laveissiere). Each fly will be identified by sex and by species. After counting and diagnosis, all fresh tsetse be dissected the different organs (proboscis, salivary glands, midgut) will be examined under a microscope to search for possible infections of SGHV and *Trypanosoma* spp. Additional flies will be sorted by species and subsamples of 50 flies will be transferred into tube containing 15-ml of 95% ethanol for the future analysis. All samples collected will be analysis using the PCR specific primers to indicate the prevalence of SGHV, *Trypanosoma* sp, *Wolbachia* spp, *Spiroplasma* and *Trypanosoma* spp. The DNA will be extracted from caught flies using the method of Bender and al. (1983). PCR reactions will be performed and PCR product will be detected with agarose (2%) gel electrophoresis and ethidium bromide staining.

The specific activities planned in the next 18 months are:

a. Continue to collect samples:

b. Use PCR to evaluate the prevalence and dynamic of SGHV, *S. glossinidius*, *Wolbachia* spp., *Spiroplasma* and *Trypanosoma* spp. in field populations of *Glossina*;

c. Determine the prevalence of *Trypanosoma* spp.

d. Determine the relation between the symbionts *S. glossinidius*, *Wolbachia* spp, SGHV, *Spiroplasma* and *Trypanosoma* spp.

**KALRO - Biotechnology Research Institute**

**Florence Wamwiri**

Collaborators: J. Maniania, S. Mekonnen, and the Seibersdorf Laboratory

Our participation in this CRP will continue to contribute to identification of the diversity and composition of tsetse symbiome in the following species: *Glossina brevipalpis*, *G. pallidipes*, *G. f. fuscipes* and *G. austeni*. In the current meeting, we have reported on the prevalence of *Wolbachia* and *Sodalis* in tsetse samples from Arabuko-Sokoke, Shimba Hills, Nguruman, Suba and Busia, based on basic PCR. However, it has been agreed that the low prevalence rates observed may be due to use of low sensitivity tools to analyse low titre samples. In light of this, we will re-analyse
the DNA samples that have already been extracted using alternative assays e.g. Variable Number of Tandem Repeats (VNTRs) protocol and/or ARM-PCR of Miller laboratory for Wolbachia diagnosis and nested PCR for Sodalis. The activities for the coming eighteen months will focus on:

(i) *Sodalis*: Using published microsatellite markers, we aim to complete the genotyping of *Sodalis* strains and investigate this bacterium’s prevalence in various tsetse host species; investigate infection in relation to dominant host species (blood meal)

(ii) *Wolbachia*: Comparative prevalence estimation using VNTR, blot PCR or ARM-PCR protocols; and to perform multi-locus sequence tag (MLST) genotyping for *G. longipennis*

(iii) *Fungi*: In collaboration with ICIPE (J. Manania, Kenya) and S. Mekonnen (Ethiopia), we propose to investigate the effect of entomopathogenic fungi (*Metarhizium anisopliae*) metabolites on the regulation of SGHV infection in *G. pallidipes*.

(iv) Investigate the presence of *Spiroplasma* in *G. fuscipes* field isolates.

**University of Florida, Gainesville, Florida, USA**

D. Boucias

Collaborators: Seibersdorf laboratory, J. Vlak, A. İnce and H. Kariithi

The overall goals of our research is a further understanding of the SGHV pathology and the impact of infection on host fitness. Specific experiments will include the following topics:

1. RNAseq data set generated from SGHV infected *Musca domestica* will be examined for additional functional assays targeting up- and down-regulated pathways. Emphasis will be placed on the significant alterations in the innate defence systems, and the down regulation of the pathways associated with oogenesis.

2. Conduct additional RNA-seq analysis on symptomatic and asymptomatic *G. pallipides* to develop a comparative analysis of the impacts of SGHV on the tsetse versus housefly transcriptomes.

**Department of Medical Microbiology, School of Medicine, Acıbadem University, İstanbul, Turkey**

İkbal Agah İnce

Collaborators: H. Kariithi, M. Bergoin, D. Boucias, S. Aksoy and Seibersdorf laboratory.

The identification of expressed genes of viral entities is one of the main advances in the recent genomic era. Expressomics, an integrated approach applying and combining transcriptome and proteome outputs for data interpretation, was used for understanding *Glossina* hytrosavirus (GpSGHV) pathobiology. The 3-prime untranslated regions (UTRs) of GpSGHV ORFs was analyzed to find active ORFs. Following to this progress the conserved mechanisms related to viral transcriptional control will be explored. In addition, an integrated approach using the transcriptome and proteome of infected cells performed to further our knowledge on interactions between different tsetse and the two GpSGHV isolates are comparatively analyzed currently by generation of genome and proteome maps of both virus and host. Knockdown of host protein factors related to viral infection will be investigated. As there is no available genetic recombination system for the host as well as for virus (e.g. bacmid), *in vitro* dsRNA production will be used for generating knockdowns of target genes for clarifying the transcriptional regulation of this new group of viruses (Hytrosavirus). Production of a genetic recombination system, such as bacmids, will greatly enhance progress of this research proposal. The ultimate aim is to unravel the key virus-host factors responsible for viral infection and latency. This research will serve to find effective mitigation strategies for infections in tsetse rearing factories.
Work plan for the next 18 months

- To unravel the evidence that *Glossina hytrosavirus* (SGHV) involves RNAi response, the total RNA and small RNA sequencing virus-infected and uninfected in tsetse flies (*Glossina pallipides*).
  - The final mapping of small RNA of *Glossina* and *Musca* hytrosaviruses is being achieved. Next is to determine targeting hot spots of host response to virus infection.
- Mapping of 3’ untranslated region of *Glossina hytrosavirus* transcript has been performed to contribute fine mapping of the viral transcriptome and to understand the termination motifs of viral transcripts. Termination motifs are important for the development of antivirals.
  - The system of amplification of 3’ untranslated region was tested and proved practical. The next is to design size and melting temperature optimized gene specific primers to perform genome wide screening of UTRs.

**Centre for Biomolecular Interactions, Faculty for Biology and Chemistry, University of Bremen: Soerge Kelm**
Collaborators: J. Van Den Abbeele, A. Mala, F. Khamis, A. Geiger, Seibersdorf laboratory and all groups sampling flies and groups analysing symbiomes

The present research aims to acquire a better understanding of the role of trans-sialylation in the transmission and colonisation of trypanosomes between the insect vector and its mammalian host. Within the CRP the focus will be on further evaluating the potential of targeting sialidases expressed in the symbiome to prevent and/or eradicate trypanosomal colonisation and maturation in tsetse flies.

The following activities are planned for the next 18 months:
- analyse field tsetse fly midgut samples collected and isolated bacterial cultures for sialidase and sialic acid degradation activities using the enzyme assays and analytical methods established in the first periods of this CRP
- provide samples for virus and symbiont diversity studies within the CRP
- investigate the binding of trans-sialidase (TS) lectin domains to endosymbionts and entomopathogenic fungi
- screen existing bacterial isolates from tsetse midguts for sialidase activity
- develop expression system for TS variants in *Sodalis*; evaluate the secretion of active enzyme
- investigate the impact of TS expression on *Sodalis* biology in culture and in tsetse flies
- analyse TS diversity in field samples
- explore the potential of TS genes to identify *T. vivax* in field samples
- prepare *T. brucei* TS as antigens for nanobody screening and characterize epitopes for nanobodies obtained
- harmonize handling of field samples
Seibersdorf, IAEA: Abd-Alla, A.
Collaborators: W. Miller, A. Heddi, L. De Vooght, M. Kaltenpoth, G. Tsiamis, S. Mekonnen, J. Vlak, A. Ince, H. Kariithi

The Seibersdorf laboratory will be involved with several of the proposed work plans by providing services to CRP partners.

Following the preliminary results suggesting a negative impact of irradiation treatment on tsetse symbionts, we plan to conduct a detailed study on the impact of irradiation on different aspects including the following:

Plan for the next 18 months:

- Analyse the impact of irradiation treatment on the refractoriness of tsetse flies harbouring modified *Sodalis* (L. De Vooght and J. Van Den Abbeele)
- Analyse the impact of irradiation treatment on CHC (M. Kaltenpoth)
- Analyse the impact of irradiation treatment on tsetse symbiont using FISH (A. Heddi)
- Analyse the impact of irradiation treatment on tsetse symbionts in tsetse hybrids using FISH (W. Miller and A. Heddi)
- Analyse the impact of irradiation treatment on tsetse gut microbiota (G. Tsiamis)
- Analyse the impact of SGHV on tsetse immune system (J. Vlak, H. Kariithi, and A. Ince)
- Analyse the impact of SGHV infection on antimicrobial peptides (A. Heddi)
- Analyse the impact of SGHV male reproductive biology (A. Malacrida)

Institut National des Sciences Appliquées de Lyon (INSA-Lyon), Villeurbanne, France
Abdelaziz Heddi & Anna Zaidman-Rémy
Collaborators: S. Aksoy, B. Weiss, W. Miller, M. Kaltenpoth, Seibersdorf laboratory, A. Malacrida, J. Vlak, H. Kariithi, G. Tsiamis

Tsetse flies harbour three symbiotic bacteria, namely *Wigglesworthia*, *Wolbachia* and *Sodalis*. The modes of symbiont transmission, and their location in the insect tissues, were investigated during the last CRP program (Balmand et al., 2013, JIP). However, several features remain unresolved. Specifically, how these three symbiotic bacteria load and distribute during host development and across host tissues, and how they are perceived and controlled by the host immune system, remains questionable (although S. Aksoy team has made impressive advance on this field). Moreover, while the SIT technology is widely used, several aspects that may be impacted by host irradiation remain to be understood (e.g. insect competence, insect competitiveness, symbiont load and reorganization).

The research aims for the next 18 months is:

1. To pursue the precise location of tsetse endosymbionts in fly somatic and germ tissues at different stages of insect development, by using qPCR and *in situ* hybridization (FISH) methodology that allows the specific screening of bacteria through the design of specific 16S rDNA probes. We will analyse embryonic and post-embryonic stages in order to monitor the dynamics and the distribution of the three endosymbionts. With this approach, we aim to determine whether endosymbiont load and repartition evolves according to insect age and physiological demands, to identify eventual critical stages that could be targeted for pest control, and to gain insight into how larval bacteriome and milk gland are ‘coordinated’ to supply metabolic nutrition to the offspring.

2. To screen symbiont location under different conditions that could be modifying the symbiont “behaviour” inside the host. Indeed, to date symbiont localization has been
characterized in laboratory-reared tsetse colonies. However, we cannot exclude that the symbiont behaviour could be significantly different in natural flies in the wild, or altered by procedures performed in the lab i.e. in hybrid colonies and, most importantly, irradiated insects. We will use similar techniques (qPCR and fluorescent microscopy imaging following in situ Hybridization (FISH)) to compare the symbiont location under these different conditions.

3. To perform comparative immunity between *Sitophilus* weevils and *Glossina* tsetse. The genome of *S. oryzae* is currently being annotated, with special attention being paid to immune pathways, including Imd, Toll, apoptosis and autophagy. Our aim here is to exploit the weevil as a model system to identify key elements of the immune response that impact the maintenance or control of symbionts, using transcriptomic data and functional analysis tools that are available in this model. We will also test the involvement of the tsetse fly orthologous genes in symbiosis regulation.

4. To analyse the role of atacin (weevil and tsetse defensin) in the response to both symbionts as well as virus (GpSGHV).

University of Patras, Agrinio, Greece
Eva Dionyssopoulou and George Tsiamis
Collaborators: P. Takac, S. Aksoy, A. Malacrida, A. Heddi, Seibersdorf laboratory

We will be using lab and natural populations from *Glossina fuscipes fuscipes*, and *G. tachinoides* in order to: (a) isolate the *Spiroplasma* strains found in the respective tsetse flies, (b) access the prevalence of *Spiroplasma*, and examine the density of the other three bacterial symbionts (*Wolbachia, Sodalis*, and *Wigglesworthia*) in these flies. A metagenomic approach will be adopted for sequencing the *Spiroplasma* genome from *G. f. fuscipes*. The location of *Spiroplasma* at different stages of insect development will be determined using in situ hybridization. Horizontal Gene Transfer events will be further characterized in different *Glossina* species and outgroups. Provide emerging information on *Spiroplasma* PCR primers to all CRP partners.

IRD, UMR 177 InterTryp « Interactions hôtes-vector-s-parasites-environnement dans les maladies tropicales négligées dues aux trypanosomatidé », France
Anne Geiger
Collaboration: F. Njiokou, S. Kelm and Seibersdorf lab

Tsetse flies transmit African trypanosomes responsible for sleeping sickness in humans and Nagana in animals. This disease, fatal when untreated, affects many people with considerable impact on public health and economy in sub-Saharan Africa. The available drugs are inefficient and have even induced trypanosome resistance. Therefore, the investigations for novel strategies must continue, among them are alternative vector-based strategies such as the engineering of insects capable of blocking the transmission of the parasite. Field-captured flies are colonized with symbionts and a taxonomically complex microbiome acquired from their environment. In other insects, such as *Anopheles* sp., the gut microbiome was shown to prevent establishment and/or transmission of pathogens.

In the light of this IAEA-CRP, our project will aim at bringing knowledge regarding the interactions between the different tsetse fly species: *G. morsitans*, *G. palpalis*, trypanosomes and symbionts (*Sodalis* and *Wolbachia*) and microbiome going on in African Trypanosomiasis foci, Adamaua, Fontem, Bipindi and Campo in Cameroon, as well as from insectary flies. This work will be analysed for the different stages of the development of the flies.
Practically, this project will first involve entomological survey, in endemic area of Trypanosomiasis in Cameroon, with specific PCR identification of trypanosomes, symbionts and microbiome. We have first screened limited number of samples and identified high bacteria diversity using metataxogenomic analyses. Then we will look at insectary flies.

**Planning:**
- Phase 1: larger entomological surveys and tsetse fly sampling
- Phase 2: identification of the pertinent *Glossina* species; evaluation of trypanosome prevalence
- Phase 3: complementary entomological prospections and sampling
- Phase 4: characterization of the bacteriome of the next samples using metataxogenomic analysis
- Phase 5: Analyse bacterial isolates from tsetse midguts for the presence of sialidase activity

**Institute of Zoology, Slovak Academy of Sciences**
**Peter Takáč**
Collaborators: J. Van den Abbeele, S. Aksoy, G. Tsiamis, A. Malacrida, M. Kaltenpoth and Seibersdorf laboratory

The overall aim of our proposed studies is to develop novel methods for reducing tsetse fecundity (and thus population size) and trypanosome vector competency. The first step, in cooperation with the other members of CRP, is to investigate the dynamics of maternal transmission of tsetse symbionts.

**Plan for the next 18 months**
We will study the molecular aspects of host-symbiont dialogue during colonization and establishment in host tissues, knowledge critical for paratransgenic applications. Transcriptomes derived from different larval developmental stages will be obtained, and genes expressed during specific stages of maturation will be identified. Along this line, we plan to use comparative genomics to determine if orthologous genes are expressed at similar time points in other tsetse species. Silencing of selected genes related to larval development will be performed by RNA interference. The siRNA will be produced by dicer siRNA kits, and the effect of RNA interference on gene expression and fecundity will be monitored. Quantitative PCR and immunohistochemistry will be used to confirm knockdown of gene expression in selected tissues. These experiments will facilitate the identification of genes that can be targeted in an effort to reduce tsetse fecundity. We will also continue to evaluate the impact of different diet additives (e.g., bacterial extracts and B-complex vitamins) that can maintain tsetse fecundity in the absence of symbiotic bacteria. We will investigate the effect of these additives on tsetse fitness and fecundity. Finally, as a mass rearing insectary facility with laboratory colonies of *Glossina morsitans morsitans*, *G. pallidipes*, *G. palpalis gambiensis* and *G. fuscipes*, we serve as a crucial supplier of material and samples for the other CRP collaborators.

**Department of Biology and Biotechnology, University of Pavia, Pavia, Italy**
**Anna Malacrida**

Our research activities will be centered on determining the impact of symbionts and SGH virus on male reproductive physiology.

Male mating performance/fertility depends on reproductive physiology/behaviour. Thus, the outcomes of our investigations will be important for SIT improvement.
In particular, we will investigate the impact that Wolbachia, Sodalis, Wigglesworthia and SGHV may have on:

1) the composition/regulation of proteins, metabolites and sperm that are present in the semen and are transferred to the female, and
2) male mating behaviour and sperm transfer/use

Using tissue specific RNA-seq (testes and male accessory glands), genomics and proteomics data (spermatophore) from a Glossina morsitans morsitans (Gmm) strain with a complete repertoire of symbionts, we identified and quantified the relative abundance of proteins that are produced by the male and are transferred to the female. In order to gain more knowledge regarding the semen composition from this tsetse strain, we will determine the composition of metabolites present in the male semen that are transferred to female reproductive tissues (spermatophore and spermathecae). This will be done with a metabolomics approach.

In order to test the effect of symbionts and SGHV, we have already produced transcriptomic data from testes and MAG of an aposymbiotic Gmm strain as well as proteomic data from the tsetse male spermatophore.

We will produce data on the metabolite composition of the semen produced by aposymbiotic males and transferred to the females.

This will allow a comparative analysis in relation to the transcripts/proteins/metabolites that are present/absent in Wolbachia/Sodalis infected male MAGs. We will similarly create libraries from MAGs from SGH males. We will study the mating behaviour Wolbachia infected/non infected males. We will analyse the tissue localization of Wolbachia/Sodalis in testes and accessory glands from males (different ages) and in sperm storage organs of females. We will investigate the effect of polyandry and migration rate on the persistence and spreading of Wolbachia in G. pallidipes populations. Collectively our results can also provide species-specific genetic markers as well as Y-specific markers.

Max Planck Institute for Chemical Ecology, Research Group Insect Symbiosis, Jena, Germany

M. Kaltenpoth and T. Engl
Collaborators: S. Aksoy, B. Weiss, J. Van Den Abbeele, Linda De Vooght, W. Miller, A. Malacrida, P. Takáč, and the Seibersdorf laboratory

So far, we have characterized the cuticular hydrocarbon (CHC) profiles and mate choice decisions of Glossina m. morsitans upon perturbation of the microbial community by antibiotic treatment (tetracycline and ampicillin). While tetracycline treatment eliminates Wigglesworthia, Sodalis, and Wolbachia, ampicillin only affects Wigglesworthia and Sodalis, but not Wolbachia. Our results reveal significant differences in overall CHC profiles and in relative amounts of female contact sex pheromone between wildtype and antibiotic-treated flies, as well as reduced mating success of treated males and females. However, at present we cannot distinguish between a direct impact of antibiotic treatment on host physiology and indirect effects via the symbionts. Thus, we will now focus on performing (i) mate choice assays with teflon dummies treated with CHCs extracted from either wildtype or tetracycline-treated flies (collaboration with Serap Aksoy and Brian Weiss, Yale), and (ii) CHC analyses of stable G. m. morsitans lines with and without Sodalis that are not treated with antibiotics anymore (collaboration with Jan Van Den Abeele). In addition, we will assess the impact of irradiation and SGHV infection on CHC profiles in male and female G. m. morsitans (collaboration with Adly Abd-Alla, Seibersdorf) and complete our existing dataset on the chemical profiles of G. m. morsitans and G. m. centralis parental and stable hybrid lines (collaboration with Wolfgang Miller and Daniela Schneider). These experiments will yield insights on the effects of symbionts, irradiation, and hybridization on chemical profiles, which can have important implications for mate choice and sexual performance in the field.
Tsetse flies transmit African trypanosomes responsible for sleeping sickness in humans and Nagana in animals. This disease, fatal when untreated, affects many people with considerable impact on public health and economy in sub-Saharan Africa. The available drugs are inefficient and have induced trypanosome resistance. Therefore, the investigations for novel strategies must continue, among them are alternative vector-based strategies such as the engineering of insects capable of blocking the transmission of the parasite. Field-captured flies are colonized with symbionts and a taxonomically complex microbiome acquired from their environment. In other insects, such as Anopheles sp., the gut microbiome was shown to prevent establishment and/or transmission of pathogens.

In the light of the new IAEA-CRP, our project will aim at bringing knowledge regarding the interactions between the tsetse flies G. tachinoides, G. m. submorsitans, trypanosomes and symbionts (Sodalis and Wolbachia) in the African Trypanosomiasis focus, Adamaoua, in Cameroon.

Practically, this project will involve entomological survey, in endemic area of Trypanosomiasis in Cameroon, with specific PCR identification of trypanosomes, symbionts and microbiome.

Preliminary results:
Entomological prospections were done in the Faro and Deo animal trypanosomiasis focus. Tsetse fly community was dominated by Glossina tachinoides (87%) followed by G. m. submorsitans (13%). Both symbionts (Sodalis glossinidius and Wolbachia) were identified in both vector species. Trypanosoma brucei sl, T. congolense forest type and T. congolense savannah type also infected both vectors, though the prevalences were low. No significant association was observed between trypanosome infections and presence of the symbionts. Sodalis and Wolbachia samples are being genotyped. Few alleles (1 to 2) are identified per locus.

Planning for next 18 months:
- Optimization of the symbionts genotyping
- Sequencing of the amplified alleles
- Testing the association between the symbionts haplotypes and the trypanosome infection
- Analysis of a new samples of tsetse flies recently collected
- Sending samples to Seibersdorf to screen for the presence of SGHV
- Screening samples for Spiroplasma infection prevalence

Agricultural Research Council - Onderstepoort Veterinary Institute, South Africa
Otto Koekemoer
Collaborators: S. Aksoy, G. Tsiamis, A. Geiger and the Seibersdorf laboratory

New Generation Sequencing (NGS) data were generated from a total DNA extract from one G. brevipalpis specimen collected from the Hluhluwe-Imfolozi National Park. This data was analysed for bacterial diversity using several bioinformatics tools. The same procedure will be followed using G. austeni collected from the Charters Creek area. Field collection will be continued to include as large as possible a fraction of G. austeni infected with T. congolense. Trypanosome infection will be examined using both PCR and microscopy. Data from the NGS analysis will be used to advise the selection of 16S rRNA PCR primers to amplify material for determination of the diversity of microbiota in infected versus uninfected tsetse. The same technique will be used to verify the time of acquisition of gut bacteria by analysing larvae from wild and colonised flies. This
will be done to determine which bacteria are acquired from the environment and which are acquired from the mother. Colonies of *G. austeni* and *G. brevipalpis* reared at Onderstepoort Veterinary Institute (OVI) and wild specimens of the same species collected in Kwazulu Natal will be used.

**Plan for next 18 months:**
1. Continue field collections
2. NGS sequencing of total DNA extract from field collected *G. austeni*.
3. Select and use 16S rRNA primers to determine bacterial diversity in both *G. austeni* and *G. brevipalpis*, infected and not infected with trypanosomes as well as colonized specimens of both species.
4. Expose field collected flies to sterilizing doses of irradiation and repeat diversity screening by 16s rRNA sequencing.
5. Screen field samples for *Spiroplasma* infection prevalence.

**Yale School of Public Health**

**S. Aksoy/B. Weiss**

**Collaborators:** A. Malacrida, M. Kaltenpoth, G. Tsiamis, J. Van Den Abbeele, I. A. Ince, F. Wamwiri, I. Malele, P. Takac, A. Heddi and the Seibersdorf laboratory

We will work on three fronts related to achieving the CRP goals: microbiota, parasite resistance mechanisms and comparative genomics. Tsetse house a taxonomically restricted population of microbes in their gut, and the microbiotas of laboratory-reared and field-captured individuals is different. The dynamics of tsetse’s microbiota can be further impacted by the presence of trypanosomes. **Over the next 18 months we will:**

1) Use next-generation sequencing techniques to characterize the microbiota from field populations of different tsetse species, and we will compare individuals that are infected with trypanosomes to their uninfected counterparts. Additionally, we will characterize the temporal and spatial dynamics of symbiont colonization of larval tsetse tissues.

2) Focus on understanding the functional association between tsetse’s microbiota and the development and proper function of fly immune barriers (e.g., the cellular immune system and peritrophic matrix). Techniques involved will include RNA-seq based analyses of gene expression and RNAi to functionally characterize genes of interest.

3) We will annotate physiologically ‘interesting’ gene loci from the genomes of *Glossina* cluster flies.

4) We will perform comparative analyses on reproductive and immune system related genes, using genomics data from the different tsetse species.

5) We will participate in experiments that aim to understand the male accessory gland physiology

6) We will determine the role of symbionts on host behaviour/mate choice.

**Medical University, Vienna, Austria**

**Wolfgang J. Miller**

**Collaborators:** B. Weiss, S. Aksoy, A. Heddi, M. Kaltenpoth, and the Seibersdorf laboratory

Maintaining homeostasis between all symbiont players and tsetse flies is essential for tsetse symbiosis. Quantitative and qualitative interactions between tsetse and bacterial symbionts will be further deciphered that restrict symbiont titer and tissue tropism during development and in adults. Symbiont gain- and loss-of-function strategies will be applied for artificially disturbing native host-symbiont equilibrium *in vivo*, i.e. in mixed genetic host backgrounds of inter-species hybrids, upon treatment with antibiotics and gamma-irradiation. Finally, we plan to assay potential alterations in
composition of gut symbionts upon such artificial perturbations and their potential impact on host pheromonal profiles and mating success.

Specific aims within the next 18 months:

1. Monitoring and quantifying symbiont titer and tropism dynamics of *Sodalis, Wigglesworthia* and *Wolbachia* by qRT-PCR, *wsp* blot PCR and Stellaris FISH, in different age classes of (i) wild-type, (ii) antibiotic-treated, (iii) gamma-irradiated tsetse flies, plus (iv) inter-species hybrids.

2. Generate antibodies against *Wolbachia, Sodalis, Wigglesworthia, Spiroplasma* and SGHV, testing and optimizing detection protocols (BW, LD, GT).

3. Profiling gut microbiota of (i) wild-type, (ii) antibiotic-treated, (iii) gamma-irradiated tsetse flies, plus (iv) inter-species hybrids (GT).

4. Monitoring CHC signatures of (i) wildtype, (ii) antibiotic-treated, (iii) gamma-irradiated tsetse flies, plus (iv) inter-species hybrids (MK).

5. Generating isofemale lines from stabilized hybrid colonies (Seib) and consequently select for candidate lines, which lack assortative mating (Seib) but induce complete post-mating isolation (Seibersdorf lab) against parental and wild-type females.

6. Comparative analyses of the respective mitochondria and *Wolbachia* genomes of *Gmm, Gmc* and the *Gmm/Gmc* hybrid line (AA, Seibersdorf).

**General issue (All)**

International exchange of biological materials is currently subjected to the rules and regulations of the Nagoya protocol. The *Nagoya Protocol* on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity is a 2010 supplementary agreement to the 1992 Convention on Biological Diversity (CBD). Exchange of biological material should be clearly documented (source, owner).

### 4. Recommendations

1. Specify standardized markers/antibodies and protocols to detect and (semi) quantify the symbiome components as soon as possible.

2. Develop a website (hosted by George Tsiamis) that provides protocols, data sets, published material and other relevant information to facilitate communication among CRP participants.

3. Implement standard protocols for the sample collection, handling and processing.

4. In depth studies on the effects of irradiation on tsetse’s microbiome and vector competency should be intensified.

5. Identify novel effector molecules for use in tsetse paratransgenic (*Sodalis*) control strategies.

6. In conjunction with SIT, explore the development of novel, emerging strategies for improving control of tsetse-transmitted trypanosomiasis.

7. Adhere to the rules and regulations of exchange of biological materials and property rights (Nagoya protocol).
5. AGENDA

THIRD RESEARCH CO-ORDINATION MEETING
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

“Enhancing Vector Refractoriness to Trypanosome Infection”

Lyon, France

6 -10 June, 2016. INSA

AGENDA

Monday, 6th June, 2016

SESSION 1

08.00-09.00 Registration and Coffee

09.00-09.10 Abdelaziz Heddi: Welcome speech

09.10-09.20 Adly Abd-Alla: Introduction and administrative details

09.20-09.50 A. Abd-Alla: Update on research activity on tsetse fly in Seibersdorf: Impact of irradiation on tsetse symbiont, mtDNA and genotyping.

09.50-10.20 De Vooght L., Caljon G., Hussain S. and Van Den Abbeele J.: Sodalis glossinidius a delivery system for Nanobodies that target the tsetse-trypomosome interplay.

10.20-10.35 COFFEE

SESSION 2

10.35-11.05 Weber, Judith; Waespy, Mario; Nilima Dinesh; Gbem, Thaddeus T; Shaida, Stephen; Thomas Haselhorst; Joe Tiralongo; Mamman, Muhammad; Nok, Jonathan A. and Sorge Kelm.: Sialic acid, trans-sialidase and sialidase in the midgut of tsetse flies

11.05-11.35 Njokou F., Kame Ngasse G., Nana-Djeunga H., Melachio-Tanekou T. & Geiger A: Relation between the symbionts Sodalis glossinidius, Wolbachia sp and trypanosomes hosted by tsetse flies in the animal trypanosomiasis focus of Faro-Deo in Cameroon.

11.35-13.00 LUNCH

SESSION 3
<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Presentation Title</th>
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<tbody>
<tr>
<td>13.30-14.00</td>
<td>George Tsiamis, Evangelos Doudoumis, Antonios A. Augustinos, Eva Dionyssopolou, Peter Takac, Adly M. M. Abd-Alla and Kostas Bourtzis</td>
<td>Detection and Characterization of a new bacterial symbiont in tsetse flies</td>
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<tr>
<td>14.00-14.30</td>
<td>Solomon Mekonnen, Adly M. M. Abd-Alla and Andrew G. Parker</td>
<td>Salivary Gland Hyperthrophy Virus Infection Screening in <em>Glossina pallidipes</em> and <em>G. f. fuscipes</em> Colony in Ethiopia</td>
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<td>14.30-15.00</td>
<td>Martin Kaltenpoth, Tobias Engl, Veronika Michalkova, Brian Weiss, Daniela Schneider, Wolfgang Miller, Serap Aksoy</td>
<td>Antibiotic treatment affects cuticular hydrocarbon profiles and mate choice in tsetse flies (<em>Glossina m. morsitans</em>)</td>
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<td>15.00-15.15</td>
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**SESSION 4**

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<th>Time</th>
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<tr>
<td>15.15-16.45</td>
<td>Daniela I. Schneider, Andrew G. Parker, Florent Masson, Adly M. M. Abd-Alla, Erica Ras, Abdelaziz Heddi, Wolfgang J. Miller</td>
<td>Symbiont-dynamics and transmission modes in tsetse fly hybrids</td>
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<tr>
<td>16.45-17.15</td>
<td>Anna Zaidman-Rémy, Abdelaziz Heddi</td>
<td>Endosymbiont control and load adjustment to insect physiological needs</td>
</tr>
<tr>
<td>17.15-17.45</td>
<td>B. Weiss</td>
<td>An endosymbiont-regulated odorant binding protein mediates tsetse hematopoiesis</td>
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<tr>
<td>17.45-18.30</td>
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<td><strong>General discussion</strong></td>
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**Tuesday, 7th June, 2016**

**SESSION 5**

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<th>Time</th>
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<tr>
<td>08:30-09:00</td>
<td>Francesca Scolari, Grazia Savini, Serap Aksoy, Geoffrey M. Attardo, Anna R. Malacridda</td>
<td>Exploring the role of endosymbionts in tsetse male reproductive physiology</td>
</tr>
<tr>
<td>09.00-09.30</td>
<td>Veronika Michalková; Joshua B. Benoit; Brian L. Weiss; Geoffrey M. Attardo; Serap Aksoy, Peter Takáč</td>
<td>Dependence of tsetse fly fertility on nutrient homeostasis and symbiosis.</td>
</tr>
<tr>
<td>09.30-10.00</td>
<td>Florence N. Wamwiri, Samuel Guya, Patrick Obore and George Kimotho</td>
<td>Some characteristics of endosymbiont infection in selected Kenyan tsetse species and populations</td>
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<td>10.00-10.30</td>
<td>COFFEE</td>
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<td>10.30-11.00</td>
<td>SESSION 6</td>
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<tr>
<td>10.30-11.00</td>
<td>Wamiti L.G., Fathiya K.H., Ekesi S., Ombura L. and Maniania N.K.: Effect of fungal infection by Metarhizium anisopliae on vector competence to transmit Trypanosoma parasite and the effect of fungal infection on fly hemocytes.</td>
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<td>11.30-12.00</td>
<td>Astan Traore: Combined effect of deltamethrin and diminazene about the trypanosomias transmission in Mali.</td>
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<tr>
<td>12.00-13.30</td>
<td>LUNCH</td>
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<td>13.30-14.00</td>
<td>SESSION 7</td>
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<td>14.00-14.30</td>
<td>Marc Ciosi Skype presentation</td>
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<td>15.30-15.45</td>
<td>COFFEE</td>
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<td>15.45-16.15</td>
<td>SESSION 8</td>
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<tr>
<td>15.45-16.15</td>
<td>İkbal Agah İnce, Adly Abd-Alla, Henry M. Kariithi: Microgenomics of Glossinnavirus: Exploring the small RNA profiles in infected vs non infected Tsetse flies</td>
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<tr>
<td>16.45-18.00</td>
<td>General discussion</td>
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Wednesday 8th June, 2016

SESSION 9

08.30-09.45  General Discussion of the Logical Framework and CRP evaluation documents

09.45-10.15  COFFEE

10.15-11.15  General Discussion of the Logical Framework

11.15-12.00  Working Group Discussions

12.10-17.00  Excursion to Lyon (Lyon city tour)

17:00- 21:00 Group Dinner

Thursday 9th June, 2016

SESSION 10

09.00-09.40  Working Group Discussions

09.45-10.15  COFFEE

10.15-12.30  Working Group Discussions

12.30-13.30  LUNCH

13.30-15.30  Drafting Report

15.30-16.00  COFFEE

16.00-17.00  Drafting Report

Friday 10th June, 2016

SESSION 11

09.00-10.30  Reports of Working Groups and CRP evaluation

10.30-11.00  COFFEE

11.00-12.30  Drafting of CRP evaluation
12.30-13.30  LUNCH

14.00-14.30  General Discussion

  Closing Remark by Host Country representative

**Working Group 1: Symbionts**
- Weiss, İnce, Heddi, Geiger, Kaltenpoth, Koekemoer, Malacrida, Miller, Njiokou, Tsiamis, Takac, Anna

**Working Group 2: Pathogens**
- Vlak, Traore, İnce, Kelm, Kariithi, Malele, Maniania, Ouedraogo, De Vooght, Mekonen, Wamwiri
6. LIST OF PARTICIPANTS

LIST OF PARTICIPANTS TO THE THIRD RCM ON ENHANCING VECTOR REFRACTORINESS TO TRYPANOSOME INFECTION
From June 6-10, 2016, Lyon, France

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*Participant did not attend the 3rd RCM in Lyon, France*
7. NEXT MEETING

Location: Pretoria, South Africa
Period: 27 November-1 December 2017
8. ANNEX I: WORKING PAPERS

Anna Zaidman-Rémy, Abdelaziz Heddi: Endosymbiont control and load adjustment to insect physiological needs.  

Francesca Scolari1, Grazia Savini1, Serap Aksoy2, Geoffrey M. Attardo2, Anna R. Malacrida: Exploring the role of endosymbionts in tsetse male reproductive physiology.  


Brian Weiss and Serap Aksoy: An endosymbiont-regulated odorant binding protein mediates tsetse hematopoiesis.  

Flobert Njiokou, Kame Ngasse G., Farikou O, Simo G. Geiger A: Improving knowledge of the interactions between Glossina tachinoides, Glossina morsitans submorsitans, its symbionts Sodalis glosinidus, Wolbachia, and its parasites Trypanosoma sp in the African Animal Trypanosomiasis focus of Adamoua, northern Cameroon  

Florence Wamwiri, Samuel Guya, Patrick Obore and George Kimotho: Some characteristics of endosymbiont infection in selected Kenyan tsetse species and populations  

George Tsiamis., Antonios A. Augustinos, Vangelis Doudoumis, Eva Dionyssopoulou, Peter Takac, Adly M.M. Abd-Alla, Kostas Bourtzis: Characterization of gut and gonadal microbiota in lab tsetse populations revealed Spiroplasma as a new symbiotic association  

Ikbal Agah Ince: Microgenomics of Glossinavirus: Exploring the small RNA profiles in infected vs non infected Tsetse flies  

Imna Malele, Hamisi Nyingilili, Eugene Lyaruu, Anne Geiger: Bacterial Diversity Associated with Glossina pallidipes from the coastal area of Tanga and their interactions with Trypanosomes.  

Linda De Vooght, Guy Caljon & Jan Van Den Abbeele: Update on the Sodalis expression of functional anti-trypanosome nanobodies in different tsetse fly tissues  


Martin Kaltenpoth, Tobias Engl, Veronika Michalkova, Brian Weiss, Daniela Schneider, Wolfgang Miller, Serap Aksoy: Antibiotic treatment affects cuticular hydrocarbon profiles and mate choice in tsetse flies (Glossina m. morsitans).  

Wamiti L.G., Fathiya K.H., Ekesi S., Ombura L. and Nguyan K. Maniania: Effect of fungal infection by Metarhizium anisopliae on vector competence to transmit Trypanosoma parasite and the effect of fungal infection on fly hemocytes  

Otto Koekemoer: Microbial diversity in tsetse in South Africa and its influence on
vector capacity

Veronika Michalkova, Joshua B. Benoit, Brian L. Weiss, Geoffrey M. Attardo, Serap Aksoy and Peter Takáč: Tsetse fly fertility dependence on nutrient homeostasis and symbiosis


Traore Astan: Combined effect of deltamethrin and diminazene about the trypanosomiasis transmission in Mali

Daniela I. Schneider, Andrew G. Parker, Florent Masson, Balmand Séverine, Adly M.M. Abd-Alla, Abdelaziz Heddi, Wolfgang J. Miller: Symbiont-dynamics and transmission modes in tsetse fly hybrids