IAEA-D4.40.05-CR1
LIMITED DISTRIBUTION

WORKING MATERIAL

"Reproductive biology of male *Aedes* mosquitoes for SIT applications"

Report of the First Research Coordination Meeting of an FAO/IAEA Coordinated Research Project

3–7 July 2023

Reproduced by the IAEA
Vienna, Austria 2023

Table of Contents

SUMMARY

The application of the Sterile Insect Technique (SIT) in Area-Wide Integrated Pest Management (AW-IPM) programmes continues to increase in response to requests from Member States. These requests include the development and refinement of the SIT package for mosquitoes as an innovative and sustainable approach, for controlling mosquito vector populations to improve human health by reducing the burden of mosquito-borne diseases. The success of a SIT pilot project and subsequently, the operational application as a key component of a control strategies against human disease vectors depend directly on the efficiency of released irradiated male mosquitoes to effectively compete with wild males in mating with wild females to suppress target populations. In the workflow process from production to release of the sterile males, several abiotic and biotic factors can affect the male sexual performance in the field that may compromise the effectiveness of the SIT. Since the SIT is based on reducing the birth rate of the target population, it is crucial to understand various aspects of the natural history and reproductive biology of *Aedes* mosquitoes under field conditions. This CRP will focus on key knowledge gaps on male mosquito reproductive biology, particularly investigating the factors that contribute to the mating success of sterile males in SIT programmes.

BACKGROUND AND SITUATION ANALYSIS

Mosquito-borne diseases are among the most critical public health problems worldwide. In recent years, mosquito-transmitted dengue has become a significant international public health burden due to the increasing spread of invasive mosquito species of the genus *Aedes*, which also transmit Zika, chikungunya, and yellow fever. According to the World Health Organization (WHO), the incidence of dengue has grown more than 30-fold during the last 50 years. In the last decade, the number of reported cases grew from 2.4 million in 2010 to 5.2 million in 2019. Simultaneously, the number of dengue-related deaths increased four times from 960 in 2000 to 4,032 in 2015. The most significant number of dengue cases reported globally was in 2019 with the American region alone reporting 3.1 million cases in 2019, of which more than 25,000 were classified as severe cases (Oliel et al., 2019).

Aedes mosquitoes are widely distributed, and nearly half of the human population is at risk from the diseases they transmit. Disease transmission by mosquitoes has the potential to emerge as an even more significant threat as the Earth undergoes climate change, population growth, and biological invasions (Lounibos, 2002; Juliano & Lounibos, 2016; Lounibos & Kramer, 2016; Lounibos & Juliano, 2018). With the notable exception of yellow fever, most viruses transmitted by *Aedes* mosquitoes have no specific drug treatments or reliable vaccines. Due to the lack of these clinical interventions, disease control heavily depends upon suppressing mosquito populations. While numerous approaches to reduce *Aedes* vectors have been deployed over the years, including chemical, environmental, educational, and genetic measures (MacGregor & Connelly 2021), controlling these mosquitoes is still challenging. Historically, vector control has relied heavily on the use of insecticides. However, this control method has not prevented the increasing number of cases and has made the problem more complex due to increasing rates of insecticide resistance in mosquitoes (Vontas et al., 2012; Francis et al., 2021).

The Sterile Insect Technique (SIT) is an environmentally friendly AW-IVM alternative. In the last two decades, the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, in collaboration with its Member States has developed and evaluated the technology for controlling mosquito vector populations as a component of the AW-IVM. This control approach relies on the systematic area-wide release of sterile insects over infested areas, where males mate with wild females, resulting in no offspring and consequently reducing the pest population. The viability of the technological package has been demonstrated in several pilot projects in various parts of the world (i.e., Brazil, China, Cuba, Germany, Greece, Italy, Mauritius, Mexico, Singapore, Spain, Sri Lanka, Thailand, and USA) and an engaged community of practitioners is emerging (Oliva et al., 2021; Gouagna et al., 2020; Lees et al., 2014, 2021). In recent years, the SIT has been deployed against three species of *Aedes* such as *Ae. aegypti*, *Ae. albopictus*, *Ae. polynesiensis.*

The SIT requires the mass-production, sterilisation, handling, transportation, release, and field monitoring of millions of male mosquitoes. The success of the SIT relies on the efficiency of released irradiated male mosquitoes to compete with wild males in mating with wild females to suppress the target populations. Maximising efficiency and insect fitness at each stage of the SIT processes is critical to maintaining male sexual competitiveness and, ultimately, programmatic success (Gómez et al., 2023). However, in between producing and releasing sterile males, several abiotic and biotic factors can affect male sexual performance in the field which may compromise the effectiveness of the SIT. Recent experiences from field projects have made it possible to identify gaps in knowledge or areas of opportunity that could make the technology more effective and at a competitive cost compared with alternatives. Knowledge about the reproductive biology and physiology of *Aedes* mosquitoes represents one such area of opportunity.

Most research on mosquito reproduction has been done under laboratory conditions. Meanwhile, the impact of mass-rearing, irradiation and handling processes on the reproductive biology and physiology, and, ultimately on the sexual performance of the sterile mosquitoes in the field is still poorly understood. Since SIT is based on reducing the birth rate of the target population, the need to understand various aspects of the natural history, reproductive biology and behaviours involved in mating of *Aedes* mosquitoes under field conditions is evident since they have implications for SIT. Therefore, the success of the SIT as an industrial process can be significantly enhanced by crucial insights derived from studying the biology and natural history of the species targeted (Dyck et al., 2021).

As in other pest and vector species against which SIT is applied, the target of interest are the males and the mating system wherein they operate. Aedine males face various challenges on their road to reproductive success (Yuval, 2006; Oliva et al., 2014). In the first 24 hours following emergence, they must erect fibrillae on their antennae to enable them to respond to female wingbeat frequencies (Roth, 1948). Concurrently, their genitalia, which at emergence are literally "twisted out of shape," must be rotated to the correct position for copulation and insemination. Sexually mature males must actively search for and encounter receptive females.

In most medically relevant genera of mosquitoes, mating occurs in swarms (Yuval, 2006). "Swarming" describes the initiation of male pre-copulatory behaviours and species-specific flight patterns associated with mating. Thus, a single mosquito can be said to be swarming (Gibson, 1985). When many individuals initiate swarming behaviour synchronously, they form an aggregation called a "swarm" with interspecific variation in aggregation sizes, swarm locations, and circadian patterns (Hartberg; 1971). Unlike anophelines, *Aedes* mosquitoes (i.e., *Stegomyia* group), do not form crepuscular stationary swarms, and may encounter females in the vicinity of the host throughout the day, along with other possible encounter sites including "smaller swarms" around attracting objects (e.g., dark objects, drainage openings, hosts, etc.). *Ae. aegypti,* for example, can form small swarms around the human host, which coincides with peak feeding activity (Hartberg; 1971; Cator et al., 2011). It is currently thought that males and females use a range of olfactory and acoustic cues for mate recognition and courtship (Cabrera & Jaffe, 2007; Fawaz et al., 2014; Pitts et al., 2014; Cator & Harrington, 2011; Andrés et al., 2020). Males perceive the flight tone produced by the beating wings of females, which elicits a strong tactic response (Roth 1948). Males pursue females in flight and copulas are formed in midair within swarms. Females can actively reject potential mates using evasive flight, kicks, and abdomen tilts (Gwadz & Craig, 1968; Jones & Pilitt, 1973; Benelli, 2015; Benelli et al., 2015; Aldersley & Cator 2019) and males are persistent and will attempt to mate with a female even after rejection (Aldersley & Cator, 2019).

In *Ae. aegypti*, copula formation is a complex and dynamic process involving the integration of multiple behaviours mediated by rapidly fluctuating acoustic interactions, as described by Aldersley & Cator (2019). Before mating, males and females can interact acoustically by harmonising their flight tones in a behaviour called "harmonic convergence", which plays a crucial role in mate choice (Cator et al., 2009) and increases matting success (Cator & Harrington, 2011). Males and females interact acoustically by shifting their flight tones to match, resulting in a courtship duet. This matching is not made at the fundamental wing beat frequency of around 400 Hz (for females) or around 600 Hz (for males) but at a shared harmonic frequency of around 1200 Hz exceeding the previously known upper limit of hearing in mosquitoes (Cator et al., 2009). Furthermore, through analysis of the structure of behavioural and acoustic interactions during mating attempts, studies also explored how different aspects of this process relate to the likelihood of female rejection, and ultimately, male mating success or failure. Females were observed to exert a high degree of influence over the eventual outcome of a given mating attempt (Aldersley & Cator, 2019). In recent studies, for example, rejection kicking has been ubiquitously observed among mating attempts (95.5% of all interactions contained female kicks). Displacement of the male through kicking resulted in his active rejection by the tethered female, which was the most recorded interaction outcome (50.4% of attempts). Although most of the mating attempts involved some degree of kicking, females' responses differed in attempts that eventually resulted in successful mating. These findings support the role of female choice as a decisive factor in determining

the male mating success of *Ae. aegypti* (Aldersley & Cator 2019). This rejection-kicking behaviour has also been observed in *Ae. albopictus* during the courtship, if females do not allow genital contact, they then perform rejection kicks and/or fly away (Benelli, 2015). Studies conducted in semi-natural conditions with this species have also evidenced the effect of daylight hours on male mating behaviour, with this behaviour less successful in the early afternoon, over the morning and late afternoon (Benelli, 2015).

Copulation and insemination processes in the field are brief yet may be relatively protracted in laboratory strains (Oliva et al., 2014). In *Aedes* mosquitoes, the copulation process is brief and overall can take less than 1 minute (Roth, 1948; Oliva et al., 2013). Mosquito males can inseminate multiple females over their lifetime (Oliva et al., 2012; Oliva et al., 2013; Boyer et al., 2011). Nonetheless, laboratorial studies report a difference in the number of females that can be successfully inseminated by a single male, with factors such as body size and "male condition" (i.e., male mating history) impacting male insemination capacity (Helinski & Harrington, 2011; Alfonso-Parra et al., 2014). During mating, males transfer an ejaculate containing sperm and male accessory gland secretions (MAGs). After mating, sperm are stored within the three spermathecae of the female (Carrasquilla et al., 2019). In inseminated females, sperm numbers vary with male age and condition but are in the range of 2000-6000 (Oliva et al., 2014). MAGs contain a cocktail of bioactive molecules that affect female postcopulatory behaviour, mainly related to subsequent receptivity and reproductive metabolism (Helinski et al., 2012a; Sirot et al., 2011; League et al., 2019; Meuti & Short, 2019). A sequence of multiple mating over short periods depletes seminal fluid components (Helinski & Harrington, 2011; Alfonso-Parra et al., 2014) that can affect female fecundity and longevity (Helinski & Harrington, 2011). In wild males, sperm and MAG products are replenished after mating, but it is unclear what happens in mass-reared and sterilised males (Ramírez-Sanchez et al., 2020).

The potential role of nutrition, and hormonal and semiochemical factors on the sexual performance of male mosquitoes has not yet been widely explored for mosquitoes as in other systems such as fruit flies. Previous work in *Ae. aegypti* found that male body size significantly affected sperm and MAGs depletion, possible affecting female fecundity and longevity. *Ae. aegypti* females mated to smaller males in sequence experienced a more rapid loss of fecundity than females mated to large males (Helinski & Harrington, 2011). High larval densities and artificial diets with different compositions are used for the mass-rearing process. This can alter male size and reproductive physiology resulting in field disadvantages for sterile mosquitoes. Besides, recent evidence suggests that *Culex pipiens* males, which consumed low sucrose diets, had significantly smaller male accessory glands, an altered metabolic profile and sired fewer offspring than males that were reared on high-nutrition diets (Huck et al., 2021). This may be significant in the context of SIT targeting mosquito species as it could impact female pots-mating behaviour.

Aedes female are, for the most part, monogamous. Nevertheless, recent studies suggest that, following mating, the window of receptivity may remain open briefly before shutting down for the duration of the female's life (Helinski et al., 2012b; Degner et al., 2019). Factors affecting male condition, such as age (Agudelo et al., 2021) and sperm depletion (Ramírez-Sanchez et al., 2020), significantly impact the propensity of females to remate. In the field, only 6% of *Ae. aegypti* females were found to have mated with multiple males (Richardson et al., 2015). The emerging scenario suggests that the onus of limiting female receptivity lies in males. Failure of mass-reared sterile males to do so may jeopardise the success of the SIT. Therefore, studying the factors that can affect the reproductive physiology of sterile males could prove crucial to manipulate (if needed) rearing conditions (e.g., larval densities, artificial diet composition) and improve the insemination capacity of sterile males in nature to reduce mosquito populations. Previous work found evidence for the role of genetic determinants of male competitive mating success and genes corresponding to the removal of sexual selection have been

identified (Wyer et al. 2023). These findings suggest that the selective environment experienced by adults plays a crucial role in shaping mating phenotypes (Qureshi et al., 2019, Wyer et al., 2023).

The underlying assumption of this proposal is that male mating success is not random, and it may be significantly enhanced by identifying the factors that determine the success of males reared and released in SIT programmes. Accordingly, we aim to determine the biological factors relevant to the SIT that affect male performance. We propose research on the precopulatory behaviour, copulation and insemination processes, and female remating behaviour, and their relevance to the SIT application, specifically:

1. **Pre-copulatory behaviour:**
    a. What are the primary mate encounter sites in the field? When are the secondary sites used? What characterizes the males using them (e.g., age, nutrition, size)?
    b. Do mass-reared males exhibit similar behaviors toward encounter sites under the same constraints?
    c. What is the role of olfaction in species and mate recognition? Can specific molecules and their receptors be identified?
    d. Are these compounds and receptors present in mass-reared males?
    e. What are the factors that affect harmonic convergence in Aedines? Can manipulation of male condition improve or disrupt their sexual performance in this task?
    f. Do sterile and fertile mass-reared males exhibit similar harmonic convergence abilities compared to wild males?

2. **Copulation and insemination**
    a. What factors affect sperm numbers and volume of MAG secretions transferred to females?
    b. Does mass-rearing and sterilization affect the insemination ability of males and the ability to replenish ejaculate?
    c. How is sperm allocated and used in repeated oviposition events?
    d. Is the sperm from mass-reared and sterilized males stored and allocated in a similar manner?
    e. Can male condition (size, larval and adult diets, microbiome) affect sperm storage and use?

3. **Female remating**
    a. What is the remating rate in the field for the various *Aedes* species? Does it vary by male condition, locality, season, or population density?
    b. Do mass-reared and sterilized males inhibit female receptivity to remating as effectively as wild males? Can this ability be manipulated?

COORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on a Consultants' Meeting which was held in Vienna, IAEA headquarter from 23-27 May (report available) to explore and discuss the need to conducting research & development activities on sexual male performance to support and improve the SIT package to mosquito control and based on the gathered information to formulate a CRP proposal on *"Reproductive Biology of male Aedes mosquitoes for SIT Applications".*

The overall objective of this new **CRP D4.40.05**, approved for the period **2023–2027,** is to characterize the mating system of *Aedes* mosquitoes from the male perspective and determine whether and how natural factors, and those related to the SIT application, affect male sexual performance. The CRP is focused on the following three thematic areas to improve the male sexual performance for SIT application against *Aedes* species: 1. Characterize pre-copulatory behaviour in laboratory and natural conditions; 2. Study copulation and insemination processes, and 3. Determine patterns of female remating and factors that control it.

FIRST RESEARCH CO-ORDINATION MEETING (RCM)

The first RCM was held in Vienna, IAEA headquarter from 3-7 July 2023, attended by 21 scientists from 13 Member States including Albania, Argentina, Burkina Faso, Cuba, China, France, Indonesia, Italy, Greece, Jamaica, Mexico, United Kingdom, and United State of America. The list of participants is given in **Annex 1**. The meeting agenda is attached in **Annex 2.**

During the meeting, participants presented research relevant to the CRP and discussed theirs proposed research plans for the entire five years of the CRP. The meeting also included general discussions on the three mentioned-thematic areas to improve the male sexual performance for SIT application against *Aedes* species. The objectives of the CRP, the Logical Framework and the proposed outcomes were reviewed and discussed with the participants to agree on minimum outputs to be achieved at the end of the CRP. Furthermore, the Chief Scientific Investigators (CSI's) were divided into two working groups **(Annex 3)** for developing a detailed technical programme to be conducted during the first 18 months of the CRP for each of them. The potential subjects for collaboration were also discussed during the meeting.

Abstracts of the presentations are compiled in **Annex 4** and a copy of all Power point presentations was made available to all participants at the end of the meeting in the TEAMS group specially created for this RCM.

The research areas were grouped into three major topics as follows:

1. **Precopulatory behaviour of *Aedes* mosquitoes (**aggregation, wingbeat, harmonic convergence, pheromones, copulation sites).
2. **Copulation and insemination process in *Aedes* mosquitoes (**ejaculate composition and storage by females, timing and place of copulation, male sexual maturity, spermatogenesis and recovery of fertility, MAGs).
3. **Patterns of female remating and factors that control it (**male condition, locality, season, population density).

LOGICAL FRAMEWORK

Table 2: Logical framework of the CRP proposal on *"Reproductive biology of male Aedes mosquitoes for SIT applications"* including the CRP's objectives and outcomes/outputs to be achieved at the end of the CRP.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective The main objective of this CRP is to characterize the mating system from the male perspective and determine how natural factors, and those related to SIT application, affect male sexual performance.	N/A	N/A	Demands by Member States about mosquito control population using the SIT are increasing. A better understanding of mosquito reproductive biology and the factors related to the SIT that affect male sexual performance will improve the SIT, ensure its successful implementation against *Aedes* species, and mitigate the risks of failures. Availability of expertise and resources required to conduct research on mating behavior of *Aedes* species.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Specific objectives 1. Characterize pre-copulatory behaviour in laboratory and natural conditions.	Pre-copulatory behaviour of wild and mass-reared strains is characterized.	Reports and published papers.	Mass-rearing process affects the pre-copulatory behaviour of male *Aedes*.

2. Study copulation and insemination processes.	Copulation, insemination, and sperm allocation patterns are determined for mass-reared sterile and wild males.	Reports and published papers.	Mass-rearing and sterilization process affects the copulation and insemination abilities of male *Aedes*.
3. Determine patterns of female remating and factors that control it.	Factors affecting female remating determined.	Reports and published papers.	Mass-rearing and sterilization process affects male ability to inhibit female receptivity.

Project Design Elements	**Objective Verifiable Indicators**	**Means of Verification**	**Important Assumptions**
Outcomes (Results) 1. Detailed understanding of factors affecting pre-copulatory behaviour of mass-reared and wild males.	Research on the effects of mass-rearing and sterilization on pre-copulatory behaviour conducted.	Technical reports and papers documenting factors affecting pre-copulatory behaviour of mass-reared and wild males published.	Results obtained can be used by Member States to improve SIT application protocols.
2. Patterns of ejaculate transfer and sperm storage are elaborated for wild and mass-reared *Aedes*.	Research on copulation and inseminations abilities of mass-reared sterile males compared with wild males conducted.	Technical reports and papers documenting patterns of ejaculate transfer and storage for wild and mass-reared *Aedes* published.	Results obtained can be used by Member States to improve SIT application protocols.
3. Patterns of female receptivity to remating determined for wild and mass-reared sterile *Aedes*.	Research on factors affecting wild and sterile males' ability to inhibit female remating conducted.	Technical reports and papers documenting patterns of female receptivity to remating determined for wild and mass-reared sterile *Aedes* published.	Results obtained can be used by Member States to improve SIT application protocols.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Outputs (Products)			
Output 1.1- Pre-copulatory behaviour patterns of wild and mass-reared males characterized.	Effects of male condition on pre-copulatory behavior determined.	Research on effects of male condition on pre-copulatory behavior is documented in the scientific literature.	Research can reveal how mass rearing affects the pre-copulatory behaviour patterns of *Aedes* males.
Output 1.2 - Role of olfaction in mate recognition by mass-reared and wild males studied.	Effects of semiochemicals on mate recognition identified.	Research on effects of olfaction in mate recognition by mass-reared and wild males is documented in the scientific literature.	Research can reveal how mass rearing affects the role of olfaction in mate recognition of *Aedes* males. Male condition affects harmonic convergence in *Aedes*.
Output 1.3 - Effects of male condition (e.g. size, larval and adult diets, microbiome, sterility) on successful harmonic convergence studied.	Conditions determining variations in male harmonic convergence are revealed.	Research on effects of male condition on harmonic convergence is documented in the scientific literature.	
Output 2.1 - Factors affecting sperm and male accessory gland secretions transfer to females determined.	Variation in sperm and male accessory gland secretions transfer by wild and mass-reared males analysed.	Research on factors affecting sperm and male accessory gland secretions transfer to females is documented in the scientific literature.	Mass-rearing and sterilization affect the outcome of ejaculate transfer and storage.
Output 2.2 - Impact of male condition (e.g. size, larval and adult diets, microbiome, sterility) on patterns of sperm allocation and use by females determined.	Effects of male condition on patterns of sperm allocation and use by females analysed.	Research on effects of male condition on patterns of sperm allocation and use by females is documented in the scientific literature.	Male condition affects sperm allocation and use by females.
Output 3.1 -Effects of male condition, locality, season, and population density on female remating determined.	Variation in female remating determined.	Research on effects male condition, locality, season, and population density on female remating is documented in the scientific literature.	Male condition, locality, season, and population density affect female remating.

Output 3.2 - Effect of mass-reared sterile male condition on inhibition of female receptivity assessed.	Optimal conditions for inhibiting female remating by mass-reared sterile males determined.	Research on effects mass-reared sterile male condition on inhibition of female receptivity is documented in the scientific literature.	Optimization of sterile male ability to inhibit females remating is achievable.

Project Design Elements	**Objective Verifiable Indicators**	**Means of Verification**	**Important Assumptions**
Activities			
1. Announce project amongst established entomologists working in mosquito area-wide SIT operational programmes.	Proposals evaluated and 10 Research Contracts, 10 Research Agreements.	Signed contract and agreements.	Suitable proposals submitted, funding available and approval of Contract and Agreements by CCRA-NA committee.
2. Organize first RCM to refine the logical framework and plan the overall activities of the CRP (Q2 2023).	1st RCM held Q2 2023.	Participants' activities and logical framework reviewed.	Contracts and Agreements signed by counterpart organizations.
3. Prepare necessary research protocols to contract holders.	Research protocols available.	Reports and protocols.	Research protocol will be implemented by qualified scientists. Methods and resources available.
4. Conduct applied research and development.	New knowledge created on mosquito reproductive biology.	Scientific papers and reports from the participants.	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
5. Organize second RCM to analyse progress in delivering research	2nd RCM held Q1 2025.	Participants and RCM Progress Reports.	Progress satisfactory.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
outputs and plan the next phase of the project (Q1 2025).			
6. Conduct applied research and development.	New knowledge created on mosquito reproductive biology.	Scientific papers and reports from the participants report.	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
7. Review the CRP after its third year.	Satisfactory progress of research agreements and technical contract	Participants and RCM Progress Reports.	Progress satisfactory and mid-CRP evaluation approved by CCRA-NA committee.
8. Organize third RCM to analyse progress in delivering the research outputs and plan the final phase of the project (Q3 2026).	3rd RCM to be held Q3 2026.	Participants and RCM Progress Reports.	Progress satisfactory.
9. Conduct applied research and development.	New knowledge created on mosquito reproductive biology.	Scientific papers and reports from the participants.	Methods and resources available.
10. Organize final RCM to assess the success of the CRP in reaching its objectives and review the final publication (Q1, 2028).	4th RCM to be held Q1 2028.	Participants and RCM Final Reports	Final reports are submitted to the Agency.
11. Evaluate the CRP and submit evaluation report.	Satisfactory completion of research agreements and technical contract	Report. Scientific publications.	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
12. Publish the results of the CRP in a special issue of a peer reviewed journal.	At least 20 publications accepted.		Consensus can be found on appropriate peer review journal and acceptance by journal obtained.

LIST OF REFERENCES

Aldersley, A. and Cator, L.J., 2019. Female resistance and harmonic convergence influence male mating success in *Aedes aegypti*. *Scientific Reports.* 9 (1), 2145.

Aldersley, A., Champneys, A., Homer, M. and Robert, D., 2016. Quantitative analysis of harmonic convergence in mosquito auditory interactions. *Journal of The Royal Society Interface*. 13 (117), p.20151007.

Alfonso-Parra, C., Avila, F. W., Deewatthanawong, P., Sirot, L. K., Wolfner, M. F., Harrington, L.C. 2014. Synthesis, depletion and cell-type expression of a protein from the male accessory glands of the dengue vector mosquito *Aedes aegypti*. *Journal of Insect Physiology.* 70, 117–124.

Andrés, M., Su, M.P., Albert, J. and Cator, L.J., 2020. Buzzkill: targeting the mosquito auditory system. *Current Opinion in Insect Science*. 40,11-17.

Agudelo, J., Alfonso-Parra, C., Avila. W. F. 2021. Male age influences re-mating incidence and sperm use in females of the dengue vector *Aedes aegypti*. *Frontiers in Physiology.* 12, 691221.

Benelli, G., 2015. The best time to have sex: mating behaviour and effect of daylight time on male sexual competitiveness in the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae). *Parasitology Research*. 114 (3), 887-894.

Benelli, G., Romano, D., Messing, R.H. and Canale, A., 2015. First report of behavioural lateralisation in mosquitoes: right-biased kicking behaviour against males in females of the Asian tiger mosquito, *Aedes albopictus*. *Parasitology Research*. 114, 1613-1617.

Boyer, S.; Gilles, J., Merancienne, D., Lemperiere, G., Fonteniele, D. 2011. Sexual performance of male mosquito *Aedes albopictus*. *Medical and Veterinary Entomology.* 25 (4), 454-9.

Cabrera, M. and Jaffe, K., 2007. An aggregation pheromone modulates lekking behavior in the vector mosquito *Aedes aegypti* (Diptera: Culicidae). *Journal of the American Mosquito Control Association*. *23* (1), 1-10.

Carrasquilla, M.C., Lounibos, L.P., Honorio, N.A. and Murr, S., 2019. Spermathecal filling in *Aedes aegypti* and *Aedes albopictus*: effects of female and male body sizes and species. *Journal of Medical Entomology*. 56 (2), 334-340.

Cator, L.J., Arthur, B.J., Harrington, L.C., Hoy, R.R. 2009. Harmonic convergence in the love songs of the dengue vector mosquito. *Science*. 323 (5917), 1077-1079.

Cator, L.J., Harrington, L.C. 2011. The harmonic convergence of fathers predicts the mating success of sons in *Aedes aegypti*. *Animal Behaviour*. 82 (4), 627-633.

Cator, L.J., Benjamin, A.J., Ponlawat, A., Harrington, L.C. 2011. Behavioral observations and sound recordings of free-flight mating swarms of *Ae. Aegypti* (Diptera: Culicidae) in Thailand. *Journal Medical Entomology*. 48, 941–946.

Cator, L.J. and Zanti, Z., 2016. Size, sounds and sex: interactions between body size and harmonic convergence signals determine mating success in *Aedes aegypti*. *Parasites & Vectors*. 9, 1-11.

Degner, E.C., Ahmed-Braimah, Y.H., Borziak, K., Wolfner, M.F., Harrington, L.C., Dorus, S. 2019. Proteins, transcripts, and genetic architecture of seminal fluid and sperm in the mosquito *Aedes aegypti*. *Molecular & Cellular Proteomics.* 18, S6-S22.

Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.), Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. CRC Press, Boca Raton, FL, USA. 1216pp.

Fawaz, E.Y., Allan, S.A., Bernier, U.R., Obenauer, P.J. and Diclaro, J.W., 2014. Swarming mechanisms in the yellow fever mosquito: aggregation pheromones are involved in the mating behavior of *Aedes aegypti*. *Journal of Vector Ecology*. 39 (2), 347-354.

Francis, S., Frank, C., Buchanan, L., Green, S., Stennett-Brown, R., Gordon-Strachan, G., Rubio-Palis, Y., Grant, C., Alexander-Lindo, R.L., Nwokocha, C. and Robinson, D., Delgoda, R. 2021. Challenges in the control of neglected insect vector diseases of human importance in the Anglo-Caribbean. *One Health.* 13, p.100316.

Gómez, M., Johnson, J. B., Bossin, C. H., Argilles-Herrero, R. 2023. Joint FAO/IAEA Coordinated Research Project on "*Mosquito Handling, Transport, Release and Male Trapping Methods*" in Support of SIT Application to Control Mosquitoes. Insects. 14 (2), 108.

Gibson, G. 1985. Swarming behaviour of the mosquito *Culex pipiens quinquefasciatus*: a quantitative analysis. *Physiological Entomology.* 10, 283–296.

Gouagna, L.C., Damiens, D., Oliva, C.F., Boyer, S., Le Goff, G., Brengues, C., Dehecq, J.-S., Raude, J., Simard, F., Fontenille, D., 2020. Strategic approach, advances, and challenges in the development and application of the SIT for Area-Wide control of *Aedes albopictus* mosquitoes in Reunion Island. *Insects.* 11(11),770.

Gwadz, R. W., Craig, G. B. Jr, 1968. Sexual receptivity in female *Aedes aegypti*. *Mosquito News*. 28, 586–593.

Hartberg, W. (1970) Observations on the mating behavior of Aedes aegypti in nature. *Bulletin of the World Health Organization.* 45, 847–850.

Helinski, M.E.H., Harrington, L.C. 2011. Male Mating History and Body Size Influence Female Fecundity and Longevity of the Dengue Vector *Aedes aegypti*. *Journal of Medical Entomology*. 48 (2), 202-211.

Helinski, M.E.H., Deewatthanawong, P., Sirot, L.K., Mariana F. Wolfner, M.F., Harrington, L.C. 2012a. Duration and dose-dependency of female sexual receptivity responses to seminal fluid proteins in *Aedes albopictus* and *Ae. aegypti* mosquitoes. *Journal of Insect Physiology*. 58 (10), 1307-1313.

Helinski, M.E.H., Valerio, L., Facchinelli, L., Scott, T.W., Rasmey, J., Harrington, L.C. 2012b. Evidence of Polyandry for *Aedes aegypti* in Semifield Enclosures. *American Journal of Tropical Medicine and Hygiene.* 86 (4), 635-641.

Huck, D. T., Klein, M.S., Meuti, M. E. 2021. Determining the effects of nutrition on the reproductive physiology of male mosquitoes. *Journal of Insect Physiology*. 129,104191.

Juliano, S.A., Lounibos, L.P., 2016. Invasions by mosquitoes: The roles of behaviour across the life cycle. *Biological Invasions and Animal Behaviour.* 245, 221–244.

Jones, J.C., Pilitt, D.R., 1973. Observations on the sexual behavior of free-flying *Aedes aegypti* mosquitoes. *The Biological Bulletin*. 144 (3), 480-488.

League, G.P., Baxter, L.L., Wolfner, M. F., Harrington, L.C., 2019. Male accessory gland molecules inhibit harmonic convergence in the mosquito *Aedes aegypti*. *Current Biology*. 29 (6), R196-R197.

Lees R.S., Knols B., Bellini R., Benedict M.Q., Bheecarry A., Bossin H.C., Chadee D.D., Charlwood J., Dabire R.K., Djogbenou L., 2014. Improving our knowledge of male mosquito biology in relation to genetic control programmes. *Acta Tropica*. 132, S2–S11.

Lees R.S., Carvalho D.O., Bouyer J., 2021. Potential impact of integrating the sterile insect technique into the fight against disease-transmitting mosquitoes. In: Dyck V.A., Hendrichs J., Robinson A.S., editors. Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. CRC Press; Vienna, Austria, 1082–1118.

Lounibos LP., 2002. Invasions by insect vectors of human disease. *Annual Review of Entomology.* 47, 233-266.

Lounibos, L.P., Juliano, S.A., 2018. Where vectors collide: the importance of mechanisms shaping the realized niche for modeling ranges of invasive *Aedes* mosquitoes. *Biological Invasions*, 20, 1913-1929.

Lounibos, L.P., Kramer, L.D., 2016. Invasiveness of *Aedes aegypti* and *Aedes albopictus* and vectorial capacity for Chikungunya virus. *Journal of Infectious Diseases*, 214, S453-S458.

McGregor, B.L., Connelly, C.R. 2021. A Review of the Control of *Aedes aegypti* (Diptera: Culicidae) in the Continental United States, *Journal of Medical Entomology*, 58 (1), 10–25.

Meuti, M.E., Short, S.M., 2019. Physiological and environmental factors affecting the composition of the ejaculate in mosquitoes and other insects. *Insects*, 10 (3), pp.74.

Oliel, S., Baldwin, A. Linn, L., 2019. OPS/OMS| VIH/SIDA. *Pan American Health Organization/World Health Organization. https://www. paho. org/hq/index. php*.

Oliva, C.F., Jacquet, M., Gilles, J., Lempérière, G., Maquart, P.-O., Quilici, S., Schoone-man, F., Vreysen, M.J.B., Boyer, S., 2012. The sterile insect technique for controlling populations of Aedes albopictus (Diptera: Culicidae) on Reunion Island: mating vigour of sterilized males. *PLOS ONE*. 7 (11), e49414.

Oliva, C.F., Damiens, D., Vreysen, M.J.B., Lempérière, G., Gilles, J., 2013. Reproductive strategies of Aedes albopictus (Diptera: Culicidae) and implications for the Sterile Insect Technique. *PLOS ONE*. 8 (11), e78884.

Oliva, C.F., Damiens, D., Benedict, M.Q., 2014. Male reproductive biology of *Aedes* mosquitoes. *Acta Tropica.* 132, S12-S19.

Oliva, C.F., Benedict, M.Q., Collins, C.M., Baldet, T., Bellini, R., Bossin, H., Bouyer, J., Corbel, V., Facchinelli, L., Fouque, F., Geier, M., Michaelakis, A., Roiz, D., Simard, F., Tur, C., Gouagna, L.C., 2021. Sterile Insect Technique (SIT) against *Aedes* species mosquitoes: A roadmap and good practice framework for designing, implementing and evaluating pilot field trials. *Insects* 12 (3),191.

Papadopoulos, N.T., Katsoyannos, B.I., Kouloussis, N.A., Hendrichs, J.P. 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata. Entomologia Experimentalis et Applicata.* 99 (2), 253-261.

Pereira, R., Yuval, B., Liedo, P., Teal, P.E.A., Shelly, T.E., McInnis, D.O., Hendrichs, J., 2013. Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. *Journal of Applied Entomology.* 137, S178-S190.

Pitts, R.J., Mozūraitis, R., Gauvin-Bialecki, A. and Lempérière, G., 2014. The roles of kairomones, synomones and pheromones in the chemically-mediated behaviour of male mosquitoes. *Acta Tropica*. 132, S26-S34.

Qureshi, A., Aldersley, A., Hollis, B., Ponlawat, A. and Cator, L.J., 2019. Male competition and the evolution of mating and life-history traits in experimental populations of *Aedes aegypti. Proceedings of the Royal Society B. 286*(1904), 20190591.

Ramírez-Sánchez, L.F., Camargo, C. and Avila, F.W., 2020. Male sexual history influences female fertility and re-mating incidence in the mosquito vector *Aedes aegypti* (Diptera: Culicidae). *Journal of Insect Physiology*. 121, 104019.

Richardson, J.B., Jameson, S.B., Gloria-Soria, A., Wesson, D.M. and Powell, J., 2015. Evidence of limited polyandry in a natural population of *Aedes aegypti. The American Journal of Tropical Medicine and Hygiene*. 93 (1), p.189.

Roth, L.M., 1948. A study of mosquito behavior. An experimental laboratory study of the sexual behavior of *Aedes aegypti* (Linnaeus). *The American Midland Naturalist*. 40 (2), 265-352.

Shelly, T.E., Holler, T.C. and Stewart, J.L., 2006. Mating competitiveness of mass-reared males of the Mediterranean fruit fly (Diptera: Tephritidae) from eclosion towers. *Florida Entomologist*. 89(3), 380-387.

Sirot, L.K., Hardstone, M.C., Helinski, M.E., Ribeiro, J.M., Kimura, M., Deewatthanawong, P., Wolfner, M.F. and Harrington, L.C., 2011. Towards a semen proteome of the dengue vector mosquito: protein identification and potential functions. *PLoS Neglected Tropical Diseases*. *5* (3), e989.

Vontas, J., Kioulos, E., Pavlidi, N., Morou, E., Della Torre, A. Ranson, H., 2012. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pesticide Biochemistry and Physiology*. 104 (2), 126-131.

Yuval, B., 2006. Mating systems of blood-feeding flies. *Annual Review of Entomlogy. 51*, 413-440.

Wyer, C.A., Cator, L.J. Hollis, B., 2023. Release from sexual selection leads to rapid genome-wide evolution in Aedes aegypti. *Current Biology*. 33 (7),1351-1357.

ANNEX 1: LIST OF PARTICIPANTS

S. No.	Authority	Personal Details
1	**IAEA**	Mr. Rui CARDOSO PEREIRA Vienna International Centre P.O. Box 100 Wagramer Strasse 5 1400 VIENNA AUSTRIA Email: r.cardoso-pereira@iaea.org
2	**IAEA**	Ms. Maylen GOMEZ PACHECO Vienna International Centre P.O. Box 100 Wagramer Strasse 5 1400 VIENNA AUSTRIA Email: m.gompez-pacheco@iaeaa.org
3	**IAEA**	Ms. Konstantinos BOURTZIS Vienna International Centre P.O. Box 100 Wagramer Strasse 5 1400 VIENNA AUSTRIA Email: k.bourtzis@iaea.org
4	**Albania**	Ms Enkelejda DIKOLLI Institute of Public Health St. Aleksander Moisiu, 80 TIRANA ALBANIA Email:dikollikela@gmail.com
5	**Argentina**	Ms Monica Graciela LOPEZ ISCAMEN -Institute of Agricultural Health and Quality of Mendoza Boulogne Sur Mer 3050 MENDOZA ARGENTINA Email:lopez.monicagraciela@gmail.com
6	**Burkina Faso**	Mr Hamidou MAIGA 399 Avenue de la liberte 01 BO 545 BOBO DIOUS-LASSO BURKINA FASO Email:
7	**China**	Mr Dongjing ZHANG Sun Yat-sen University Zhongshan School of Medicine No.74 Zhongshan Road 2 510080 GUANGZHOU CITY GUANGDONG PROVINCE CHINA Email:D.Zhang@iaea.org
8	**Cuba**	Mr Rene GATO ARMAS Calle 310 # 2924 e/ 29 y 31. Rpto Fraga. La Lisa La Habana HAVANA CUBA Email:rene1968gato@gmail.com
9	**France**	Ms Clelia OLIVA Terratis Halle de l'Innovation – 10 Place Françoise Héritier 34000 MONTPELLIER FRANCE Email:clelia.oliva@terratis.fr
10	**Greece**	Mr Antonios MICHAILAKIS Laboratory of Insects & Parasites of Medical Importance Benaki Phytopathological Institute 8, Stefanou Delta street ATHENS GREECE Email:a.michaelakis@bpi.gr
11	**Greece**	Mr Nikolaos PAPADOPOULOS Laboratory of Entomology and Agricultural Zoology University of Thessaly 38446 VOLOS GREECE Email:nikopap@uth.gr

12	**Indonesia**	Mr Beni ERNAWAN National Nuclear Energy Agency (BATAN); Centre for Application of Isotopes and Radiation Technology (PATIR); Department of Agriculture Pusat Aplikasi Isotop dan Radiasi (PAIR) Jl. Lebak Bulus Raya No. 49 P.O.Box 7002 12440 JAKARTA INDONESIA Email:beni003@brin.go.id
13	**Italy**	Ms Francesca SCOLARI Institute of Molecular Genetics Luigi Luca Cavalli-Sforza, IGM-CNR Via Abbiategrasso 207 27100 PAVIA ITALY Email: francesca.scolari@igm.cnr.it
14	**Italy**	Mr Giuliano GASPERI Universita degli Studi di Pavia Via Strada Nuova, 65 27100 PAVIA ITALY Email:gasperi@unipv.it
15	**Italy**	Ms Anna MALACRIDA Dipartimento de Biologia e Biotechnologie; Universita degli Studi di Pavia Via Ferrata 9 27100 PAVIA ITALY Email:malacrid@unipv.it
16	**Italy**	Mr Marco SALVEMINI Department of Biology University of Naples Federico II Via Cinthia, 26 80126 NAPLES ITALY Email:marco.salvemini@unina.it
17	**Jamaica**	Ms Sheena Aster Marie FRANCIS University of the West Indies Mona Road 7 KINGSTON JAMAICA Email:Sheena.francis02@uwimona.edu.jm
18	**Mexico**	Mr Juan Guillermo BOND COMPEAN Regional Center of Public Health Research National institute of Public Health 19 Calle Poniente esq. 4a Avenida Norte s/n TAPACHULA, CHIAPAS MEXICO Email:gbond@insp.mx
19	**Mexico**	Ms Diana PEREZ STAPLES Fondo de Empresas de la Universidad Veracruzana AC Edificio Centro Estudiantil Universitario Circuito Gonzalo Aguirre Beltran S/N 91000 XALAPA MEXICO Email:diperez@uv.mx
20	**United Kingdom**	Ms Lauren CATOR Imperial College London Exhibition Road SW7 2BX LONDON SOUTH KENSINGTON UNITED KINGDOM Tel: Email:l.cator@imperial.ac.uk
21	**United States of America**	Ms Megan MEUTI NICOL Ohio State University 2001 Fyffe Rd. 43210 COLUMBUS OH UNITED STATES OF AMERICA Email:meuti.1@osu.edu

ANNEX 2: AGENDA

FIRST RESEARCH COORDINATION MEETING

Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture

"Reproductive biology of male Aedes mosquitoes for SIT applications"

Room M7 Vienna International Centre
Vienna, Austria
3 – 7 July 2023

Project Officers: Maylen GOMEZ and Rui Pereira

Monday, 3 July 2023 Room M7

08:00 – 09:00	VIC/ Registration at Gate 1 (by subway U1 station - Kaisermühlen VIC)
09:00 – 09:10	**Rui Cardoso Pereira/ Section Head/ Insect Pest Control/ FAO/IAEA** Welcome & opening remarks
09:10 – 09:20	Introduction of the participants
09:20 – 09:40	**Maylen Gomez/ FAO/IAEA** Objectives of the meeting, agenda, and CRP goals.

SESSION I: Presentations by participants (Chairperson: *Ms. Diana Perez-Staples)*

09:40 – 10:10	Mating behaviour of *Aedes albopictus* males in natural conditions: Implications for SIT/ **Ms. Enkelejda Dikolli**
10:10 – 10:40	Evaluation of *Aedes aegypti* in their mating and remating sexual behaviour through sperm transfer in urban areas under different environment conditions/ **Ms. Monica Lopez**
10:40 – 11:00	**Coffee Break**
11:00 – 11:30	Assessing swarming and mating behaviour of sterile male *Aedes aegypti* under large cages and field conditions / **Mr. Hamidou Maiga**
11:30 – 12:00	A*edes aegypti* sterile male mating success: inbreeding depression relevance and management/ **Mr. Rene Gato**
12:00 – 13:30	**Lunch Break**
13:30 – 14:00	Enhancing the mating performance of mass-reared sterilized males to increase the efficacy of Sterile Insect release programs/ **Mr. Nikos Papadopoulos**
14:00 – 14:30	Investigating sexual maturity of radio-sterilized male *Aedes aegypti* and female receptivity for re-mating/ **Mr. Beni Ernawan**

14:30 – 15:00 Genetic, molecular and biochemical approaches to identify traits of *Aedes albopictus* reproductive biology which may impact the adoption of SIT/ **Ms. Anna Malacrida**

15:00 – 15:30 Coffee Break

15:30 – 16:00 Sperm energy metabolism in the Asian tiger mosquito: A combined autofluorescence imaging and micro spectrofluorimetric analysis approach/ **Ms. Francesca Scolari**

16:00 – 17:00 General Discussion

<u>Tuesday, 4 July 2023</u> Room M7

SESSION I: Presentations by participants (Chairperson: *Mr. Nikos Papadopoulos*) (cont'd)

09:00 – 09:30 Investigating the Main Factors that Influence the Copulatory Behavior of Male Aedes Mosquitoes for the Sterile Insect Technique/ **Mr. Dongjing Zhang** (online presentation)

09:30 – 10:00 Characterizing male reproductive behavior of Aedes albopictus through a gradient of ecological conditions and following the evolution of this behavior during the upscaling process of a mass-reared SIT colony / **Ms. Clelia Oliva** (online presentation)

10:00 – 10:30 Group Discussion

10:30 – 11:00 Coffee break

11:00 – 11:30 Transcriptomics and functional genomics to uncover male reproductive biology in *Aedes* mosquitoes / **Mr. Marco Salvemini**

11:30 – 12:00 Evaluation of Nutrient on Survival, Sexual Competitiveness and Precopulatory Behaviour in Male Aedes Mosquitoes for SIT Applications/ **Ms. Sheena Francis**

12:00 – 13:30 Lunch Break

13:30 – 14:00 Mating in Aedes aegypti: towards understanding sex specific short-range interactions for the benefit of SIT programmes / **Ms. Diana Perez-Staples**

14:00 – 14:30 Harnessing Sexual Selection for Mass-reared *Aedes aegypti*/ **Ms. Lauren Cator**

14:30 – 15.00 Coffee Break

15:00 – 15:30 Mating and insemination capacity of wild and mass-reared irradiated *Aedes aegypti* mosquitos using stable isotopes/ **Mr. Juan Guillermo Bond** (online presentation)

15:30 – 16:00 Male mating harassment on female *Aedes* mosquitoes and potential impact on the efficiency of the Sterile Insect Technique /**Mr Jeremy Bouyer**

16:00 – 17:00 General Discussion

Wednesday, 5 July 2023 Room M7

SESSION II: Review of Individual Proposals (*Chairperson: Maylen Gomez and Group(s) Leader*)

09:00 – 10:30	Working Groups: Discussion, reviewing and planning individual work plans for the 5 year of the CRP and for the next 18 months.
10:30 – 11:00	**Coffee break**
11:00 – 12:00	Working Groups: Discussion, reviewing and planning individual work plans for the 5 year of the CRP and for the next 18 months.
12:00 – 13:30	**Lunch Break**
13:30 – 15:00	Working Groups: Discussion, reviewing and planning individual work plans for the 5 year of the CRP and for the next 18 months.
15:00 - 15:30	**Coffee break**
15:30 – 17:00	Working Groups: Discussion, reviewing and planning individual work plans for the 5 year of the CRP and for the next 18 months.

Thursday, 6 July 2023

SESSION II: CRP documents, drafting and compiling and RCM report (*Chairperson: Maylen Gomez and Group Leaders*)

09:00 – 10:30	Review and adjustment of the logical framework matrix **(LFM)**
10:30 – 11:00	**Coffee break**
11:00 – 12:00	Review and agreement on content of RCM report
12:00 – 13:30	**Lunch Break**
13.30 – 15:00	Review and agreement on content of RCM report
15:00 – 15:30	**Coffee break**
15:30 – 17:00	Review and agreement on content of RCM report

Friday, 7 July 2023

SESSION II: CRP documents, drafting and compiling and RCM report (Chairperson: *Maylen Gomez and Group Leaders*)

09:00 – 10:30	Finalizing RCM report
10:30 – 11:00	**Coffee break**
11:00 – 12:00	Finalizing RCM report
12:00 – 13:30	**Lunch Break**

13.30 – 15.00 Presentation of draft RCM report including main goals and future work.

ANNEX 3: WORKING GROUPS

Table 4: Working groups by research topics.

RESEARCH TOPICS		
Topic 1_ Pre-mating behaviour	**Topic 2_** Copulation and Insemination patterns	**Topic 3_**Female remating and factors that control it
GROUP 1	**GROUP 2**	
Ms Enkelejda DIKOLLI	Ms Monica Graciela LOPEZ	
Mr Hamidou MAIGA	Ms. Aba MALACRIDA	
Mr Rene GATO ARMAS	Mr Beni ERNAWAN	
Mr Nikolaos PAPADOPOULOS*	Ms Diana PEREZ STAPLES *****	
Mr Marco SALVEMINI	Ms Francesca SCOLARI	
Mr Antonios MICHAILAKIS	Mr Giuliano GASPERI	
Ms Sheena Aster Marie FRANCIS	Mr. Guillermo Bond	
Ms Lauren CATOR	Ms. Maylen GOMEZ**	
Mr Dongjing ZHANG	Mr Dongjing ZHANG	
Mr. Konstantinos BOURTZIS**	Mr. Rui C. PERERIRA**	

***Suggested Chair per group**

****IAEA support staff**

ANNEX 4: ABSTRACTS OF PRESENTATIONS

ABSTRACTS

"Mating behaviour of *Aedes albopictus* males in natural conditions: Implications for SIT"

AUTHOR (S): Enkelejda Dikolli[1], Përparim Kadriaj[1], Eugena Tomini[1], Silva Bino[1]

ORGANIZATION: [1]Institute of Public Health, Tirana, Albania

SHORT SUMMARY OF PAPER

Abstract:

Aedes albopictus, is established in Albania for at least 45 years, longer than in any other country in Europe. So far passive and active targeted surveillance on the presence, bio-ecology, seasonal activity, geographical and altitudinal dispersal, have been carried out. Studies on vector competence has shown that of *Aedes albopictus* local strain, could serve as vector for dengue virus. Populations of *Ae. albopictus*, from high altitudes in Albania are used to estimate how far north Europe DENV and CHIKV transmission could occur.

The control strategies implemented in Albania are non – chemical larvicide based. A mark release-recapture experiment was carried out within a highly urbanized area in Tirana in 2017. The dispersal capacity, probability of daily survival and competitiveness, and the size of the target population have been estimated, under field conditions. Irradiated males dispersed, survived long enough and competed well with their wild male counterparts.

Swarming and mating in mosquitoes remains a poorly understood process. Many culicine species, characteristically, mate in swarms when males aggregate in sometimes large numbers. Successful mating is critical for the success of proposed strategies for vector-borne-disease control using SIT. The knowledge on mating behaviour of *Aedes albopictus* in natural conditions are lacking. Here we propose to study and characterize the mating system of *Ae. albopictus* in the field i.e., encounter sites; swarming place, composition, the onset, the altitude, duration; the pairs movements, position related to the observer, ground, breeding and resting sites and characterize copulation and insemination processes of *Ae. albopictus* sterile males. Data provided from this study will be used to predict the number of released individuals required for effective SIT applications and to adjust the release rate over time to have optimal mating efficiency.

"Evaluation of *Aedes aegypti* in their mating and remating sexual behaviour through sperm transfer in urban areas under different environment conditions"

AUTHOR (S): Lic. Baldoni, Juan Cruz-Tech. López, Mónica – Tech. Rovere, Lucas- Agr Eng. Cordero, Joel

ORGANIZATION: ISCAMEN – "Instituto de Sanidad y Calidad Agropecuaria de Mendoza"

SHORT SUMMARY OF PAPER

Abstract:

In a SIT program it is essential that sterile male mosquitoes produced under mass rearing conditions will be able to achieve success mating. The sterile males must be competitive against wild males and also compatible with wild females.

The effect of colonization could be cause of changes on the behaviour of the sterile males in the field, which could be reflected in the maturation time for mating, changes on courtship behaviour, quality and quantity of pheromone produced by sterile males (Cayol 2000) and affecting the capacity of mating trough out compatibility with wild females and competitiveness against wild males.

Biology factors such as adult size and sperm transfer mechanism and abiotic factors such as temperature, lighting, relative humidity, or location of mating, could influence the effectiveness of couples' interaction.

Two different strains are reared at Iscamen´ s labs: Misiones strain transferred from CNEA´s labs and Mendoza strain from local wild mosquitoes collected two seasons ago from the field. For the study of the compatibility, competitiveness and behaviour of Mendoza and Misiones strains labs and field test will be performed according to the protocols available from bibliography and adapted from International Quality Control Handbook IAEA-FAO 2020 adapted to Aedes aegypti. It will be evaluated in the two different seasons and also different environment conditions.

The compatibility test shall be used for the following specific purposes:

- To quantify the effect of colonisation of such strains
- To assess colony refreshment requirements and stablish a protocol by incorporating wild individuals into colony rearing.
- To apply the test as a mechanism of evaluation to keep the colony through subsequent generations and averaging eggs per female produced.
- Evaluation of differential sizes in pupae and then in adults reared differential feeding in larvae.
- Influence of the size on the success of mating.
- From the sperm transfer test, the location of sperm and the incidence on the mating between Mendoza and Misiones and wild strains will be observed.

For compatibility tests, sugary food with rhodamine shall be used to identify the type of treatment to which the copulations belong, as well as the location and use of the semen.

"Assessing swarming and mating behaviour of sterile male *Aedes aegypti* under large cages and field conditions"

AUTHOR (S): Maiga H, NS Bimbile Somda, SP Sawadogo, A Diabate, R.K Dabire

ORGANIZATION: Institut de Recherche en Sciences de la Sante_ Direction Regionale de l'Ouest (IRSS/DRO), Bobo-Dioulasso, Burkina Faso

SHORT SUMMARY OF PAPER

Abstract:

The sterile insect technique (SIT) is the most effective strategy of vector control used to control insect pests of agricultural and medical/veterinary importance. This technique is based on repeatedly releasing large numbers of sterile male insects to reduce the reproduction in a target population of the same species. However, its success depends on the ability of released sterile males to survive, disperse, compete with wild males and inseminate the wild females. Success of an SIT programme will depend on understanding parameters underlying male mating success such as age, body size, mating acoustics, swarms, nutritional reserves, hormones and breeding conditions that can be used to inform control programs to reduce cost of production and increase success of released males. Semi-field and laboratory studies designed to dissect the mating biology and characterize essential factors that enhance mating competitiveness in mosquitoes are needed to provide a foundation for predicting the potential utility of genetic control. The main objective in this proposal, is to assess the swarming and mating behaviour of sterile male *Aedes aegypti* under large cages and field conditions. Specifically, we will address the effect of male: female ratio on male mating performance and sexual harassment, the swarming/mating behaviour in semi-field and field conditions and male attraction to ovitraps.

"Aedes aegypti sterile male mating success: inbreeding depression relevance and management"

AUTHOR (S): René Gato Armas

ORGANIZATION: Institute Pedro Kourí, IPK, La Habana, Cuba

SHORT SUMMARY OF PAPER

Abstract:

The efficacy of the sterile insect technique depends to a large extent on the competitiveness of the released mosquitoes to mate with the wild females. A common approach to evaluating the sterile mosquito's fitness is the ability of irradiated males to reduce the offspring of fertile females. The interpretation of the results of caged experiments is restricted by the bias of an abnormal rate of physical encounters, due to space limits and the absence of hiding sites. Additionally, the mating behavior is influenced by the mosquito density in the cages.

The study of mating behavior in the field is not practical because of uncontrollable mosquito movements and complex environmental factors. An intermedia approach is a simulated field environment, where a mosquito population could be observed for a long period. Here, we propose to mark mosquitoes by means of a non-invasive method based on quantum dots and to record the mating behavior by a multi-spectral image acquisition system.

When a wild population is compared to a long-established laboratory population, the results could be confounded by adaptation to rearing rather than biological phenomena like inbreeding. Since mass rearing is the first basic step of the technological process of SIT, the biological properties of the strains, including the genetic variability are one of the most important components to be controlled.

An important goal of this research is to improve the knowledge background for the development of comprehensive protocols for the maintenance of high-quality mosquito strains, as the base of the massive production of mating-competitive sterile males for SIT.

"Enhancing the mating performance of mass-reared sterilized males to increase the efficacy of Sterile Insect release programs"

AUTHOR (S): Nikos T. Papadopoulos[1] and Antonios Michaelakis[2]

ORGANIZATION: *University of Thessaly, Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environments, Fytokou St. N. Ionia (Volos), Magnisia Greece (nikopap@uth.gr)*
Benaki Phytopathological Institute, Laboratory of Insects & Parasites of Medical Importance, Scientific Department of Entomology and Agricultural Zoology, 14561 Kifissia, Greece (a.michaelakis@bpi.gr)

SHORT SUMMARY OF PAPER

Abstract:

The success of the Sterile Insect Technique (SIT) depends directly on the performance of the sterilized released males. However, factors such as mass rearing, adaptation to artificial conditions, irradiation, pre-release handling and/or transportation may deteriorate the quality of males. To "rescue" part of, or even enhance the necessary qualities of the released males several options have been considered in model systems for SIT. For instance, in the case of the Mediterranean fruit fly the sexual performance of sterile males can be boosted with exposure to semiochemicals ginger root oil and orange oil. In fact, exposure to ginger root oil has been adopted by many SIT programs as a standard prerelease treatment of male medflies. To develop prerelease treatments, a comprehensive and detailed knowledge of the reproductive physiology and sexual behavior of males of the respective insect pests should be known. The current paper summarizes existing prerelease treatments that have been developed and applied in Tephritidae for enhancing the performance of released males. In addition, we present recent data regarding the biology of the Asian tiger mosquito, *Aedes albopictus* that have been collected in the Laboratory of Entomology and Agricultural Zoology at the University of Thessaly, Greece and the Benaki Phytopathological Institute regarding life history and sexual behavior, including male mating performance. We discuss concepts and approaches regarding the adoption, in the Asian tiger mosquito SIT, of prerelease treatments developed in tephritids, and the development of novel prerelease handlings of males for mosquito releases. Last, we propose a list of studies to explore basic aspects of the sexual behavior of *Ae. albopictus* with emphasis on males.

"Investigating sexual maturity of radio-sterilized male *Aedes aegypti* and female receptivity for re-mating"

AUTHOR (S): Beni Ernawan, Hadian Iman Sasmita, Titik Kartika, Ade Lestari Yunus, Murni Indarwatmi, Indah Arastuti

ORGANIZATION: National Research and Innovation Agency of Indonesia (BRIN)

SHORT SUMMARY OF PAPER

Abstract:

An effective SIT application against Aedes mosquito relies on the ability of sterile males to mate with females. For successful mating, the males should be able to recognize the mate, initiate courtship, undergo genitalia contact, and transfer their functional sperm. Handling, irradiation treatment, or other modifications may potentially impair their ability to mate. Identifying what changes in the reproductive system and mating behavior of sterile males relative to untreated males would provide insightful information to determine factors affecting female receptivity for re-mating. In the present study, we are interested in observing male antennae fibrillar hairs as an indicator of sexual maturity in Aedes aegypti. This study will document the morphology and motion of fibrillar hair antennae and see their association with pre-mating activities including recognition of the pair. The effect of semen and seminal fluid from irradiated males on the female receptivity for re-mating will be studied, as well as other factors such as interval mating and the density of males. Stable isotope labelling will be used to detect the mixed semen from re-mating incidences using isotope-ratio mass spectroscopy (IRMS). Data expected in this study will be useful in the development and refinement of mosquito control programs using SIT.

"Genetic, molecular and biochemical approaches to identify traits of *Aedes albopictus* reproductive biology which may impact the adoption of SIT"

AUTHOR (S): Malacrida AR[1], Forneris F[1], Fiorenza G[1]; Foffano G[1], Gasperi G[1,2], Gomulski LM[1], Mancini MV[1], Piccinno R[1]

ORGANIZATION: [1]Dept. Biology and Biotechnology, Univ. of Pavia, Pavia, Italy; [2]CNR-Institute of Molecular Genetics, Pavia, Italy

SHORT SUMMARY OF PAPER

Abstract:

The goal of our group is to analyse the reproductive biology of Diptera of economic and medical importance in order to understand their invasive potentials and to control their populations. We have accumulated a strong experience in the analyses of the male and female reproductive biology of fruitflies, tsetse flies, biting midges and mosquitoes at the behavioural, physiological, genomics/functional genomics, proteomic and metabolic levels. Great efforts have been devoted to the analyses of mating and remating behaviour and to the identification of the genetic and molecular repertoires controlling the male ejaculate components.
We will use this experience to fill the gaps in two aspects of *Aedes albopictus* reproductive biology which may create constraints in SIT application:

1) presence of polyandry in populations and strains of *Aedes albopictus*
The presence of remating and the possibility that females could store sperm from different males, including sterile males, for egg fertilization and perhaps use the sperm from different males differentially, may constitute a critical factor for the success of eradication programmes.

2) Disentangling the components and factors (biotic/abiotic) which determine and modulate the mating success of wild and sterilized males.

We will analyse the key cues affecting male mating success (insemination). This is one of the key points both in mass rearing and for the success of sterile-male releases in SIT programmes. We will examine the chemical and molecular machinery which enable a male to recognize suitable females for successful copulation. We will investigate the relationships between contact pheromones and the chemosensory apparatus involved in this process.

“Sperm energy metabolism in the Asian tiger mosquito: A combined autofluorescence imaging and microspectrofluorimetric analysis approach”

AUTHOR (S): Croce A.C., Moyano A., Soldano S., Scolari F.

ORGANIZATION: Institute of Molecular Genetics Luigi Luca Cavalli-Sforza, IGM-CNR

SHORT SUMMARY OF PAPER

Abstract:

In insects, investigation of the efficiency of sperm energy metabolism, which is essential for sperm motility and male fertility, is still in a pioneering phase, and it has not been approached yet in the Asian tiger mosquito *Aedes albopictus*.
In this species body size has a strong impact on adult male fitness, but the role of larval rearing conditions on sperm energy metabolism has not been investigated yet.
To address this gap, we will take advantage of the knowledge and techniques generated by our research on the reproductive biology of fruit flies and tsetse flies, especially in terms of sperm transfer and use, and of the potential of autofluorescence as an intrinsic biomarker of cell metabolism.
In particular we will employ a combined imaging and spectrofluorometric approach to attempt tracing the ubiquitous coenzymes NAD(P)H and FAD as intrinsic biomarkers of sperm energy metabolism. These coenzymes are key players in cell energy metabolism, and they can be assayed optically by exploiting their autofluorescence properties in a label-free manner.
Our research activities will be articulated in the following tasks: 1) develop a protocol for the collection of sperm samples from male testes and female sperm storage organs suitable for autofluorescence imaging and microspectrofluorimetric analysis, 2) collect autofluorescence-derived data on sperm metabolism in adult males and mated females reared on a) standard larval diet, and b) under different larval feeding regimes.
Our findings will be the basis for the development of a novel approach to evaluate sperm function in a mass-rearing context, thus providing new tools to evaluate the fitness of the produced mosquitoes.

“Investigating the Main Factors that Influence the Copulatory Behavior of Male Aedes Mosquitoes for SIT”

AUTHOR (S): Dongjing Zhang, Jiatian Guo, Dilinuer Paerhande, Xiaoying Zheng, Yu Wu

ORGANIZATION: Chinese Atomic Energy Agency Center of Excellence on Nuclear Technology Applications for Insect Control, Key Laboratory of Tropical Disease Control of the Ministry of Education, Sun Yat-sen University, Guangzhou, China.

SHORT SUMMARY OF PAPER

Abstract:

Since the SIT relies on reducing the birth rate of the target population, it is crucial to understand various aspects of the sexual performance of male mosquitoes, especially for Aedes mosquitoes, the primary vectors of dengue, Zika and Chikungunya. Irradiation has been found to reduce the mating competitiveness of Aedes male mosquitoes. The reduced mating competitiveness of sterile males might have negative impacts on the efficiency, and even the success of SIT. Thus, it is crucial to understand the reproductive biology of Aedes mosquitoes. The goal of this proposal is to investigate the main factors that influence the copulatory behaviors of Aedes male mosquitoes. Toward this goal, we will compare the copulatory behaviors between the wild and the sterile male mosquitoes, via comparing a serious of pre-mating (e.g. fast-response time for mating, flight ability and female capture) and mating behaviors (copulation time, number of sperm ejaculate, insemination rate). In addition, specimens will also be collected for genomics and neurobiology research to find related genes or neurological pathways that involving to the behaviors which might affect the sexual performance. The aim of the study will help to improve our knowledge on the sexual behaviors of Aedes mosquitoes and provide alternative approaches to increase the mating competitiveness of sterile male mosquitoes, thus improving the efficiency of SIT for Aedes mosquito control.

“Characterizing male reproductive behavior of Aedes albopictus through a gradient of ecological conditions and following the evolution of this behavior during the upscaling process of a mass-reared SIT colony”

AUTHOR (S): Clelia Oliva

ORGANIZATION: Terratis, France

SHORT SUMMARY OF PAPER

Abstract:

In order to ensure the efficiency of control strategies such as SIT of *Aedes mosquitoes*, filling the gaps currently present in their reproductive biology is crucial, from the lab all the way to the field, where the sterile males will ultimately need to compete successfully to access mates.
In this study, we characterize key points of male reproductive behavior of *Aedes albopictus* that are necessary for industrial monitoring of sterile male quality. This is done by studying released males in the field as well as by identifying indicators of performance in the laboratory and semi-field conditions that can convey crucial information as regard to the upscaling process of a mass-reared SIT colony.
Altogether, these results will allow us to further our understanding of sterile males behavior in the field, and on how adaptation to mass-rearing conditions and irradiation affect these traits. This work will have direct implications for SIT applications, by allowing the development of targeted releases, as well as by giving insight into how the upscaling process of an SIT colony alters SIT efficacy.

"Transcriptomics and functional genomics to uncover male reproductive biology in *Aedes* mosquitoes"

AUTHOR (S): Varone, M.[1], Lucibelli, F.[1], Di Lillo, P.[1], Fulgione, C.[1], Aceto, S.[1], Saccone, G.[1], and Salvemini, M.[1,*]

ORGANIZATION:
[1]Department of Biology, University of Naples Federico II, Via Cinthia 26 – 80100 – Naples, ITALY

SHORT SUMMARY OF PAPER

Abstract:

Aedes mosquitoes are vectors of important arboviruses, including those responsible for severe diseases such as Zika, dengue, and chikungunya. Despite their crucial role as disease vectors, there remains a paucity of knowledge regarding the reproductive biology and physiology of male mosquitoes. The field performance of male mosquitoes plays a pivotal role in population expansion and species perpetuation. At the same time, a comprehensive understanding of male-specific traits in mosquitoes, along with the underlying genetic factors, could be extremely useful to improve the sexual performance in the field of *Aedes* sterile males to be used in eco-friendly control programs based on the sterile insect technique (SIT). Through the utilization of a transcriptomic approach, the objective of this research project is to generate and validate a list of potential candidate genes in *Aedes* mosquitoes, which may be implicated in the regulation of male pre-copulatory behaviour, encompassing aspects such as male-to-female courtship, social interactions, male lifespan, and the induction of mating-related changes in females. During the first eighteen months of the research activity, we will produce by RNA-seq, sexed developmental transcriptomes of immature stages in *A. albopictus* (early embryos, late embryos, first-, second-, third, and fourth-instar larvae) and sex-specific larval brain transcriptomes. *In silico* differential gene expression analysis will result in the first catalogue of sex-specific transcripts during development in immature stages of the Asian tiger mosquito. Furthermore, we will investigate genes implicated in pre-copulatory social interactions between male and female *Aedes albopictus* mosquitoes utilizing adult whole-body RNA-seq. Samples will be obtained and Illumina-sequenced from solitary males, males with females, antennae-ablated solitary males, and antennae-ablated males with females. Genome-wide analysis of differential expression will be conducted to identify genes involved in male responses to the presence of conspecific females. Lastly, a functional analysis will be performed *in vivo* through RNA interference (RNAi) and CRISPR-Cas9 knockdown techniques to examine the impact on the lifespan and fitness of adult male mosquitoes of the silencing of a candidate gene, we recently discovered, which exhibits robust male-specific expression in all developmental stages of *Aedes albopictus*, including early embryos, as well as in *Aedes aegypti* larvae, pupae, and adults.

"Evaluation of Nutrient on Survival, Sexual Competitiveness and Precopulatory Behaviour in Male Aedes Mosquitoes for SIT Applications"

AUTHOR (S): Sheena Francis[1], Charles Grant[2], Dwight Robinson[3], Sherine Huntley-Jones[4]

ORGANIZATION: *[1]Natural Products Institute, 6 Belmopan close, University of the West Indies, Jamaica, [2]International Centre for Environmental and Nuclear Sciences, University of the West Indies, Jamaica, [3]Department of Life Sciences, University of the West Indies, Jamaica, [4]Vector Unit, Ministry of Health Wellness, Jamaica*

SHORT SUMMARY OF PAPER

Abstract:

Within the past decade, Jamaica and the Caribbean observed increased frequencies of Aedes-borne illnesses. Management of Aedes spp. population is crucial to mitigate the transmission of Aedes-borne diseases in Jamaica. However, management is deemed unattainable when combined factors that drive vector population growth exist. Factors include but are not limited to the endemicity of the vectorial agent, climate changes that influences human behaviours that unknowingly create ideal breeding environment for the vectorial agent, the increase in global travel, which results in the transportation of an infected host to a region where the vectorial agent is present.

The control of mosquitoes relies mainly on the use of chemical insecticides. Thus, vector control is further stymied by emergence of insecticide resistance within a given population. Techniques that seek to decrease mosquito population without the use of insecticides, such as Incompatibility Insect technique (IIT), Genetically Engineered Mosquitoes carrying self-limiting genes (GM) or Sterile Insect Technique via radiation or chemosterilisation (SIT), are become more favourable. Of all the techniques, SIT has been in use since the 1950s, and evidence of its success in non-mosquito insects exist. SIT does not require the pre-infection of insects with a biological agent, nor does the technique require the use of gene-drivers. However, the sterilisation process in mosquitoes appear to reduce the overall robustness of the mosquitoes and their ability to compete with males in the wild for wild-type females, and thus limits the success of the program.

Studies that examine factors that enhance fitness, survival and sexual competitiveness in sterile male mosquitoes, and lead to the over effectiveness and long-term success of SIT projects are valuable. This proposal plans to evaluate the effect of biotic factor, nutrition, and abiotic factors, temperature, and humidity, on survival, pre-copulatory behaviour, mating fitness and mating competitiveness in rearing colony Aedes aegypti mosquitoes for SIT application compared to wild-type Aedes aegypti male mosquitoes. We will also determine whether these optimised sterile males can suppress female fertility, preventing their further copulation with wild-type males. The studies will be conducted initially under laboratory conditions, and then under semi-field conditions to validate laboratory findings for semi-real application.

“Mating in Aedes aegypti: towards understanding sex specific short-range interactions for the benefit of SIT programmes”

AUTHOR (S): Diana Pérez-Staples, Laura Sirot, Dinesh Rao

ORGANIZATION: Universidad Veracruzana, Mexico; Wooster College, USA.

SHORT SUMMARY OF PAPER

Abstract:

Mosquitoes in the genus *Aedes* are noxious pests as vectors of parasites and pathogens. A specific and environmentally friendly method of control against mosquitoes, the Sterile Insect Technique (SIT), is being used in various regions of the world. The SIT consists in the mass-rearing, sterilization and release of sterile insects in affected areas. The SIT relies on sterile insects being able to find and mate with wild females, so knowledge on the mating behaviour, including the processes that occur during mating and the factors that inhibit female sexual receptivity, is key for its success. However, despite many years of study, we still lack basic information, for example on the short-range interactions that occur before and during mating. *Aedes aegypti* mates in swarms, males recognize females by wing beats, and subsequently coordinate their wingbeats in a behaviour known as harmonic convergence. However, since mating is very fast (~10-20 seconds), we still do not have a complete understanding of the factors that result in variation in pair formation, ejaculate transfer, and the initiation and the termination of mating. Here, following Aldersley and Cator (2019) with the use of high speed cameras we aim to: i. Quantify differences in behavior between of unmated and mated females towards males; ii. Quantify differences in behavior of sterile and fertile males in response to unmated and mated females; and iii. Quantify differences in behavior between unmated and mated females towards sterile and fertile males. We will examine behavior before, during, and after pair formation. Furthermore, following Carrasquilla and Lounibos technique that allows for the visualization of live sperm within the *A. aegypti* female spermathecae without killing her, we aim to visualize and record the speed at which sperm is moving. These studies would provide us with a potential quality control measure of the ejaculate. This can be used for example to test differences between fertile and sterile sperm, or between conspecific and heterospecific sperm. The comparison of the motility between conspecific and heterospecific sperm in *Ae. aegypti* and *Ae. albopictus* matings may shed some light on the process of satyrization and ultimately understanding female sexual refractoriness. Thus we aim to: i. Compare motility between sperm from sterile and fertile males; and ii. Compare motility between conspecific and heterospecific sperm in *Ae. aegypti* and *Ae. albopictus.* Finally we will test for associations between sperm motility and female sexual refractoriness.

"Harnessing Sexual Selection for Mass-reared *Aedes aegypti*"

AUTHOR (S): Lauren Cator[1]*, Claudia Wyer[1], Laura Harrington[2] and Brian Hollis[3]

ORGANIZATION: [1]Imperial College London, [2]Cornell University, [3]University of South Carolina

SHORT SUMMARY OF PAPER

Abstract:

Within a swarm of *Aedes aegypti* there is competition between males for females and females exhibit rejection-based mate choice. The result is a mating arena with intense sexual selection. While adaptation to the laboratory environment has long been known to select for different traits such as development time and reproductive schedules in mosquitoes, it has only more recently been shown to effect mating related phenotypes. Our recent work has highlighted the key role of the selective environment experienced by adults, and specifically the intensity of sexual selection, in shaping male mating success. We compared the competitive mating performance of males from populations of *Ae. aegypti* evolved under experimental conditions which either allowed for or eliminated sexual selection. We observed evidence of rapid loss of mating competitiveness in populations released from sexual selection (<5 generations). Using whole genome sequencing we have identified candidate genes underlying these observed changes in mating performance. These candidates will provide insight into male traits important for mating success. An important component of our working group contribution will be functionally characterizing key candidates. Reproductive control programmes in other insect species have repeatedly demonstrated that changes in male mating behaviour due to long-term mass rearing can significantly decrease mating competitiveness. We propose to explore maintaining the intensity of sexual selection in the mass-rearing environment as an avenue for maintaining line competitiveness.

"Mating and insemination capacity of wild and mass-reared irradiated *Aedes aegypti* mosquitos using stable isotopes"

AUTHOR (S): J.G. Bond, Pablo Liedo, Julio C. Rojas, Carlos F. Marina, Ariane Dor, Trevor Williams.

ORGANIZATION: Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México; El colegio de la Frontera Sur, Unidad Tapachula, Tapachula, Chiapas, México; Instituto de Ecología, AC (INECOL), Xalapa, Veracruz, México.

SHORT SUMMARY OF PAPER

Abstract:

The Sterile Insect Technique is a promising and environmentally-benign strategy for the control of Aedes aegypti. SIT is based on the release of large numbers of sterile male insects that compete with wild males for mating opportunities with female insects. The success of SIT programs depends on the degree to which mass-reared sterile males are capable of conferring sterility on the target female population. The present study will examine basic aspects of male reproductive biology, focusing specifically on behaviors that lead up to mating and the potential role of chemical signals involving in the localization of receptive females. Stable isotope techniques will then be used to determine the copulation and insemination capacity of irradiated males compared to fertile wild males. Finally, the tendency for inseminated females to remate after one or two cycles of oviposition will be evaluated using stable isotopes to determine the fraction of the female population that engage in this behavior, and to determine whether this tendency differs for females that have mated previously with sterile or fertile males. As the success of SIT-based control revolves around the production of high-quality sexually competitive sterile male insects, the findings of this study will provide unique information on the ability of males to locate and inseminate females in the presence of fertile male competitors. It will also provide clarity in our understanding of female remating behavior, which might jeopardize the efficacy of SIT-based programs for area-wide vector control.