TARGET MALARIA Uganda

A Vector Control Research Alliance

The use of naturally occurring colour variant mosquitoes to support facilities readiness

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Overview:

Background

- The malaria challenge
- Target Malaria

Site readiness: Target Malaria capacity development

- Infrastructural upgrades: UVRI ACL2 Insectary
- Facility operational testing: color variant studies

Future studies

- Contained use studies
- Regulatory requirements

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Background

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Malaria: the problem

The burden

- **Globally:** More than 200 million infections & half a million deaths each year, ~90% in Africa, affects mainly the poor, infants & children
- Economic losses in Africa **~$12 billion** a year
- The progress in the fight against malaria has slowed significantly since 2015

Uganda

- ~15 million cases & rising fatalities over the past 5 yrs (WHO, 2021);
- Accounts for up to 50% out-patient visits; up to 20% hospital admissions;
- ~95% of the country falls under high transmission areas.
- ~Large portion of health budget spent on tackling malaria

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Malaria: current interventions

Current methods of control are good but **insufficient**

Due to various challenges:

- Emergence of resistance in mosquitoes against insecticides
- Emergence of resistance in the parasites against drugs
- Behavioral changes in mosquitoes to avoid nets and biting outdoors

New tools are needed if we are to eradicate the disease

- **Genetic modification offers promise to combat disease in novel ways**
  - WHO advocates for research & exploration of its potential
  - AUDA-NEPAD's High-Level Panel on Emerging Technologies (APET) 's recommendation
  - Uganda NDP-III advocates Science/Technology/ Innovation (ST-I) for health, development & prosperity

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Project team members who attended our Project Team Meeting (PTM) in Uganda, Feb 2017

- CDC Foundation, USA
- Imperial College London, UK
- Institut de Recherche en Sciences de la Santé, Burkina Faso
- Instituto Nacional da Saúde Pública – INSP, Programa nacional de controlo do paludismo (*National Institute of Health, National Malaria Programme*), Cabo Verde
- Keele University, UK
- Malaria Research & Training Center, Université des Sciences, des Techniques et des Technologies de Bamako, Mali
- Polo d'Innovazione di Genomica, Genetica e Biologia, Italy
- Uganda Virus Research Institute, Uganda
- University of Ghana, Ghana
- University of Notre Dame, USA
- University of Oxford, UK

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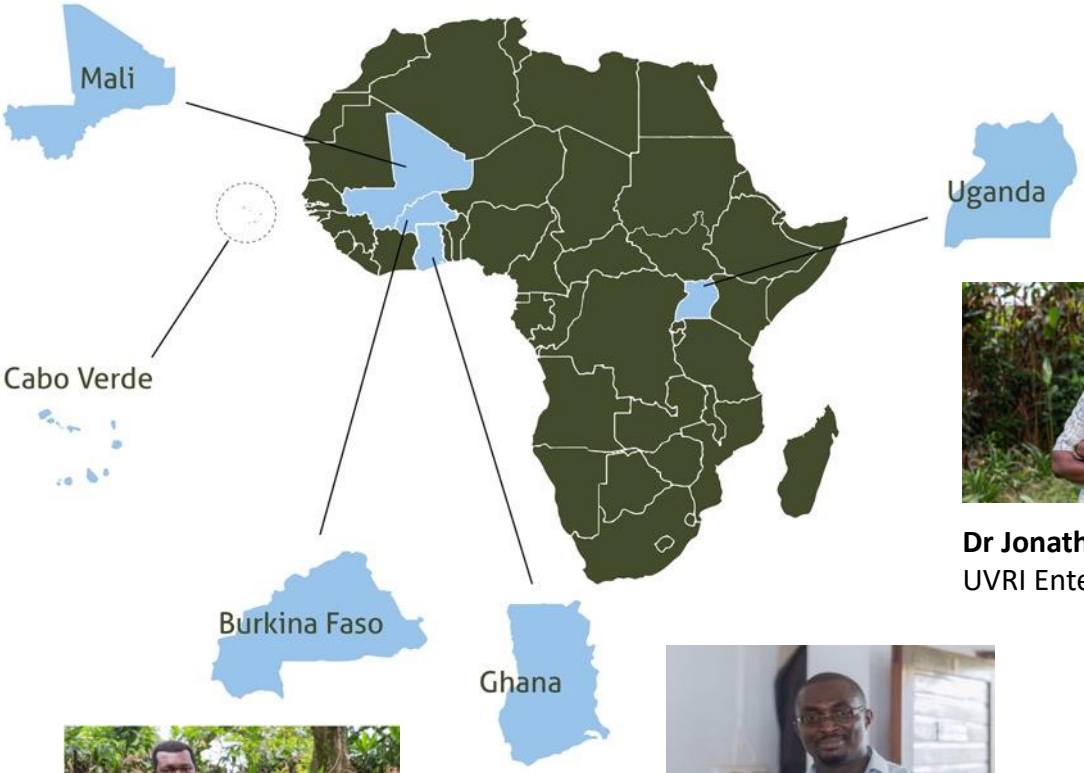
Target Malaria in Africa

Dr Mamadou Coulibaly
MRTC Bamako



Dr Jonathan Kayondo
UVRI Entebbe

Dr Adilson de Pina
Instituto Nacional da Saúde Pública – INSP

Dr Abdoulaye Diabate
IRSS Bobo Dioulasso

Dr Fred Aboagye-Antwi
University of Ghana, Accra

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Our objectives

- **To develop and share a novel genetic technology** for vector control of *Anopheles* mosquitoes to contribute to reducing the burden of malaria in Africa
- To use an approach which is **complementary to existing methods, sustainable, long term, and cost-effective**
- To **reduce the population of the mosquitoes that transmit malaria**, and therefore reduce transmission of the malaria parasite, through genetic modification of malaria mosquitoes
- **Acquire and develop the necessary knowledge, competence and infrastructure** in order to develop and implement this technology in Africa.

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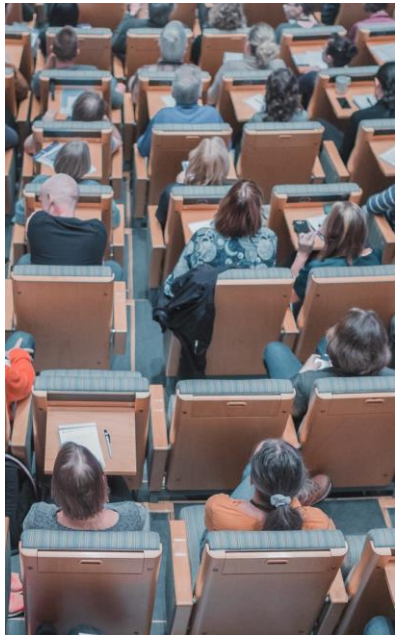
Built on three pillars

Science + **Stakeholder engagement** + **Regulation**



Infrastructural Upgrades

UVRI ACL2 Insectary

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The UVRI Contained Use Facility

Built in 2019 to arthropod containment level 2 (ACL2) specifications

The ACL2 features

TABLE 1. SUMMARY OF ARTHROPOD CONTAINMENT LEVELS

Arthropod containment level:	1		2	3	4
Arthropod distribution, escaped arthropod fate	Exotic, inviable or transient	Indigenous	Exotic with establishment, indigenous, and transgenic		
Infection status	Uninfected or infected with non-pathogen		Up to BSL-2	Up to BSL-3	BSL-4
Active VBD cycling	No	Irrelevant			
Practices	ACL-1 Standard Arthropod-Handling Practices		ACL-1 plus more rigorous disposal, signage, and limited access	ACL-2 with more highly restricted access, training and record-keeping	ACL-3 with high access restriction, extensive training, full isolation
Primary Barriers	Species-appropriate containers		Species-appropriate containers	Escape-proof arthropod containers, glove boxes, BSC	Escape-proof arthropod containers handled in cabinet or suit laboratory
Secondary Barriers			Separated from laboratories, double doors sealed electrical/ plumbing openings. Breeding containers and harborages minimized	BSL-3	BSL-4

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Facility Operational Testing

Colour Variant studies

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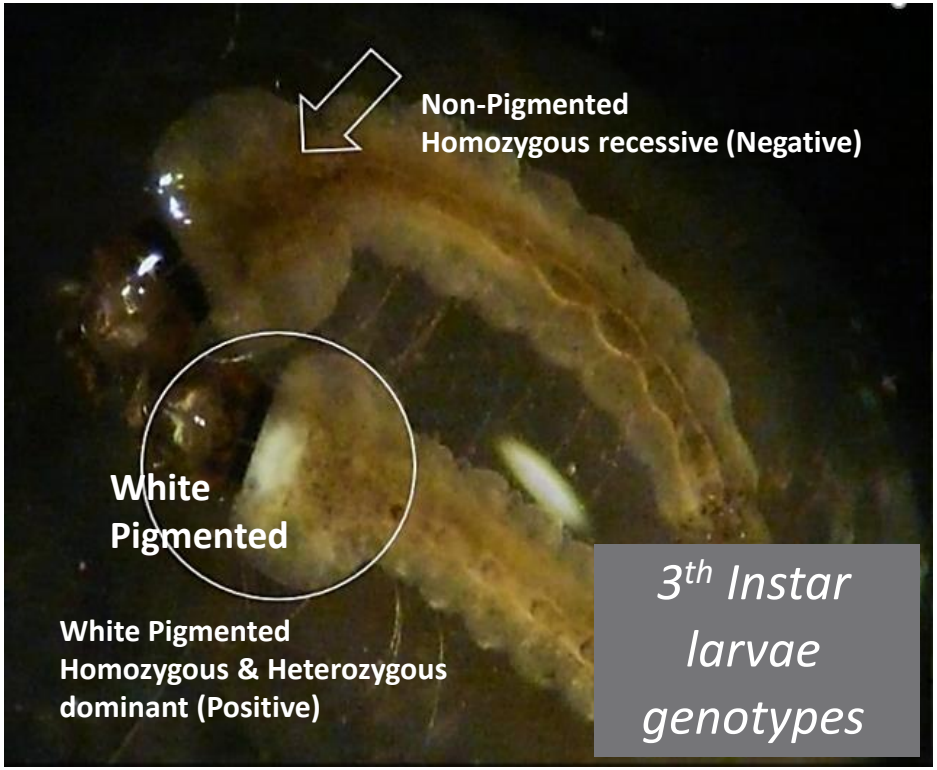
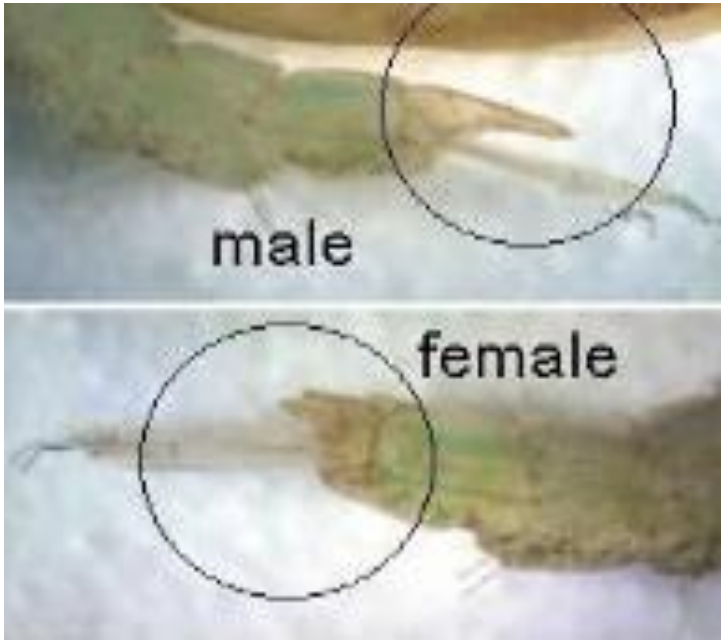
Colour variant mosquito strains

(Common in natural wild populations)





Sex structures (Pupal stage)

Maintaining the phenotypes in the respective strains is similar to what will be needed when handling future genetically modified mosquitoes.

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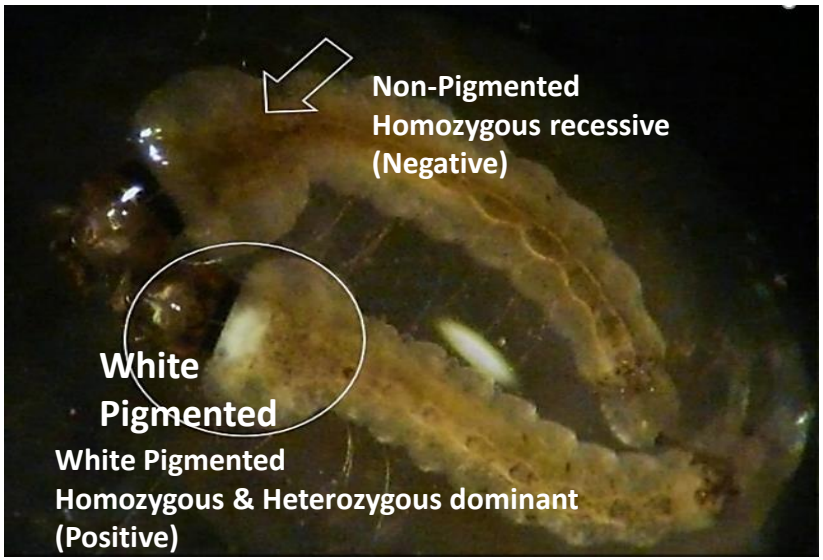
Study objectives

- To test the capacity of the facility (physical & practices) to operate under ACL2 standards
- To test team ability to handle genetic crosses
  - Using **natural** color variant mosquitoes obtained from the PMI Kisumu colony at Gulu University.



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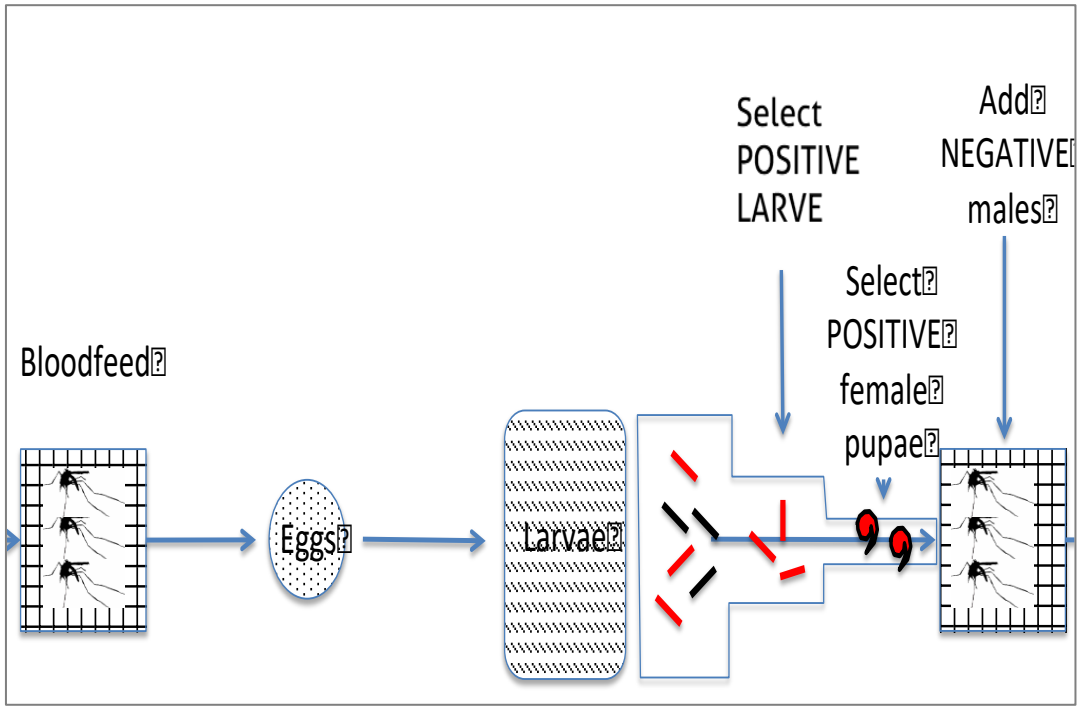
Methods-1

Creation of colour variant strains

- Basing on presence or absence of the pigmentation at 4th instar larval stage, two color variant strains were isolated
- Sexing was conducted at pupal stage
- Strain colonies formed in separate cages, allowed to produce the next generation



Results-1: Mendelian trait ratios

Results

- Larval ratio of 1:1 of pigmented to non-pigmented strains.
- The male:female sex ratio was 1:1 regardless of strain and backcross.
- Chi Square: observed progeny ratios in backcrosses not significantly different (3df, Pvalue >0.05) from the expected mendelian ratios

49.93% (SEM = 0.36, n = 16) were pigmented

Colour variant trait screen at larvae		Positive		Negative	
	Expected	0.5		0.5	
	Observed	0.5		0.5	
Sex at pupal screen		Positive		Negative	
		Male	Female	Male	Female
	Expected	0.5	0.5	0.5	0.5
	Observed	0.5	0.5	0.5	0.5
Progeny category		Positive Male	Positive Female	Negative Male	Negative Female
	Expected	0.25	0.25	0.25	0.25
	Observed	0.25	0.25	0.25	0.25

Table 1: Results of the colour variants studies showing the resultant mendelian ratios.

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Results-2: Fecundity

Results

- Each backcross produced an average of 4747.3(±2254) eggs
- Average hatch rate of 86%(±2)

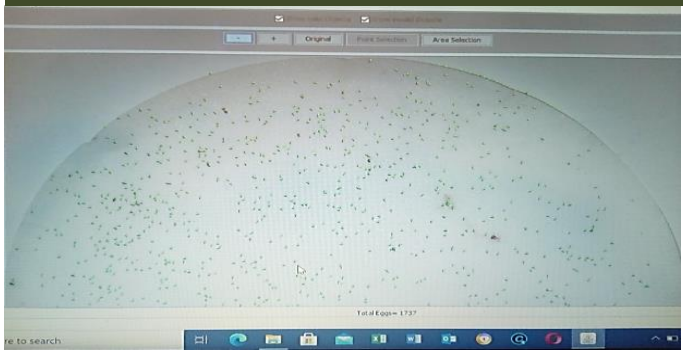
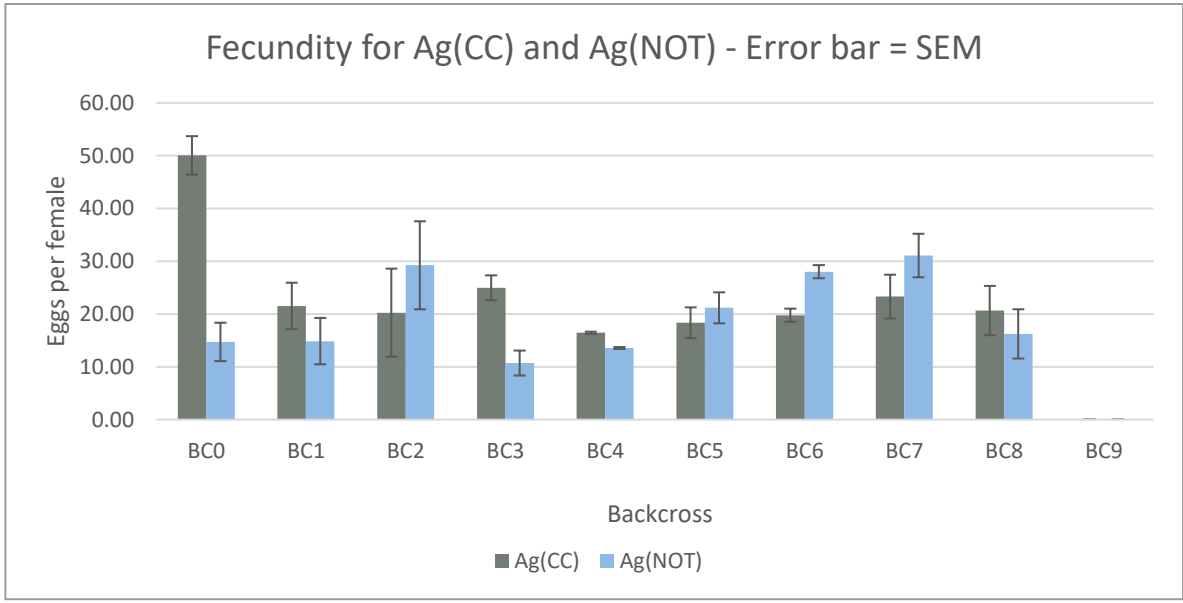


Figure 2: A screen short of the Egg counting software showing a filter paper image with eggs for counting in the facility.



Pairwise T-test showed no significant difference between the Ag(NOT) backcross colony and the Ag(CC) maintenance colony, $t(14) = 0.017$, $p = 0.9887$

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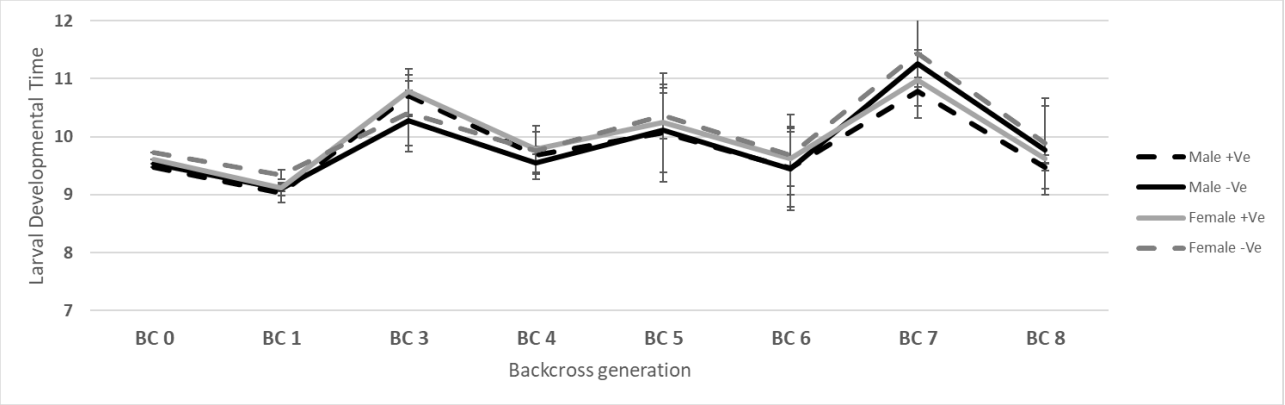
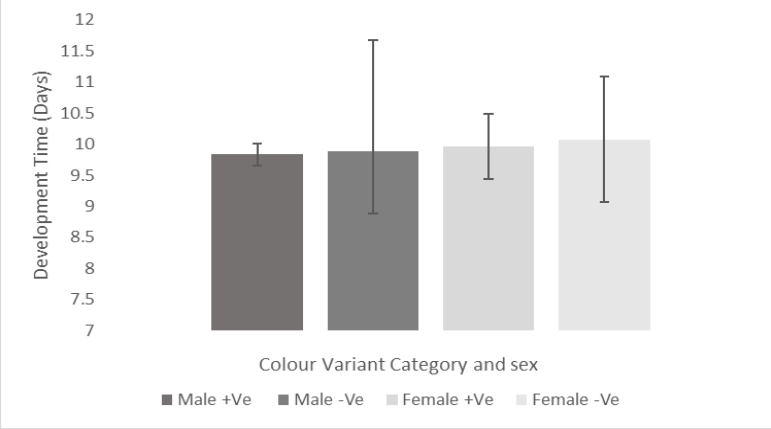
Results-3: Development times



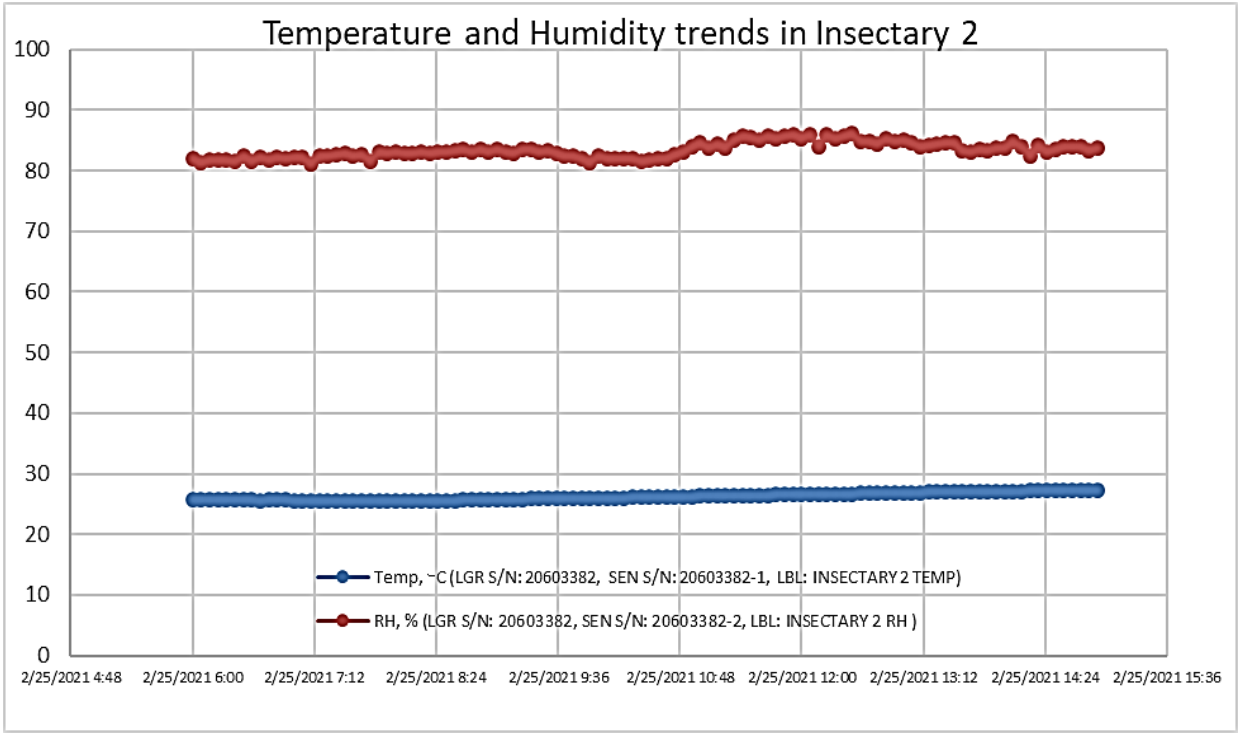
Mean larval development time (days) for each backcross for all categories of larvae. Error bars = SEM



Mean larval development time for all classes of larvae. Error bars = SEM

Results-4: Rearing Environmental controls

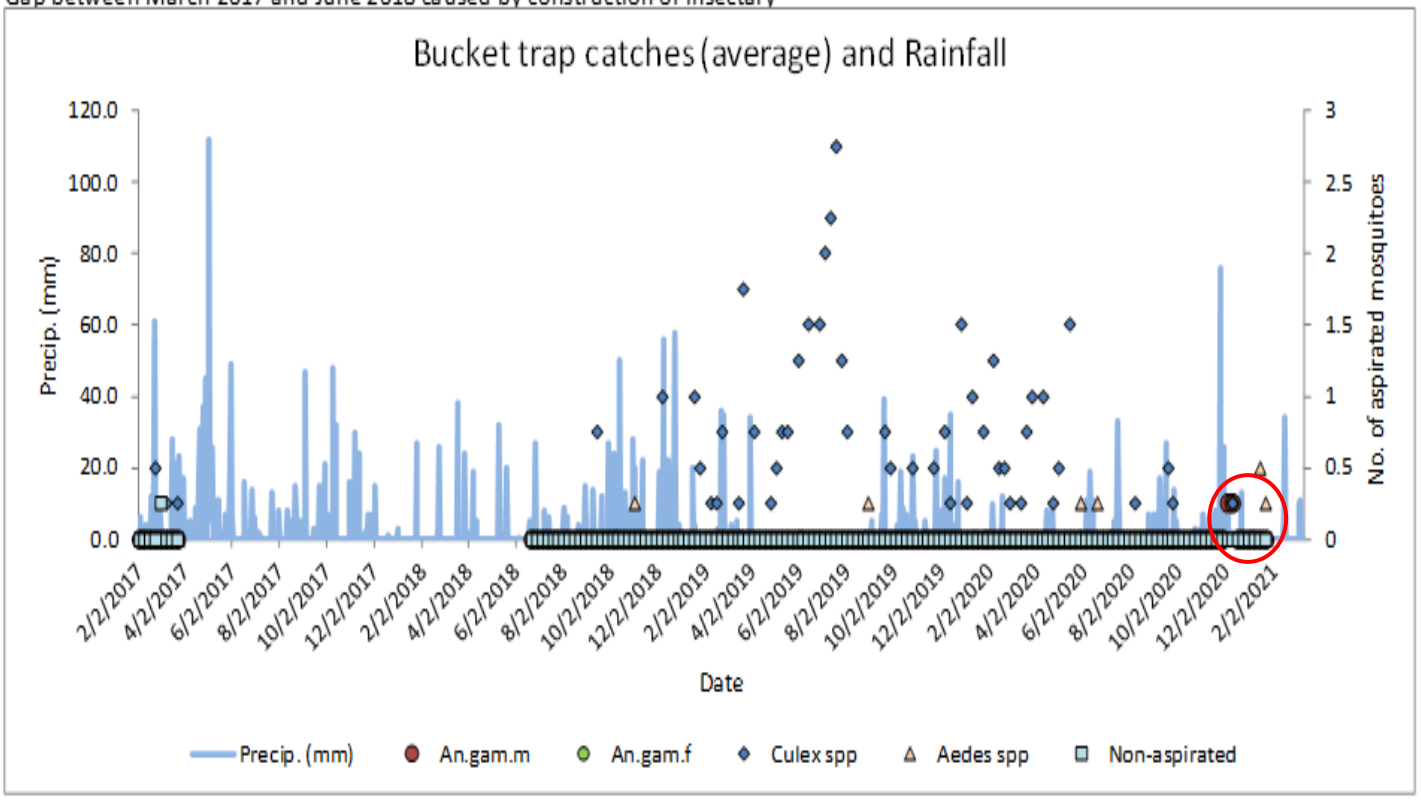
- Consistent environmental conditions (temperature & Humidity) within the facility



Results-5: Around facility mosquito surveillance

Graphs

Gap between March 2017 and June 2018 caused by construction of insectary



Very few *An gambiae* around facility, presenting reduced risk if there was accidental release mosquitoes surviving and mating with local population

Conclusion on facility readiness

Isolation and maintenance of Color variant mosquito strains shows ACL-2 facility operational readiness at the new UVRI Insectary

Conclusion

- Successful isolation and maintenance of colour variant strains under ACL-2 standards
- The use of the colour variant lines as a training opportunity also establishes documented evidence of the readiness of both the team and the facility for the next step in research leading to genetic strategies against malaria

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Future Studies

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Our long-term approach

- Our goal: a self-sustaining tool that enables a reduction in the population of malaria-carrying mosquitoes in Africa.

- Our genetically modified approach investigates 2 possible strategies:

Male bias

A modified mosquito that spreads a modification from generation to generation so that **the sex ratio of male to female in the population is changed**. As the population becomes nearly all male, the malaria-carrying mosquito population would reduce to the point where transmission is interrupted.

Female fertility

A modified mosquito that spreads a modification from generation to generation so that the **females in the population become infertile**. As the proportion of infertile females increases, the malaria-carrying mosquito population would reduce to the point where transmission is interrupted.

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Regulatory oversight is essential to ensure trust and safety

UNCST-NBC

National Research and Biosafety regulators

REC/IBC

Institutional oversight

NEMA

Environmental bodies

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Acknowledgements

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BILL & MELINDA GATES foundation

Open Philanthropy Project

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CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION


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London


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MALI-USA
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Malaria Research and Training Center (MRTC-E)
Dept.of Vectors and Vector Borne Diseases


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Thank you

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