Optimization of assays to determine the fitness competence of *An. stephensi*

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Introduction

Anopheles stephensi (*An. stephensi*) is an established and emerging malaria vector mainly present in southern Asia. Since 2012 it has invaded several countries of eastern Africa, being an emerging risk. Although insecticide-based measures targeting this vector have proven to be the most effective for malaria control, current measures are insufficient to achieve complete eradication.

The release of engineered mosquitoes has been proposed as a viable alternative. The efficiency of these systems depend on the **mating competitiveness** of these transgenic strains.

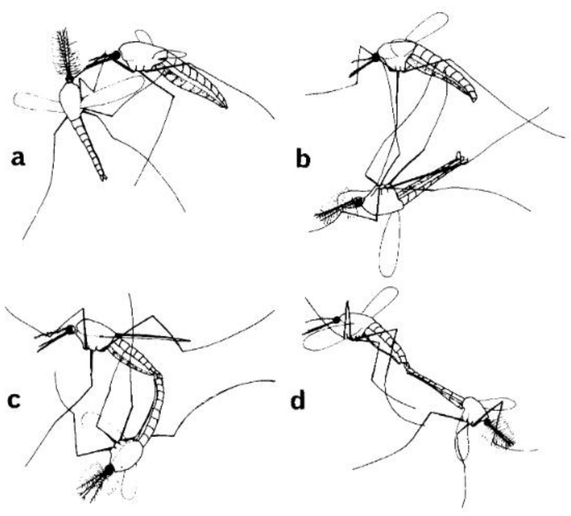


Fig 1. Diagrammatic representation of mating sequence in *Anopheles* mosquitoes. (a) A male locates and approaches a flying female; (b) One of the male's legs hooks onto a female's leg; (c) The male swings to align its genitalia to the ones of the female; and, (d) The males releases his hold and vibrates for 10-15s when he flies away. Adapted from Charlwood, *et al.* 1979.

Objectives

1. To review fitness assays from the published literature.
2. To establish and optimize the following fitness aspects in *An. stephensi:*
   - Male and female fertility and fecundity.
   - Mating competitiveness.
   - Male and female ability to fly

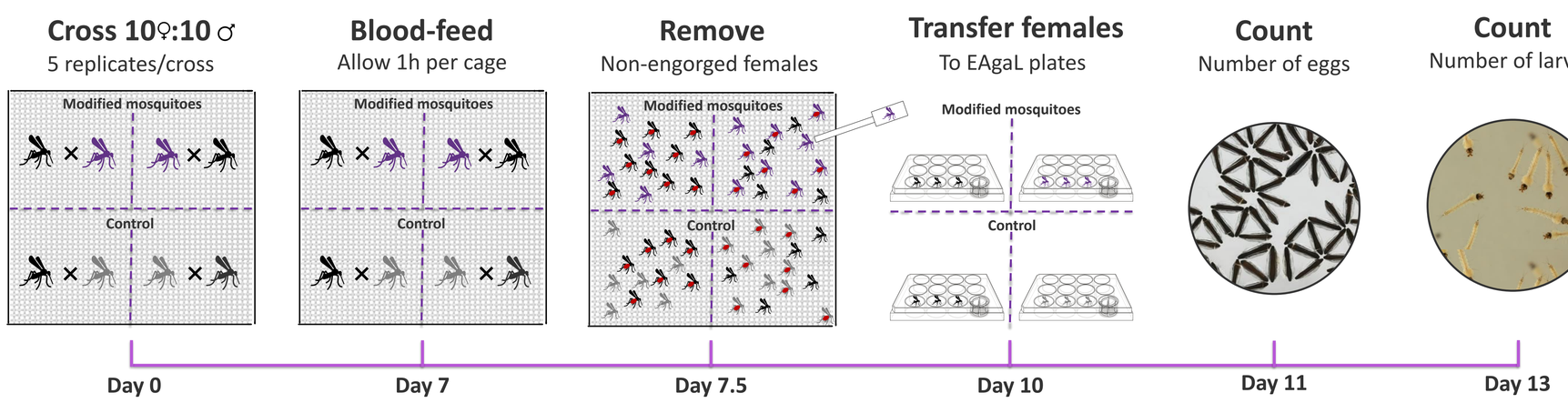
Conclusions

In summary, we established three different methods to assess *An. stephensi* lab-reared mosquitoes' reproductive fitness, serving as a first step to determine their mating competitiveness in the field. Although the presented methods are species-specific, these could easily be adapted to different lab-reared insect species, being of great interest for the entomological field.

Methods and results

Cross: female:male  WT population  WT sibling  Engineered sibling

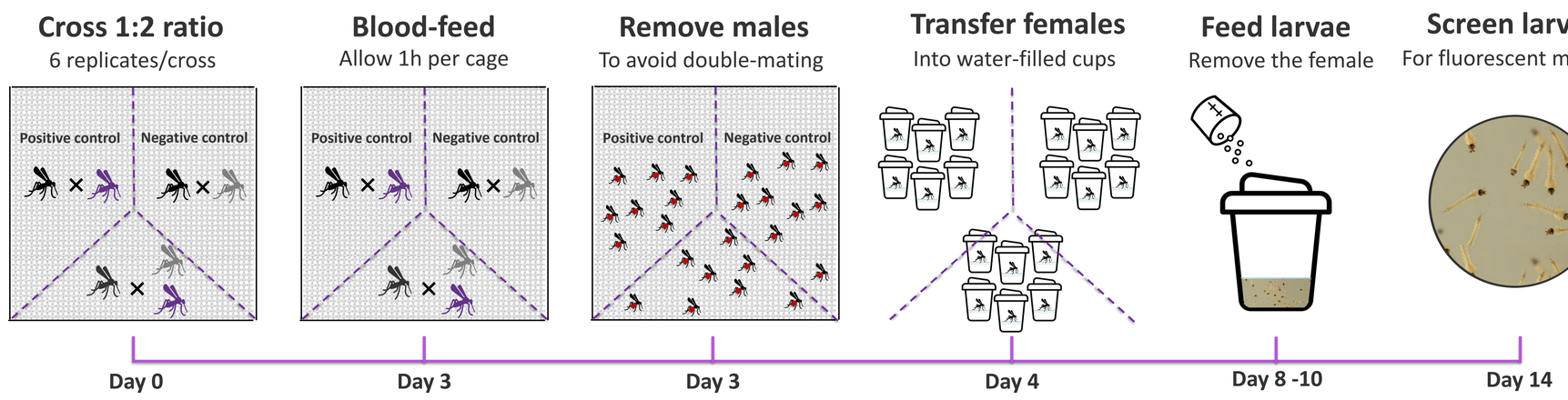
Fecundity and fertility assay



Highlights

- The EAgal plates allow all the females from a replicate cross to lay eggs in one plate which improves the efficiency of the assay without impairing the obtained data in comparison to protocols with larger laying area.
- This assay allows to obtain data on the blood-feeding rate, death after blood-feeding, fecundity and fertility.
- Adding the females to individual wells allows to use each female as an independent replicate.

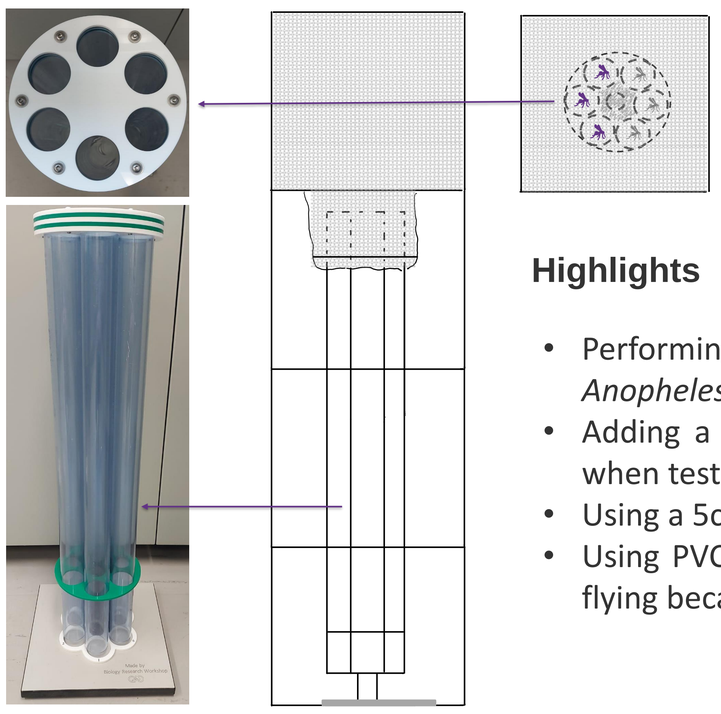
Male competitiveness assay



Highlights

- Allowing the females to mate for 72h increased the mating efficiency in comparison to 24h.
- Crossing females to males in a 1:2 ratio increases the proportion of mated females since the allowed mating time is considerably short.
- Adding the females to individual cups allows to use each female as an independent replicate.

Flight test



Procedure

- Add 30 mosquitoes/replicate to the bottom of the tubes.
- Count the number of mosquitoes remaining in the tubes every 30min during 3.5h.

Highlights

- Performing the experiment at dusk in comparison to daytime increased *Anopheles* mosquitoes' activity.
- Adding a UV light worked as an incentive to increase the number flyers when testing for females' flight ability.
- Using a 5cm diameter tubes gave enough width for *Anopheles'* to fly.
- Using PVC tubes guarantees that mosquitoes can only get to the cage by flying because PVC is too slippery for them to crawl.

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