

Lab cage mating competitiveness

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Introduction:

The purpose of these experiments is to determine mating compatibility and competitiveness of OX513A males with local *Aedes aegypti* strain(s) under small cage laboratory conditions. There are several acceptable ways to perform these experiments however the following is our preferred method.

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Mating competitiveness experiment:

For these experiments use 10 ♂ (5 RIDL, 5WT) with 5 ♀, i.e. 5:5:5 ratio.

Preparing the mosquitoes: rear WT mosquitoes without tetracycline (off-tet) and RIDL mosquitoes on-tetracycline (tet), separate the male and female pupae and determine their size¹. The pupae are then transferred to individual 15 ml plastic specimen tubes, allowed to eclose and sexed. Adults are aged 3-5 days. It is extremely important that all mosquitoes used are as equivalent and comparable as possible – reared under equivalent conditions (male size is a test of this), handled the same way, aged for the same period of time, etc. Even small differences can introduce systematic errors and biases in this type of experiment, and consequently misleading results.

Mating: Conduct mating experiments in a suitable small cage (i.e. 30x30x30cm; larger is better if available). Place female mosquitoes into the cage first followed by the males. The males (WT and RIDL) should be treated identically before addition into the cage and should both be added at the same time, e.g. pre-mixed and added together. All adults should be 3-5 days old. All adult mosquitoes are maintained off-tet i.e. no tet was added to the sugar solution or the blood. This is to simulate the post-release field conditions. Sugar water is provided as normal. Mating is for a pre-determined time, e.g 24h or 48h. Mosquitoes are then aspirated from the cage and

¹ Several methods are available for doing this. Pupal size is a good proxy, or adult wing length. Adult wing length can be measured after the mating experiment, if the males are retrieved.

separated by sex, i.e. males from females. This ends the mating period.

Post-mating analysis:

Females: pool and blood-feed (this can alternatively be done in the mating cage).

Females are then separated. Allow the females to lay eggs individually on a suitable ovipositing medium and record the number of eggs laid. Examine the spermathecae of females that did not oviposit for sperm. After conditioning the eggs for 3 days vacuum hatch off-tet (In principle, tet at this stage does not matter, but for OX513A scoring fluorescence is much easier if larvae are reared off -tet) and then record the hatch rate and fluorescence status (yes/no) of the larvae/pupae. Paternity is inferred from the fluorescence status of these larvae (fluorescent larvae had a RIDL father, non-fluorescent larvae had a wild type father).

Males: wing length and symmetry can be recorded, but genotype is not so easy to determine unless the males were marked prior to mixing, which can potentially introduce other biases. We typically measure other males from the population, i.e. not the ones used for the actual mating experiment, and select male pupae of equivalent size for the actual experiment.

At least 100 females should be used, i.e. at least 20 cages. Clearly, these do not all need to be performed simultaneously; 3 repeats of 7 cages, or 4 repeats of 5 cages may be more practical.

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Expected outcomes:

If males are equivalent, expect half of the females that mate to mate a wild type male and half to mate a RIDL male. The actual number of females that mate, and the proportion of these that blood feed and lay viable eggs, varies from strain to strain. Only 50% or so of the females used may give useful data on male competitiveness i.e. mate and lay viable eggs; this proportion may be lower for wild or recently colonised strains. Double mating (detected as females with both fluorescent and non-fluorescent progeny) should be rare.

Calculate a relative mating index as; [number of females mating a RIDL male] / [total number of females for whom mate genotype could be determined]

Clearly, if the males are equally competitive, this number should be 0.5. Statistically significant reduction below 0.5 indicates that the RIDL males are not fully competitive in this assay. However, they do not have to be fully competitive, just good enough. For Medfly SIT, a value higher than 0.2 is acceptable. Previous experiments with OX513A have given an index around 0.5, i.e. no evidence of any reduction in competitiveness relative to wild type.

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Mating isolation:

If the mating index is significantly below 0.5, one possibility is that the two strains show some reproductive isolation. This is theoretically somewhat unlikely, given the relatively recent dispersal of *Aedes aegypti* from its African origin. It can be tested by repeating the test above but using RIDL females instead of wild type ones. If the observed reduction in mating index was due to (reciprocal) mating barriers, the RIDL males should do better, and the wild males worse, against RIDL females. It is unlikely that this test will be necessary. Interpretation is also not entirely clear-cut except for strong effects, as it is also possible that females of one strain are simply less choosy – the RIDL males would have to do significantly better than the wild males, not merely better than they did against wild females (but not better than the wild males) to provide evidence of mating isolation.

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References

1. Koenraadt, C.J.M. Pupal Dimensions as Predictors of Adult Size in Fitness Studies of *Aedes aegypti* (Diptera: Culicidae). *Journal of medical Entomology* **45**, 331-336 (2008).