# Density and colonization dynamics of gut bacteria essential for development of the African malaria mosquito *Anopheles gambiae*

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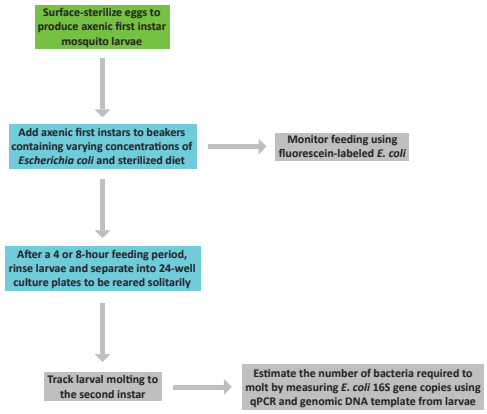
## Abstract
Gut bacteria are ubiquitous among animals and are known to play important roles in the immunity, nutrition, and overall health of their hosts. The gut bacterial community of mosquitoes has received attention due to results showing that some bacterial community members in the mosquito midgut can alter competency of the mosquito to transmit a number of important infectious pathogens. More recently, we showed that axenic (i.e. bacteria-free) mosquito larvae do not molt past the first instar. However, axenic larvae colonized by a single bacterial species such as *Escherichia coli* develop normally. Subsequent work using *Aedes aegypti* mosquitoes indicates that a particular density of bacteria must be reached in the larval gut to initiate molting. Here, we extend these studies to the African malaria mosquito *Anopheles gambiae*. We report a robust protocol for colonizing axenic *An. gambiae* larvae and the number of bacteria required for normal development. We also show that colonization of the larval gut occurs within 8 hours after hatching. These results further demonstrate a fundamental dependence by mosquitoes on their gut bacteria for development. Furthermore, the reported protocol has important implications for future studies characterizing the mechanism by which gut community members modulate mosquito development.

## Background
- Mosquitoes vector many dangerous pathogens
- Bacteria in the midgut of mosquitoes interact with the various pathogens that mosquitoes vector
- Gut bacteria are vital for mosquito development
- Larvae do not molt in the absence of bacteria
- Axenic (i.e. bacteria-free) larvae will molt after bacteria are introduced into their aquatic environment
- Work done with *Aedes aegypti* larvae shows that molting relies on colonization of the gut by living bacteria
- Concentration of bacteria in the aquatic environment is key to colonization

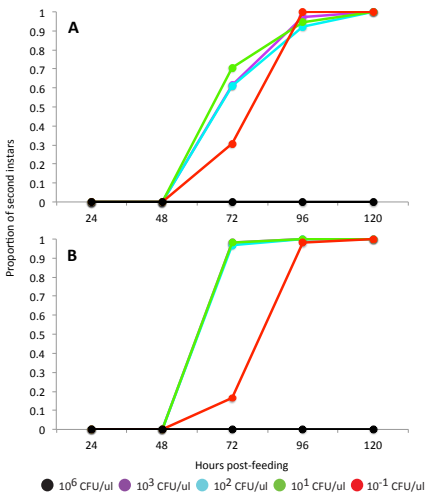
## Objectives
(1) Establish a reliable procedure for rescuing axenic *Anopheles gambiae* larvae
(2) Determine the number of bacteria necessary to restore normal development
(3) Assess *An. gambiae* larval feeding behavior

## Experimental Approach



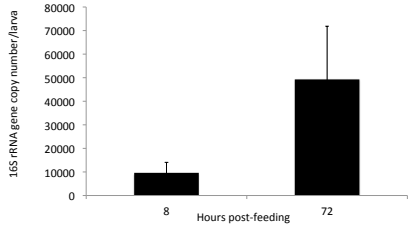

## Results

### 1. Molting success depends on the concentration of bacteria in the larval aquatic environment




Proportion of axenic first instars that molted to the second instar after being fed concentrations of $10^6$ (black), $10^3$ (purple), $10^2$ (blue), $10^1$ (green), or $10^{-1}$ (red) CFU/ul *E. coli* and sterilized diet for 4 (A) or 8 hours (B). A minimum of 60 larvae were assayed per treatment. Concentrations at or above $10^6$ CFU/ul resulted in 100% death by 48 hours post-feeding. Concentrations at or below $10^{-1}$ CFU/ul were not sufficient to facilitate colonization and minimal molting occurred. There was no difference in molting success between the $10^3$, $10^2$, and $10^1$ treatments (ANOVA, $p > 0.05$).

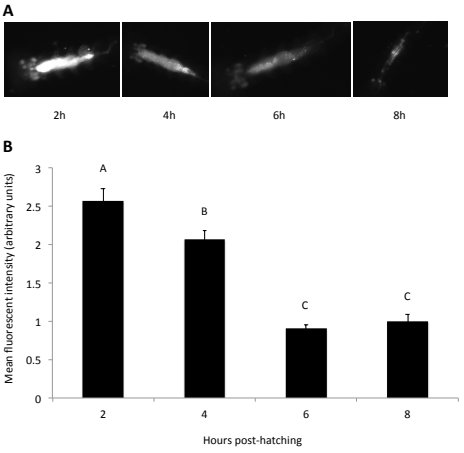
### 2. A particular threshold of bacteria is required to initiate molting




Bacterial load in larvae measured by qPCR (16S rRNA gene copy number). Axenic larvae were allowed to feed for 8 h in beakers containing 1,000 CFU/ul *E. coli* and sterilized diet. Genomic DNA was isolated from larvae immediately following the feeding period and from larvae that had molted to the second instar (72 h post-feeding). Graph shows means and standard error of the means.

## Results (continued)

### 3. Colonization takes place during the first 8 hours of feeding




(A) Epifluorescent images of individual larvae 2-8 h after placing fluorescein-labeled *E. coli* into well. (B) Mean fluorescent intensity in larvae 2-8 h after placement of fluorescein-labeled *E. coli* with larvae. Columns present mean values with 95% confidence intervals for each time point. A minimum of 15 larvae were measured per time point. Means with the same letter are not significantly different from each other (ANOVA, $p > 0.05$).

## Significance
- This work highlights a fundamental dependency of mosquitoes on their gut bacteria for development
- Future work will characterize the mechanism by which bacteria in the midgut colonize and rescue larval development

## Acknowledgements
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## References
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