Transgenic mosquitoes expressing a phospholipase A_2 gene have a fitness advantage when fed *Plasmodium falciparum*-infected blood

Ryan C. Smith[1], Christopher Kizito[1], Jason L. Rasgon[2], Marcelo Jacobs-Lorena[1]*

[1]Department of Molecular Microbiology and Immunology, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States of America.

[2]Department of Entomology, Center for Infectious Disease Dynamics and the Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, Pennsylvania, USA.

*E-mail: mlorena@jhsph.edu

Supporting Information

Figure S1. Outline of procedures used to conduct cage experiments.

Figure S2. Oocyst numbers in transgenic cage experiments.

Figure S3. Genotyping of PLA_2 transgenic lines.

Table S1. Primer sequences used for spPCR.

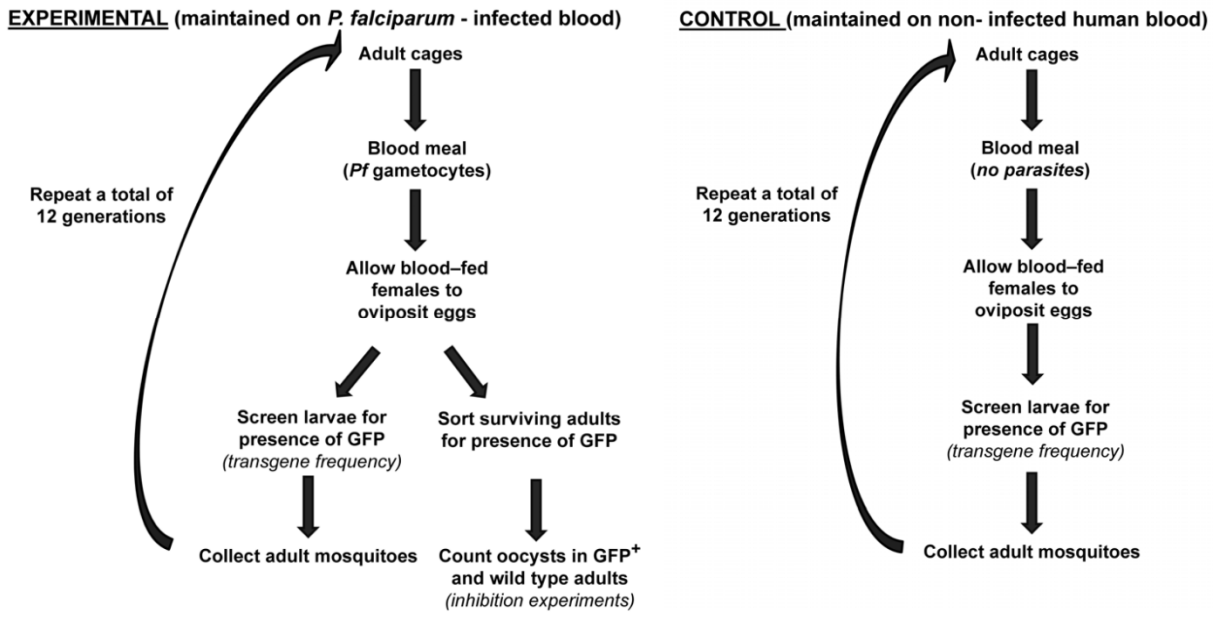
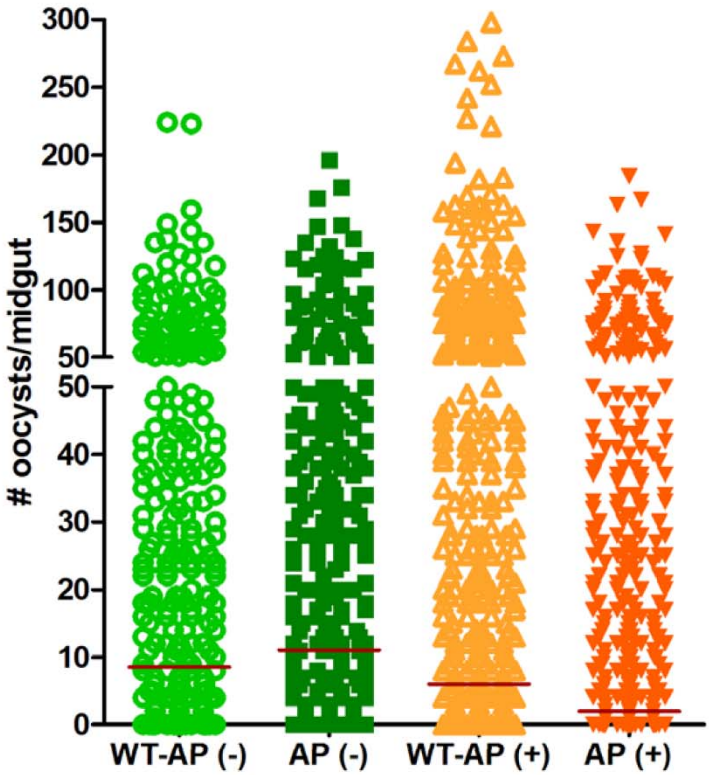


Figure S1. Outline of procedures used to conduct cage experiments.



N	370	370	571	558
Prevalence	70%	71%	69%	64%
Mean	26	26	31	19
% Inhibition	/	/	/	39%
Median	8.5	11	6	2
% Inhibition	/	/	/	66%
P-value		0.9843		<.0001

Figure S2. Oocyst numbers in transgenic cage experiments. At each generation, adult female mosquitoes from each cage were individually assessed for GFP fluorescence (to distinguish transgenic from non-transgenic mosquitoes), followed by determination of *P. falciparum* oocyst numbers. Combined data for all cage experiments demonstrate that only the AP (+) transgenic line inhibited *P. falciparum* development, similar to the results in Figure 2B. The horizontal red line denotes the median value for each data set. WT-AP (-): wild type control mosquitoes for the AP (-) infections; WT-AP (+): wild type control mosquitoes for the AP (+) infections.

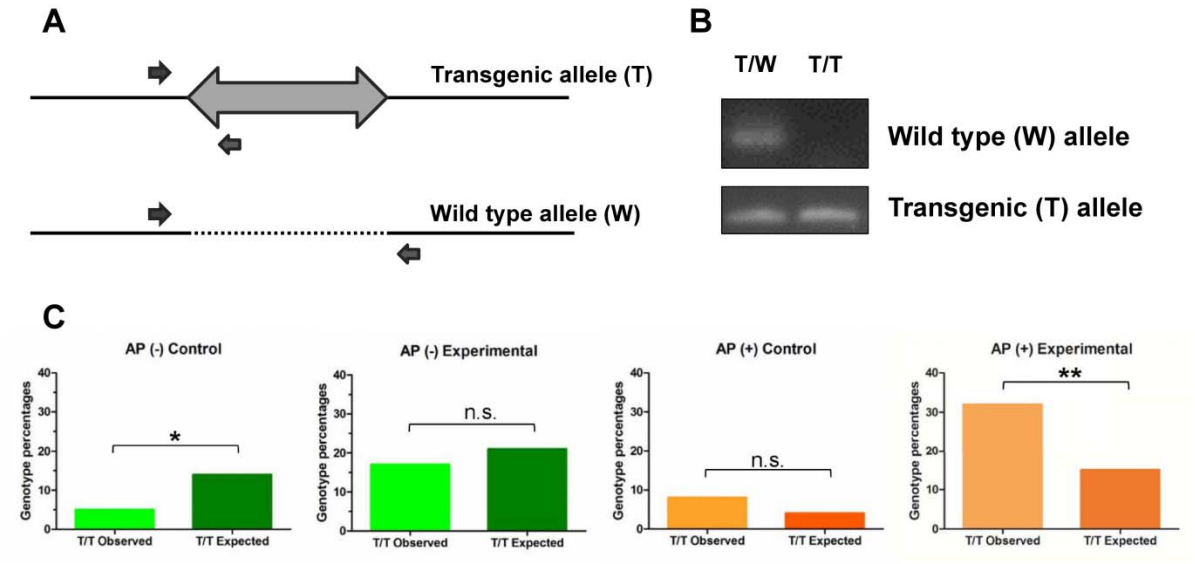


Figure S3. Genotyping of PLA_2 transgenic lines. Transgenic mosquitoes from each cage were individually genotyped by PCR to determine if the mosquito was hetero- or homozygous for the transgene. Using primers (grey arrows) that flank each insertion (A), individual GFP+ mosquitoes (containing the transgene) were genotyped by the presence/absence of the wild type allele and verified by the presence of the transgenic locus to distinguish heterozygous (T/W) or homozygous (T/T) individuals (B). Genotyping results for each transgenic cage experiment demonstrate the observed and expected percentages of mosquitoes homozygous for the transgene (C). Only the AP (+) transgenic line maintained on *P. falciparum* infected blood shows an increase in transgene prevalence as determined by Hardy-Weinberg analysis and was tested for significance by Chi-squared analysis. *= (P<0.05), **= (P<0.01), n.s.: not significant.

Table S1. Primer sequences used for spPCR.

Primer Use	Name	Sequence
spPCR Adaptor	Splink-Bottom (Universal)	CGAAGAGTAACCGTTGCTAGGAGAGACCGTGGCTGAATGAGACTGGTGTCGACACTAGTGG
	Splink-GATC-Top (*BstYI/BglII*)	GATCCCACTAGTGTCGACACCAGTCTCTAATTTTTTTTTTCAAAAAAA
	Splink-CGG-Top (*MspI*)	CGGCCACTAGTGTCGACACCAGTCTCTAATTTTTTTTTTCAAAAAAA
	Splink-AATT-Top (*EcoRI*)	AATTCCACTAGTGTCGACACCAGTCTCTAATTTTTTTTTTCAAAAAAA
Adaptor primers	Splink #1	CGAAGAGTAACCGTTGCTAGGAGAGACG
	Splink #2	GTGGCTGAATGAGACTGGTGTCGAC
***piggyBac* primers**	pBac LE #1	CAGTGACACTTACCGCATTGACAAGC
	pBac LE #2	GCGACTGAGATGTCCTAAATGCAC
	pBac RE #1	CGATATACAGACCGATAAAACACATGCGTC
	pBac RE #2	ACGCATGATTATCTTTAACGTACGTCAC