SUPPLEMENTARY MATERIALS

Variation in tolerance to parasites affects vectorial capacity in natural Asian tiger mosquito populations

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Contents

Figures

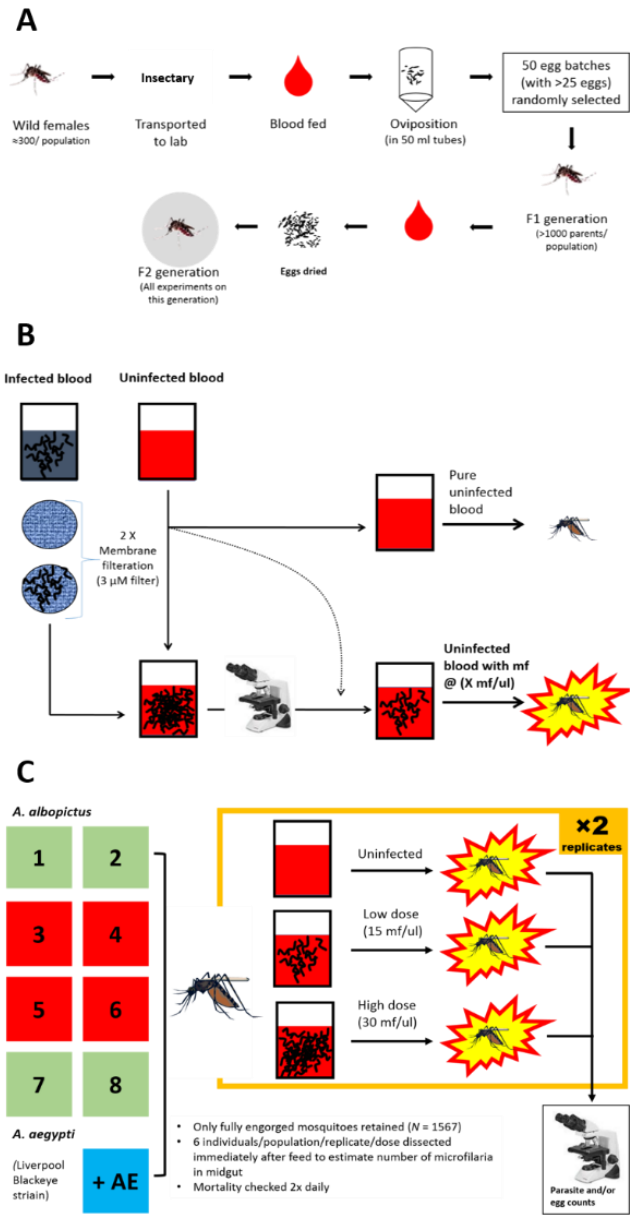

Figure S1. The basic study design used to: (A) Establish mosquito lines from field populations; (B) Produce uninfected and infected blood meals (at known microfilarial density) utilizing the same uninfected blood to control for differences in blood meal quality; (C) Study effects of *D. immitis* infection on mosquito survival, resistance and tolerance (see text for details).

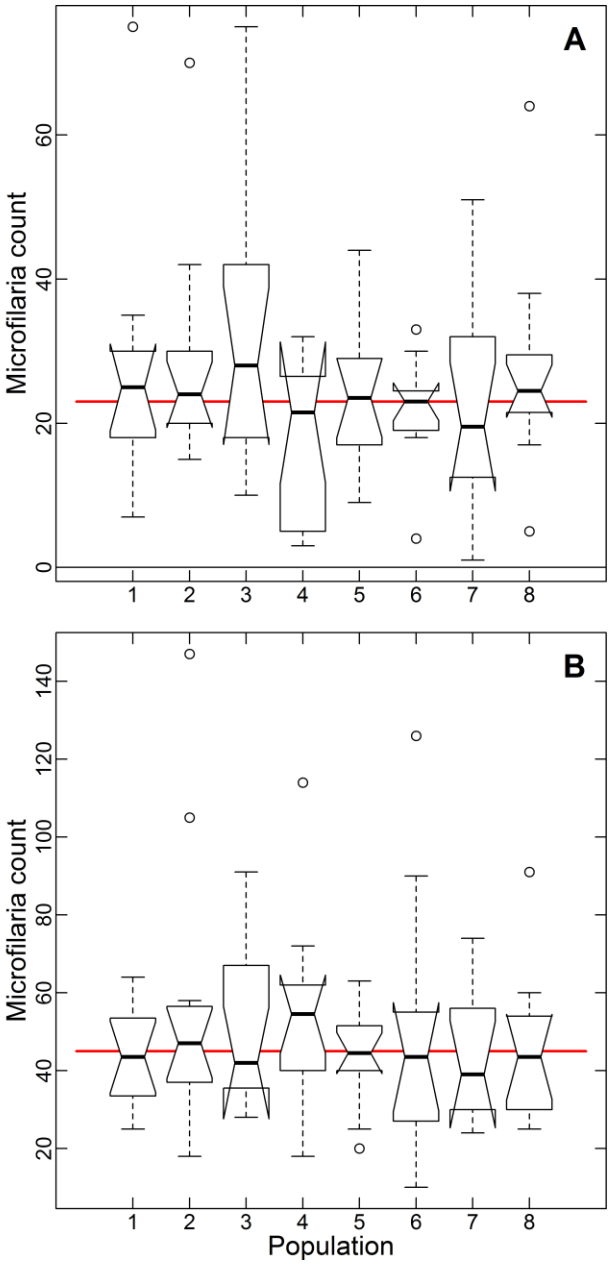

Figure S2. Initial ("Zero hour") microfilaria (mf) counts. A subset of six mosquitoes per population, dose and replicate were dissected after blood feeding to estimate initial microfilaria infection dose. The "zero-hour" counts are represented for two infection doses: (A) 15 mf/µl blood; (B) 30 mf/µl. Population numbers follow Fig. 1A in main text. We found a significant effect of Dose on initial mf load (F = 24373.23; df = 1; P <0.001), but no significant effects of Replicate (F = 177.65; df = 1; P = 0.501), Population (F = 2469.88; df = 7; P = 0.506) or

interaction between Population and Dose (F = 2231.80; df = 7; P = 0.576).

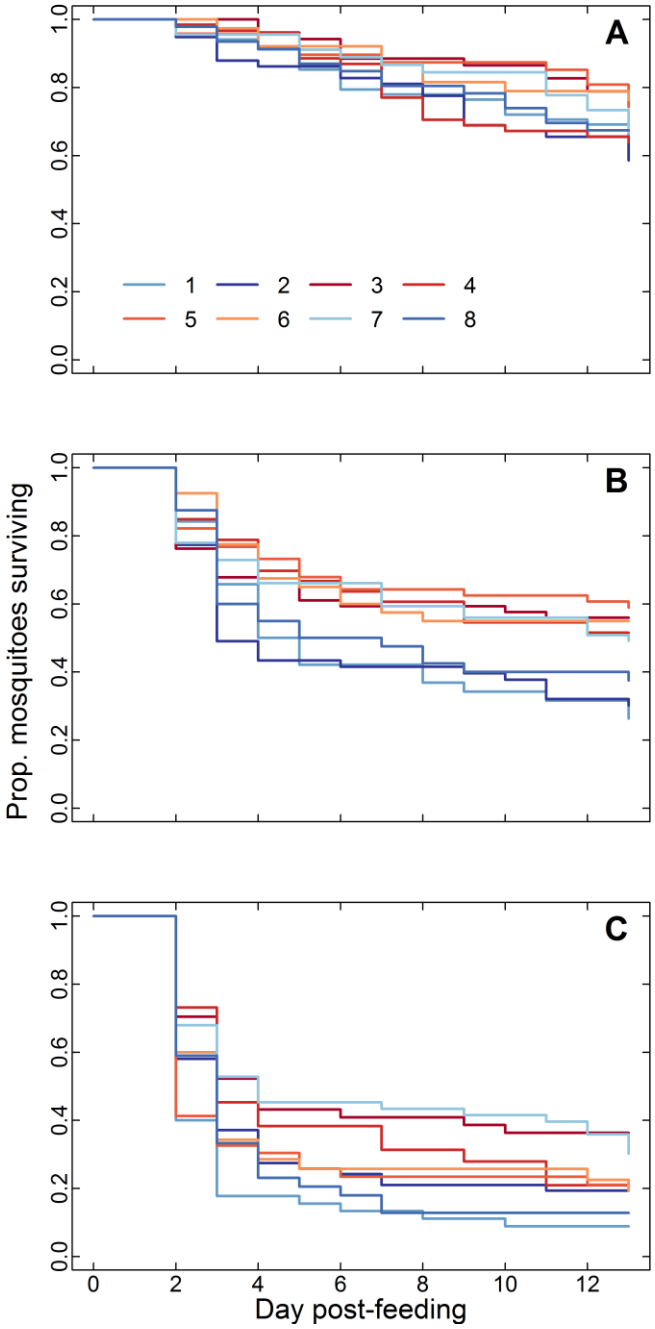

Figure S3. Survival curves for each of three *D. immitis* doses: (A) 0 mf/µl blood; (B) 15 mf/µl blood; (C) 30 mf/µl blood. The survival curves are shown for the eight different *A. albopictus* populations (See legend; Population numbers follow Fig. 1A in main text).

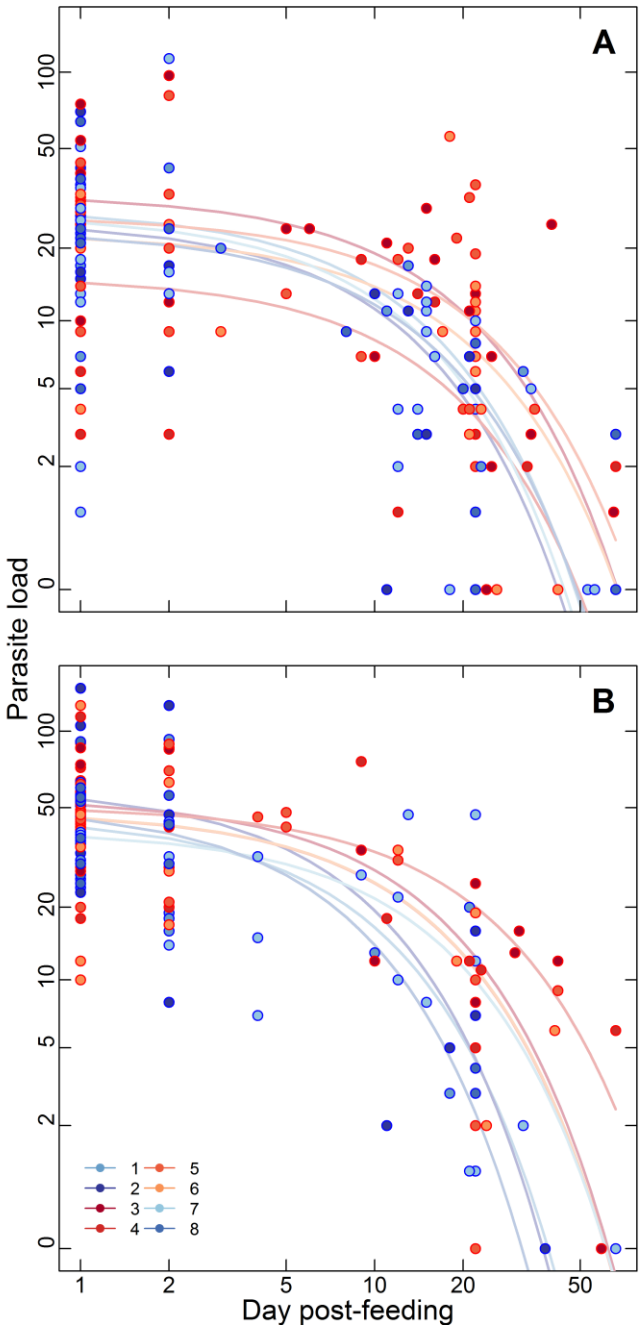

Fig. S4. Mosquito parasite loads at mortality for two *D. immitis* infection doses: (A) 15 mf/µl blood; (B) 30 mf/µl blood. The symbols represent individual mosquitoes and the lines are the best-fit curves from the GLMER regression (see Methods). Parasite loads are shown for the eight different *A. albopictus* populations (See legend; Population numbers follow Fig. 1A in main text).

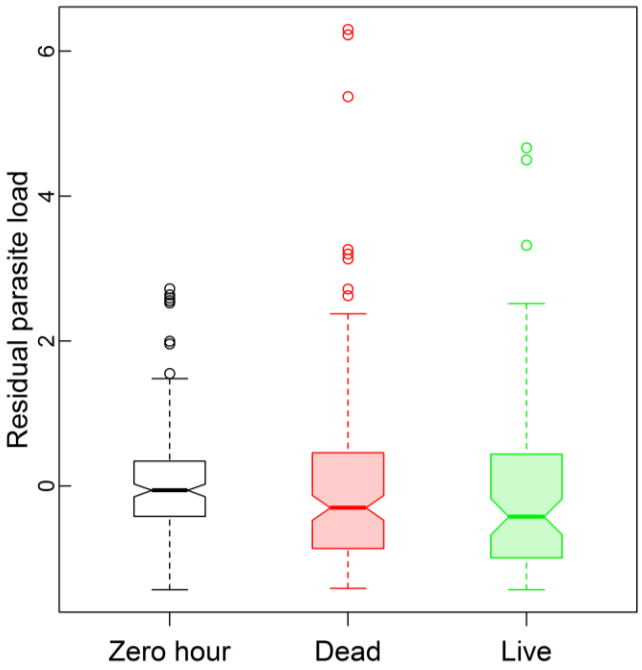

Fig. S5. Differences in parasite load amongst dead and live mosquitoes. We calculated the Pearson residuals of parasite loads based on the negative binomial regression (Table S3) to control for effects of Population, Dose and Day. We then tested if there were significant differences in residuals of parasite loads based on whether the number of parasites were determined from mosquitoes at the point of exposure (Zero hour), those that died prior to dissection (red symbols) and those that were alive but euthanized prior to dissection (green symbols). We found no significant effect of mortality status on the residual parasite load on the of the mosquito ($F_{2,409}$ = 0.815; P = 0.443). However, we note that there was a slight decrease in parasite loads amongst live mosquitoes dissected vs. dead mosquitoes.

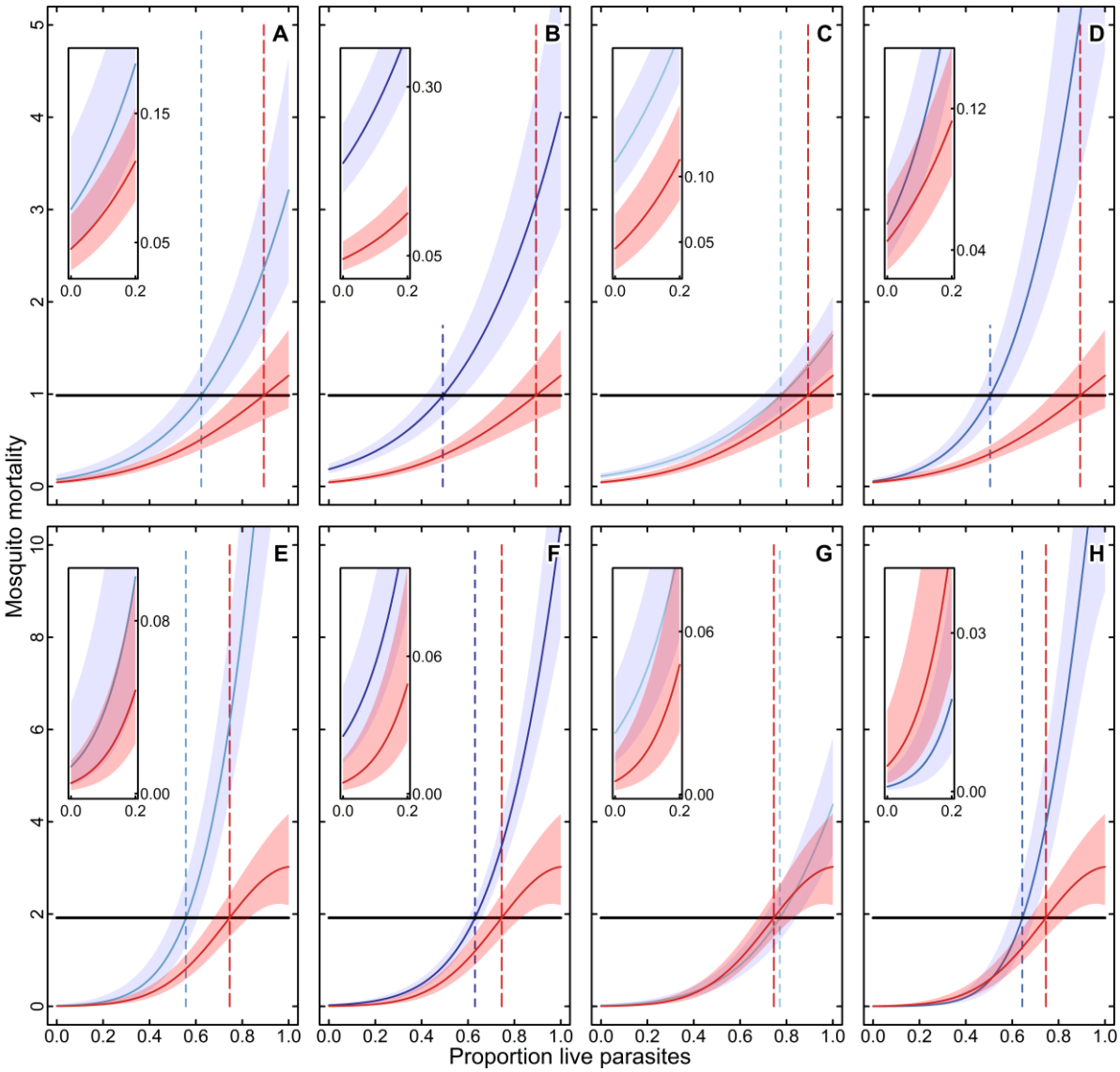

Fig. S6. Tolerance to *Dirofilaria immitis* infection demonstrated as between-population variation in fitness of *Aedes albopictus* infected with the same load of live parasites, accommodating their resistance. Differences in mortality hazard as the proportion of live parasites increases for mosquitoes infected at two *D. immitis* infection doses: 15 (**A-D**) and 30 (**E-H**) microfilaria/µl of blood. The graphs compare patterns of mortality between one high exposure population (Pop 4; red symbols) and the four low exposure populations (blue symbols): Pop 1 (**A** and **E**), Pop 2 (**B** and **F**), Pop 7 (**C** and **G**) and Pop 8 (**D** and **H**). Error bands are standard errors of the mean and population numbers follow Fig. 1A. Also represented are the maximum live parasite loads (dashed vertical lines) at which the population-specific mortality hazard is below average mortality hazard across all populations for a given infection dose (horizontal lines). The insets show mortality curve details at the highest levels of resistance (i.e., proportion of live parasites $\leq$ 0.2).

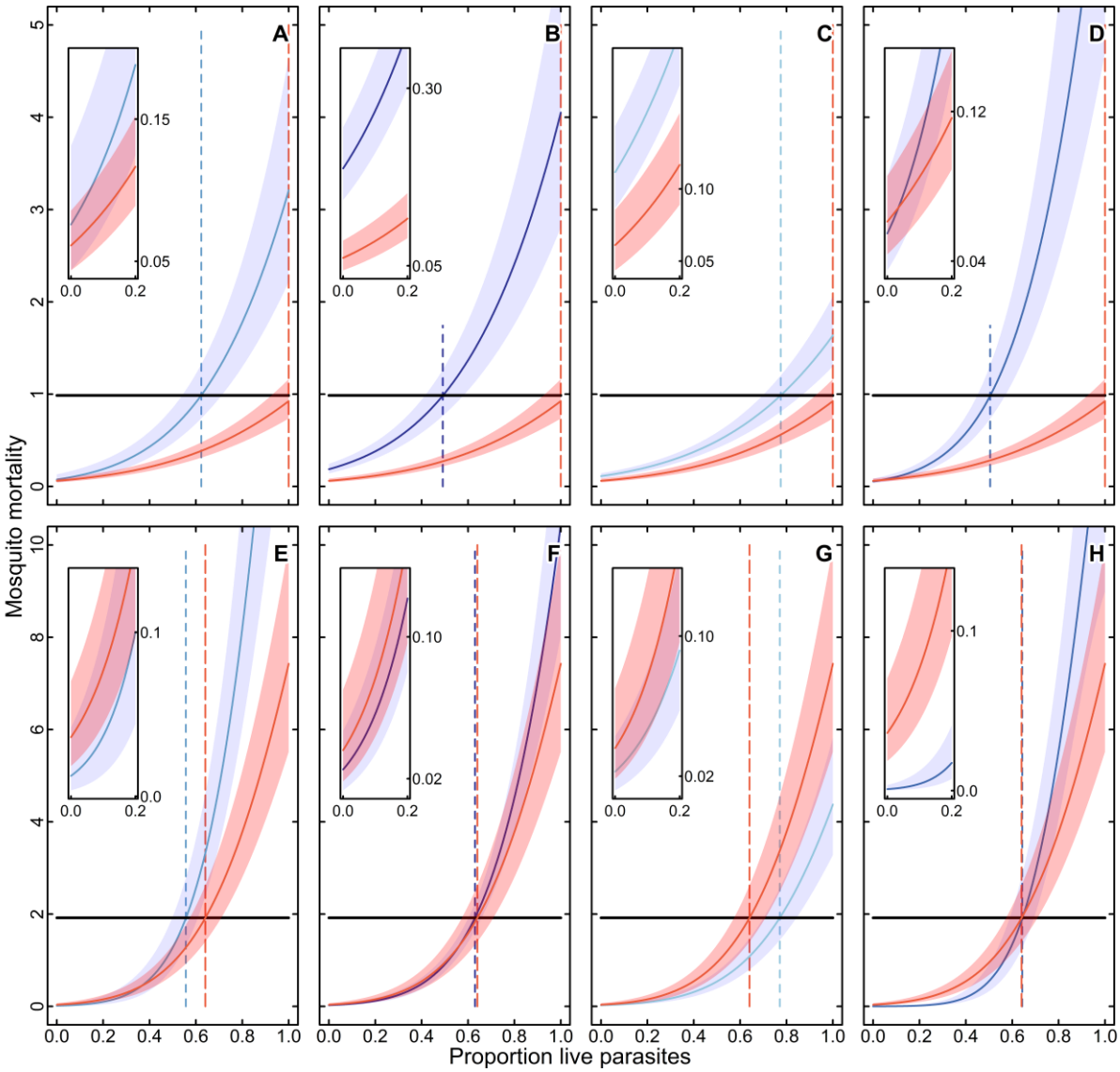

Fig. S7. Tolerance to *Dirofilaria immitis* infection demonstrated as between-population variation in fitness of *Aedes albopictus* infected with the same load of live parasites, accommodating their resistance. Differences in mortality hazard as the proportion of live parasites increases for mosquitoes infected at two *D. immitis* infection doses: 15 (**A-D**) and 30 (**E-H**) microfilaria/µl of blood. The graphs compare patterns of mortality between one high exposure population (Pop 5; red symbols) and the four low exposure populations (blue symbols): Pop 1 (**A** and **E**), Pop 2 (**B** and **F**), Pop 7 (**C** and **G**) and Pop 8 (**D** and **H**). Error bands are standard errors of the mean and population numbers follow Fig. 1A. Also represented are the maximum live parasite loads (dashed vertical lines) at which the population-specific mortality hazard is below average mortality hazard across all populations for a given infection dose (horizontal lines). The insets show mortality curve details at the highest levels of resistance (i.e., proportion of live parasites ≤ 0.2).

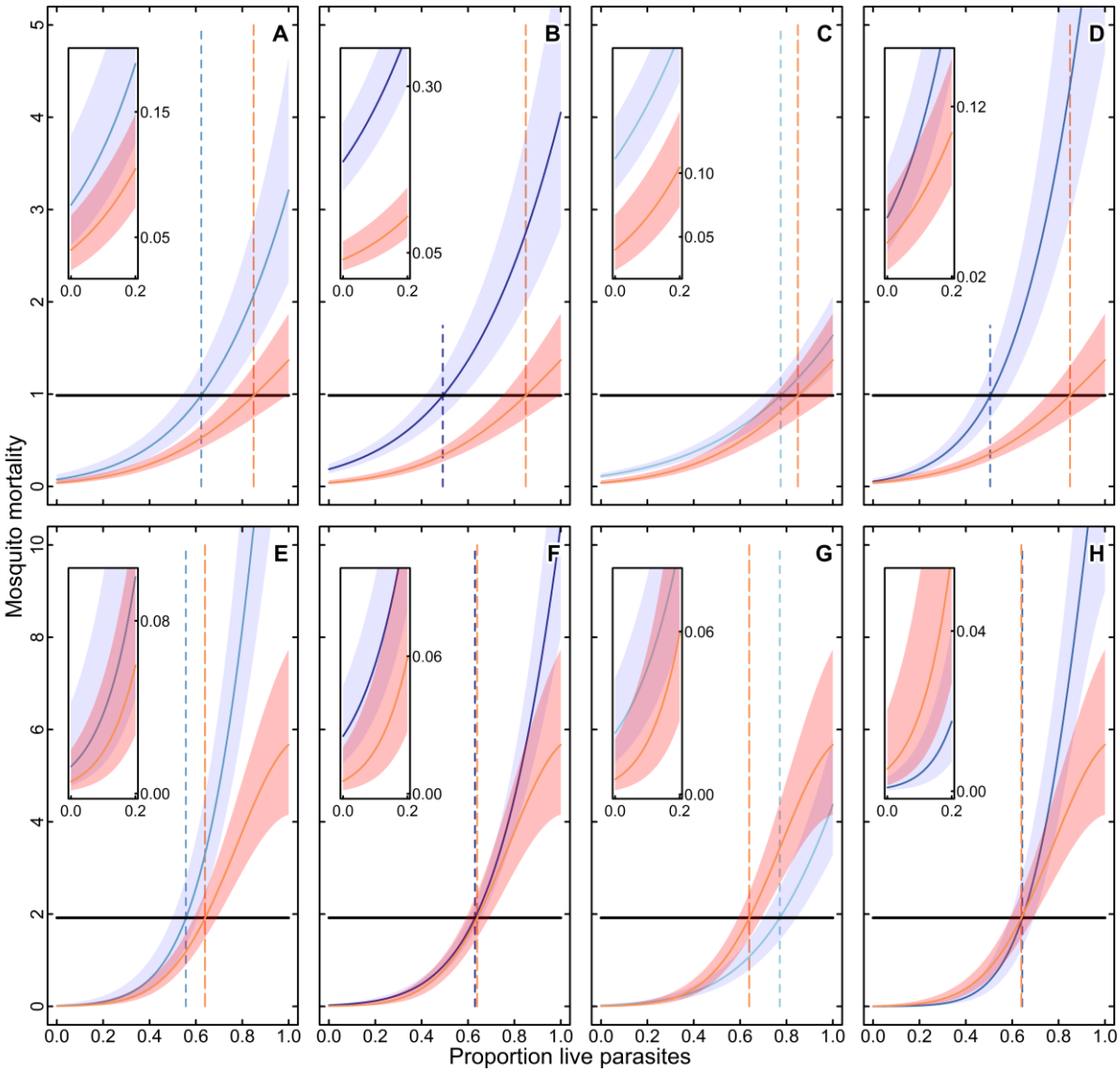

Fig. S8. Tolerance to *Dirofilaria immitis* infection demonstrated as between-population variation in fitness of *Aedes albopictus* infected with the same load of live parasites, accommodating their resistance. Differences in mortality hazard as the proportion of live parasites increases for mosquitoes infected at two *D. immitis* infection doses: 15 (**A-D**) and 30 (**E-H**) microfilaria/µl of blood. The graphs compare patterns of mortality between one high exposure population (Pop 6; red symbols) and the four low exposure populations (blue symbols): Pop 1 (**A** and **E**), Pop 2 (**B** and **F**), Pop 7 (**C** and **G**) and Pop 8 (**D** and **H**). Error bands are standard errors of the mean and population numbers follow Fig. 1A. Also represented are the maximum live parasite loads (dashed vertical lines) at which the population-specific mortality hazard is below average mortality hazard across all populations for a given infection dose (horizontal lines). The insets show mortality curve details at the highest levels of resistance (i.e., proportion of live parasites $\leq$ 0.2).

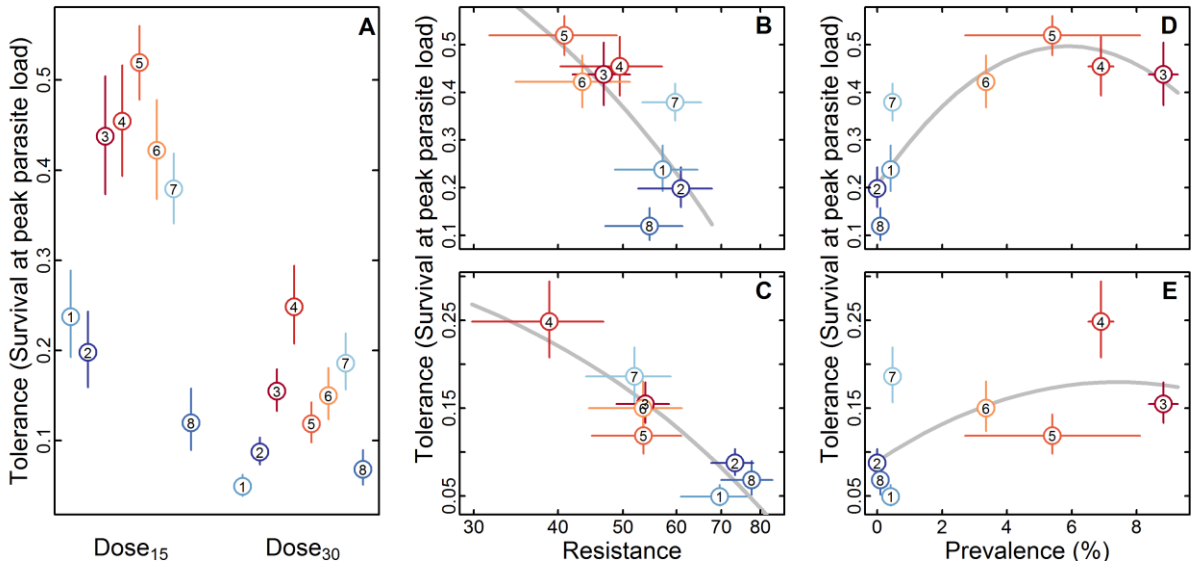

Fig. S9. Tolerance to *Dirofilaria immitis* infection amongst the eight *Aedes albopictus* populations. **(A)** Population differences in tolerance, measured as the relative survival of each population at peak infection load compared to the baseline survival, in mosquitoes infected using 15 and 30 *D. immitis* microfilaria/µl of blood ($Dose_{15}$ and $Dose_{30}$, respectively). **(B and C)** Relationship between resistance and tolerance in mosquitoes infected with 15 (B) and 30 (C) microfilaria/µl of blood. Regression model predictions are also shown (gray line; (Resistance: $F_{1,12} = 11.870$, P = 0.005; Dose: $F_{1,12} = 16.0170$, P = 0.002; Resistance × Dose: $F_{1,12} = 9.699$, P =0.009). **(D and E)** Relationship between prevalence of *D. immitis* in dogs and tolerance in mosquitoes infected with 15 (B) and 30 (C) microfilaria/µl of blood. Regression model predictions are also shown (gray line; Prevalence: F = 9.5, P = 0.012; Prevalence2: F = 4.353, P = 0.064; Dose: F = 38.855, P = <0.001). Error bars are standard errors of the mean and population numbers follow Fig. 1A

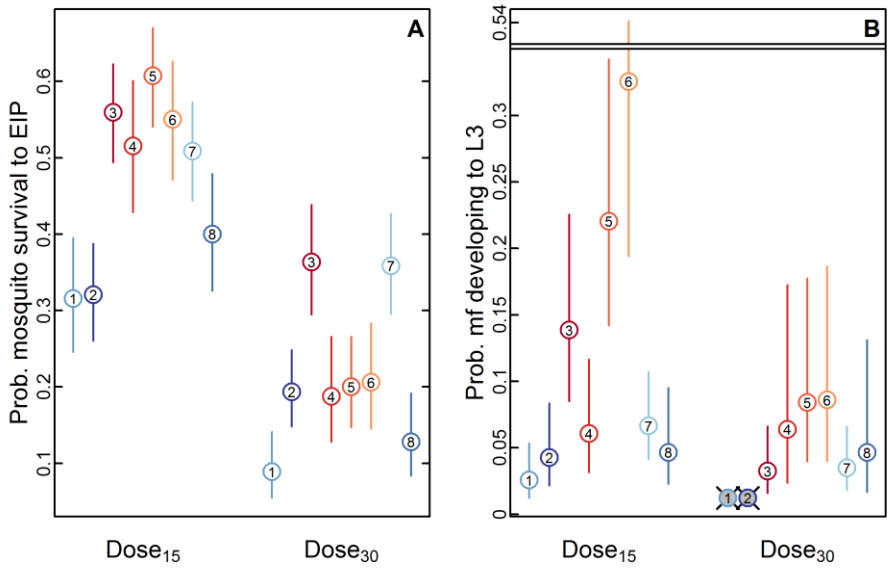

Fig. S10. Estimation of parameters used to calculate vector competence. The overall risk of parasite transmission is affected by the probability of a mosquito surviving to EIP (i.e., non-survivors have no infective parasites) and the number of mf that develop to infective L3 larvae in the surviving mosquitoes. **(A)** Population differences in the probability of a mosquito surviving to EIP in mosquitoes infected using 15 and 30 *D. immitis* microfilaria/µl of blood ($Dose_{15}$ and $Dose_{30}$, respectively); **(B)** Population differences in the infective L3 larvae in head and proboscis of mosquitoes surviving to EIP infected at $Dose_{15}$ and $Dose_{30}$, respectively.

Tables

Table S1. Details of the sampling locations including county and state, latitude and longitude. Also given are details of seroprevalence surveys for *Dirofilaria immitis* antibodies in dogs, the first reported year of *A. albopictus* presence and the number of generations/year.

Pop. No	County (State)	Lati-tude	Longi-tude	No. dogs sampled[1]	No. sero-positive[1]	Sero-prev. (±SE) [1]
1	Broward (FL)	26.01	-80.31	9398	39	0.42 (0.01)
2	Martin (FL)	27.19	-80.24	359	0	0.00 (0.00)
3	Escambia (FL)	30.43	-87.20	895	79	8.83 (0.11)
4	Gadsden (FL)	30.58	-84.58	768	53	6.90 (0.10)
5	Richmond City (VA)	37.53	-77.47	37	2	5.41 (0.40)
6	Prince George's (MD)	38.93	-76.94	1163	39	3.35 (0.05)
7	Montgomery (MD)	39.01	-77.02	5926	28	0.47 (0.01)
8	Dauphin (PA)	40.27	-76.88	1024	1	0.10 (0.01)

[1]Data from Bowman et al. [*18*]

Table S2. Sample sizes for the analyses of patterns of resistance and tolerance to *Dirofilaria immitis* in the eight *A. albopictus* (AA) populations infected using three infection doses (0, 15 and 30 mf/µl of blood; $Dose_{00}$, $Dose_{15}$ and $Dose_{30}$, respectively). Also included are the sample sizes for the *A. aegypti* (AE) positive control lab-strain.

Population	Dose	No. individuals[1]	No. censored intentional (and accidental)[2]	No. with parasite data (and zero counts)[3]	No. surviving to EIP[4]	No. with L3 data (and zero counts)[5]
1	$Dose_{00}$	68	24 (1)	--	--	--
	$Dose_{15}$	48	16 (0)	22 (0)	38	11 (9)
	$Dose_{30}$	57	14 (0)	21 (1)	45	4 (4)
2	$Dose_{00}$	58	20 (0)	--	--	--
	$Dose_{15}$	65	21 (0)	26 (2)	53	13 (9)
	$Dose_{30}$	74	17 (0)	24 (3)	62	11 (11)
3	$Dose_{00}$	52	11 (0)	--	--	--
	$Dose_{15}$	71	26 (0)	40 (4)	59	25 (16)
	$Dose_{30}$	56	19 (0)	29 (5)	44	15 (12)
4	$Dose_{00}$	61	23 (0)	--	--	--
	$Dose_{15}$	45	20 (0)	25 (1)	33	16 (10)
	$Dose_{30}$	53	14 (9)	19 (0)	32	6 (4)
5	$Dose_{00}$	48	14 (2)	--	--	--
	$Dose_{15}$	68	24 (0)	33 (0)	56	25 (13)
	$Dose_{30}$	58	16 (1)	25 (1)	45	9 (8)
6	$Dose_{00}$	38	17 (0)	--	--	--
	$Dose_{15}$	52	21 (1)	26 (2)	40	17 (9)
	$Dose_{30}$	45	12 (1)	19 (0)	34	7 (3)
7	$Dose_{00}$	45	14 (0)	--	--	--
	$Dose_{15}$	71	21 (0)	34 (3)	59	25 (16)
	$Dose_{30}$	63	18 (0)	27 (1)	53	14 (11)
8	$Dose_{00}$	46	15 (0)	--	--	--
	$Dose_{15}$	52	19 (0)	23 (4)	40	13 (10)
	$Dose_{30}$	51	15 (0)	19 (0)	39	5 (3)
AA total		1345	431 (15)	412 (27)	732	216 (148)
AE	$Dose_{00}$	58	39 (0)	--	--	--
	$Dose_{15}$	74	19 (0)	42 (2)	62	31 (6)
	$Dose_{30}$	87	13 (0)	27 (0)	75	11 (1)
AE total		219	71 (0)	69 (2)	137	42 (7)
Grand total		1564	502 (15)	481 (29)	869	258 (155)

[1] Number of adult females that blood-fed; [2] Intentionally censored individuals include those that were sacrificed at the beginning (zero-hour) or end of experiment; accidental censors include escapees and accidental mortality; [3] The total number of individuals dissected that provided good quality data for parasite counts in the mid-gut, Malpighian tubules, body and head/proboscis; Individuals with poor quality parasite counts (e.g., due to desiccation) in any of the above tissue were considered to be missing data (see text). Also reported are the number of individuals with no visible parasites; [4] Number of individuals surviving to the extrinsic incubation period (13 days post-feeding); [5] Number of individuals with high quality infective L3 larval counts in the head and proboscis amongst mosquitoes surviving to EIP. Also reported are the number of individuals with zero L3 counts; [6] Individuals in this population were dropped from the analysis of vector competence because no individual surviving to EIP had infective L3 larvae in head/proboscis.

Table S3. Population differences in survival in infected and uninfected mosquitoes. Reported are the results of the best fit Cox (proportional hazard) Mixed Effect model testing for differential mortality between the eight *A. albopictus* populations infected using three infection doses (0, 15 and 30 mf/µl of blood). The best fit model for *A. aegypti* lab strain also is reported. All models were fit with Replicate as a random effect, and levels of variability between replicates was low (see footnote).

Variable	*A. albopictus*[1]		*A.aegypti*[2]	
	χ^2 (df)	*P*	χ^2 (df)	*P*
Dose	191.473 (2)	<0.001	110.766 (2)	< 0.001
Population	24.485 (7)	0.001	--	--
Dose × Population	35.566 (14)	0.001	--	--

[1] N = 1345; Random: Replicate (σ^2 = 0.0004);
[2] N = 219; Random: Replicate (σ^2 = 0.0004);

Table S4. Population differences in resistance to infection. Reported are the results of the best fit Generalized Linear Mixed Effect Regression model (with negative binomial error distribution) testing for effects of parasite load and the rate of reduction in parasite load among the eight *A. albopictus* populations infected using two infection doses (15 and 30 mf/µl of blood). The best fit model for *A. aegypti* lab strain also is reported. All models were fit with Replicate as a random effect, and levels of variability between replicates was low (see footnote).

Variable	*A. albopictus*[1]		*A.aegypti*[2]	
	χ^2 (df)	*P*	χ^2 (df)	*P*
Day	398.840 (1)	<0.001	91.118 (1)	< 0.001
Dose	62.335 (1)	<0.001	7.923 (1)	0.005
Population	15.701 (7)	0.028	--	--
Day × Dose	3.156 (1)	0.076	--	--
Day × Population	18.820 (7)	0.009	--	--
Dose × Population	15.325 (7)	0.032	--	--
Day × Dose × Population	8.832 (7)	0.265	--	--

[1] N = 412; Random: Replicate ($\sigma^2 < 0.0001$);
[2] N = 69; Random: Replicate ($\sigma^2 < 0.0001$); Status ($\sigma^2 < 0.0001$);

Table S5. Population differences in effects of the number of live (N_{LIVE}) and killed (N_{KILLED}) parasites on mosquito survival. Reported are the results of the best fit Cox (proportional hazard) Mixed Effect model testing effects of the number of live and killed parasites on mortality in the eight *A. albopictus* populations. The best fit model for *A. aegypti* lab strain also is reported. All models were fit with Replicate as a random effect, and levels of variability between replicates was low (see footnote).

Variable	*A. albopictus*[1]		*A.aegypti*[2]	
	χ^2 (df)	*P*	χ^2 (df)	*P*
N_{LIVE}	185.289 (1)	<0.001	61.680 (1)	< 0.001
N_{KILLED}	2.427 (1)	0.119	0.751 (1)	0.386
Population	65.095 (7)	<0.001	--	--
$N_{LIVE} \times N_{KILLED}$	126.514 (1)	<0.001	--	--
N_{LIVE} × Population	22.845 (7)	0.002	--	--
N_{KILLED} × Population	31.959 (7)	<0.001	--	--
$N_{LIVE} \times N_{KILLED}$ × Population	22.266 (7)	0.002	--	--

[1] N = 929; Random: Replicate ($\sigma^2 = 0.0124$);
[2] N = 161; Random: Replicate ($\sigma^2 = 0.0627$);

Table S6. Population differences in probability of survival to the extrinsic incubation period (EIP). Reported are the results of the best fit Generalized Linear Mixed Effect Regression model (with binomial error distribution) testing for differences in the survival to EIP eight *A. albopictus* populations infected using two infection doses (15 and 30 mf/μl of blood). The best fit model for *A. aegypti* lab strain also is reported. All models were fit with Replicate as a random effect, and levels of variability between replicates was low (see footnote).

Variable	*A. albopictus*[1]		*A.aegypti*[2]	
	χ^2 (df)	*P*	χ^2 (df)	*P*
Dose	45.188 (1)	<0.001	18.245 (1)	< 0.001
Population	25.462 (7)	0.001	--	--
Dose × Population	7.743 (7)	0.356	--	--

[1] N = 732; Random: Replicate ($\sigma^2 < 0.0001$);
[2] N = 137; Random: Replicate ($\sigma^2 < 0.0001$);

Table S7. Population differences in mosquito vector efficiency. Reported are the results of the best fit Generalized Linear Mixed Effect Regression model (with negative binomial error distribution) testing for differences in the number of infective (L3) larvae in the head and/or proboscis among the eight *A. albopictus* populations infected using two infection doses (15 and 30 mf/µl of blood). The best fit model for *A. aegypti* lab strain also is reported. All models were fit with Replicate as a random effect, and levels of variability between replicates was low (see footnote).

Variable	*A. albopictus*[1]		*A.aegypti*[2]	
	χ^2 (df)	*P*	χ^2 (df)	*P*
Day	19.307 (1)	<0.001	0.335 (1)	0.563
Dose	5.569 (1)	0.018	0.524 (1)	0.469
Population	17.455 (7)	0.015	--	--
Dose × Population	2.032 (5)	0.845	--	--

[1] N = 201; Random: Replicate ($\sigma^2 < 0.0001$);
[2] N = 42; Random: Replicate ($\sigma^2 < 0.0001$);