Supporting Online Material for

Wolbachia Establishment and Invasion in an *Aedes aegypti* Laboratory Population

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MATERIALS AND METHODS

Insect Strains

The *A. aegypti* Waco strain is naturally uninfected. *A. albopictus* Houston (Hou) strain is naturally superinfected with both the *w*AlbA and *w*AlbB *Wolbachia* types (*S1*). Waco and Hou were provided by George Craig and Scott O'Neill, respectively. Mosquito strains were maintained following standard procedures as described previously (*S2, S3*).

Microinjection

Embryo injection was based upon techniques successfully used for prior mosquito and *Drosophila* transfection (*S4, S5*). Microinjection needles were prepared from quartz microcapillaries (#QF100-70-7.5; Sutter Instrument Co., Novato, CA) using a P2000 micropipette puller (Sutter Instrument Co). Approximately ten blood fed females (Hou or Waco) were allowed to oviposit on a wet filter paper funnel. Waco embryos to be injected (recipient embryos) were collected after allowing females to oviposit for ≤90 minutes. Following a brief desiccation to provide space for injected material, grey embryos were aligned on double sided tape (Scotch 665; St. Paul, MN) and covered with halocarbon 700 oil (Sigma-Aldrich Co.). Donor Hou embryos were treated similarly but not desiccated. Cytoplasm was withdrawn from donor Hou embryos and injected into the posterior of recipient Waco embryos using an IM300 microinjector (Narishige Scientific; Tokyo, Japan) as previous described (*S6*). After injection, the embryos were incubated at 80% relative humidity and 27°C for approximately 40 minutes. Embryos were then removed from oil and transferred to wet filter

paper. Embryos were allowed to develop for 5 days on wet filter paper. Subsequently, the eggs were hatched (G_0) and reared using standard maintenance conditions as above. Females (G_0) were isolated as pupae to assure virginity and were subsequently mated with Waco Aedes aegypti males. Following blood feeding and oviposition, G_0 females were assayed for *Wolbachia* infection via PCR using primer sets specific for *w*AlbA and *w*AlbB (*S7*). G_0 females testing negative for *Wolbachia* infection were discarded along with their progeny. WB1 individuals were sibling mated in G_1 and G_2. Beginning in G_3, 50 virgin WB1 females were out crossed with 50 Waco males in every generation.

INJECTION RESULTS

As shown in Table S1, thirteen of 248 injected embryos survived to adult (G_0) in two injection experiments. Of the five PCR tested G_0 females, one was infected with the *w*AlbB single infection (Table S2). PCR assays detected *Wolbachia* DNA in the remaining females and in six of seven G_0 males. Line 2.3 was subdivided into superinfected 2.3a and *w*AlbB infected 2.3b based upon PCR results (Table S2).

PCR screening of G_1 females failed to detect *Wolbachia* in Line 2.2; thus it was discarded. Lines 1.2 and 2.3a were not established due to the failure of G_2 embryo hatch. Line 1.1 was selected for subsequent characterization and named 'WB1' [Waco strain; *w*AlbB infection; Line # 1].

SUPPLEMENT REFERENCES

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Table S1. Survival rate of *A. aegypti* embryos injected with *Wolbachia* from *A. albopictus* eggs

	Percent Survival			Sex ratio
Experiment	Hatch (Larvae/ eggs)	Pupation (Pupae/ larvae)	Eclosion (Adult/ pupae)	(Female/ Total)
1	13.2% (11/83)	72.7% (8/11)	75.0% (6/8)	33.3% (2/6)
2	6.1% (10/165)	70.0% (7/10)	100.0% (7/7)	57.1% (4/7)

Table S2. *Wolbachia* infection in the *A. aegypti* transfection studies

	Name of Isofemale line	G_0 PCR Test	G_1 infection Frequency	Note
	1.1 (ŌWB1Ō)	*w*AlbB only	*w*AlbB only; n=22	'WB1' Line
	1.2	*w*AlbA and *w*AlbB	*w*AlbA and *w*AlbB; n=4 *w*AlbB only; n=1	G_2 embryos did not hatch
	2.1	Not tested	Not tested	G_0 female was accidentally killed
	2.2	*w*AlbA and *w*AlbB	No infection detected n=14	Discarded
	2.3	*w*AlbA and *w*AlbB	*w*AlbA and *w*AlbB; n=2 2.3a *w*AlbB only; n=8 2.3b	None of G_2 embryos from superinfected G_1 hatched; 'WB2' Line established from *w*AlbB infected progeny
	2.4	*w*AlbA and *w*AlbB		G_0 female did not produce eggs

† n = the number of PCR tested G_1 females

Figure S1. Egg hatch rate resulting with introgression crosses [WB1 females x Waco males]. Generation three (G_3) is the cross results shown in Table 1. Subsequent generations result from outcrosses with Waco males.