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Generation of a novel *Wolbachia* infection in *Aedes albopictus* (Asian tiger mosquito) via embryonic microinjection

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Abstract

Genetic strategies that reduce or block pathogen transmission by mosquitoes are being investigated as a means to augment current control measures. Strategies of vector suppression and replacement are based upon intracellular *Wolbachia* bacteria, which occur naturally in many insect populations. Maternally inherited *Wolbachia* have evolved diverse mechanisms to manipulate host insect reproduction and promote infection invasion. One mechanism is cytoplasmic incompatibility (CI) through which *Wolbachia* promotes infection spread by effectively sterilizing uninfected females. In a prior field test, releases of *Wolbachia*-infected males were used to suppress a field population of *Culex pipiens*. An additional strategy would employ *Wolbachia* as a vehicle to drive desired transgenes into vector populations (population replacement). *Wolbachia*-based population suppression and population replacement strategies require an ability to generate artificial *Wolbachia* associations in mosquitoes. Here, we demonstrate a technique for transferring *Wolbachia* (transfection) in a medically important mosquito species: *Aedes albopictus* (Asian tiger mosquito). Microinjection was used to transfer embryo cytoplasm from a double-infected *Ae. albopictus* line into an aposymbiotic line. The resulting mosquito line is single-infected with the *w*AlbB *Wolbachia* type. The artificially generated infection type is not known to occur naturally and displays a new CI crossing type and the first known example of bidirectional CI in Aedes mosquitoes. We discuss the results in relation to applied mosquito control strategies and the evolution of *Wolbachia* infections in *Ae. albopictus*.

Keywords: *Wolbachia*; *Aedes albopictus*; Microinjection

1. Introduction

Wolbachia is a genus of obligate, intracellular, maternally inherited bacteria that occur in many insect species (O'Neill et al., 1997a). Cytoplasmic incompatibility (CI) is one of several reproductive manipulations caused by *Wolbachia*. CI occurs in matings between individuals that differ in their *Wolbachia* infection type and results in early embryonic death. Although the CI mechanism is unknown, a proposed modification/rescue model serves to explain much of the observed CI phenomena (Charlat et al., 2001; Poinsot et al., 2003; Dobson, 2004). In this model, *Wolbachia* in the male acts to 'modify' the sperm, such that karyogamy failure occurs following fertilization, resulting in embryo death. If the female (and resulting fertilized egg) have the same *Wolbachia* type as her mate, *Wolbachia* acts to 'rescue' the modification, resulting in normal embryo development. Thus, matings between uninfected females and infected males are incompatible, but the reciprocal cross is compatible (unidirectional CI). Unidirectional CI provides *Wolbachia*-infected females with a reproductive advantage relative to uninfected females, promoting the spread of maternally inherited *Wolbachia* into uninfected host populations (Hoffmann et al., 1990). The

Abbreviation: CI, cytoplasmic incompatibility

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ability to spread into host populations has led to the proposed use of *Wolbachia* in population replacement strategies. Specifically, a desired transgene that is linked to *Wolbachia* could be 'seeded' into a mosquito disease vector population. The *Wolbachia* infection would then serve as a vehicle, driving the linked transgene into the targeted population.

Bidirectional CI can occur when two or more *Wolbachia* types infect the same host population. An example is provided by the parasitoid wasp *Nasonia vitripennis* (Perrot-Minnot et al., 1996). Crosses between *N. vitripennis* strains that are infected with divergent *Wolbachia* types (A type or B type) result in incompatibility in both cross directions. Theory predicts that bidirectionally incompatible *Wolbachia* types cannot persist within a panmictic host population (Rousset et al., 1991; Dobson et al., 2002). Bidirectional CI causes a 'battle' between the *Wolbachia* types, resulting in the elimination of infections until only one *Wolbachia* type predominates. The host population is a victim during this battle, as bidirectional incompatibility sterilizes many matings. The CI-induced suppression of the host population is transient however, lasting only until one *Wolbachia* infection type dominates the host population (Dobson et al., 2002). Therefore, known examples of bidirectional CI have been either artificially generated or isolated from allopatric populations.

Vector population suppression strategies are based upon artificially prolonging the bidirectional CI battle (Dobson et al., 2002). In a prior field test of the strategy, releases of bidirectionally incompatible males successfully eliminated a *Culex* mosquito vector population from a village in Burma (Myanmar) (Laven, 1967). However, the availability of naturally occurring bidirectionally incompatible strains that permitted the *Culex* strategy remains unique. Therefore, the use of the suppression strategy in additional mosquito vector populations requires the ability to artificially generate incompatible strains. Similarly, population replacement strategies also require an ability to generate novel infections. Although the artificial transfer of *Wolbachia* (transfection) has been successfully accomplished in other insect systems (Boyle et al., 1993; Sasaki et al., 2002; Hartmann et al., 2003; Kang et al., 2003), prior efforts to generate novel infections in mosquitoes have not proven successful (Sinkins and O'Neill, 2000).

Aedes albopictus (Asian tiger mosquito) is a medically important disease vector of multiple arboviruses and filaria (Francy et al., 1990; Moore and Mitchell, 1997; Cancrini et al., 2003). This mosquito is also an important invasive species, frequently spread by human transport (Reiter, 1998). Since its introduction to the United States, *Ae. albopictus* has spread to become a leading biting nuisance (Moore and Mitchell, 1997). *Ae. albopictus* individuals are naturally co-infected with two *Wolbachia* types (*w*AlbA and *w*AlbB) (Sinkins et al., 1995; Zhou et al., 1998). This type of co-infection is known as 'superinfection' and is commonly observed in insects, representing 34.6% of *Wolbachia* infections in one survey (Werren and Windsor, 2000). Superinfection results in additive unidirectional CI: superinfected females express both the A and B rescue and are compatible with all males in the population; superinfected males express both the A and B modification and are compatible only with superinfected females (Sinkins et al., 1995).

Although a majority of *Ae. albopictus* populations are superinfected (Armbruster et al., 2003), laboratory colonies of single-infected (*w*AlbA) strains have been established from the islands of Koh Samui and Mauritius (Sinkins et al., 1995). Crosses demonstrate that the superinfection is unidirectionally incompatible with the *w*AlbA infection (Sinkins et al., 1995). Two hypotheses have been proposed for the observation of the single-infected strains. The single-infected populations may represent an ancestral infection type, protected by geographic isolation from replacement with the superinfection (Sinkins et al., 1995; Dutton and Sinkins, 2004). An alternative hypothesis is that the single-infected lines are an experimental artifact and result from loss of the *w*AlbB infection during colony establishment (Kittayapong et al., 2002a, b).

The ability of *w*AlbB to induce CI has been speculated based upon crosses of superinfected and *w*AlbA-infected strains. Crosses of *w*AlbA-infected females and superinfected males are incompatible, resulting in high embryo mortality. Since the mates in the latter cross differ only by the *w*AlbB infection present in males, this suggests that the *w*AlbB infection is capable of inducing CI. However, the prior crosses cannot exclude an interaction between the *w*AlbA and *w*AlbB infections within superinfected males.

Here we demonstrate the use of embryonic microinjection to transfer *Wolbachia* from a naturally superinfected *Ae. albopictus* strain into an artificially generated aposymbiotic strain. The design was chosen due to concern that prior attempts to transfer *Wolbachia* in mosquitoes have failed due to an unsupportive host background or maladaptation of the *Wolbachia* infection to the recipient host (Sinkins and O'Neill, 2000). The results show that transfection efforts have generated an artificial *Wolbachia* infection type (*w*AlbB single infection) in *Ae. albopictus*. Crossing experiments with the artificial infection show a new CI crossing type, providing the first example of bidirectional incompatibility in Aedes. We discuss the results in relation to the evolution of *Wolbachia* infection in *Ae. albopictus* and to applied strategies for the control of mosquitoes and mosquito-borne disease.

2. Materials and methods

2.1. Mosquito strains

The Koh Samui strain of *Ae. albopictus* (Koh; Thailand, pre-1970) is infected with the *w*AlbA *Wolbachia* type (Sinkins et al., 1995). The Houston strain (Hou; Texas 1986) is superinfected with both *w*AlbA and *w*AlbB *Wolbachia* types (Sinkins et al., 1995). HT1 and UjuT are uninfected strains that were artificially generated by tetracycline treatment (Otsuka and Takaoka, 1997; Dobson and Rattanadechakul, 2001). Mosquitoes were maintained as previously described (Dobson et al., 2001).

2.2. Microinjection

Embryo injection was based upon techniques successfully used for mosquito transgenesis (Morris, 1997; Coates et al., 1998). Injection needles (Quartz with filament, O.D.: 1.0 mm, I.D.: 0.70 mm) were pulled with a P2000 micropipette puller (Sutter Instrument Co.; Novato, CA). Approximately ten blood-fed females (Hou or HT1) were held in Drosophila vials (Fisher Scientific) containing a wet filter paper funnel. HT1 embryos to be injected (recipient embryos) were collected after allowing females to oviposit for $\leqslant$90 min. Following a brief desiccation, gray 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 HT1 embryos using an IM300 microinjector (Narishige Scientific; Tokyo, Japan) as previously described (Morris, 1997). After injection, the embryos were incubated at 80% relative humidity and 27 °C for approximately 40 min. Embryos were then removed from oil and transferred to wet filter paper. Embryos were allowed to develop for 5 days on wet egg paper. Subsequently, the eggs were hatched (G_0) and reared using standard maintenance conditions as above.

2.3. Crosses of transfected lines

To ensure a compatible mating, G_0 females were isolated as virgins and mated with HT1 males. Following oviposition, G_0 females were assayed for *Wolbachia* infection using PCR. G_0 males were also PCR assayed for *Wolbachia* infection. G_0 females testing negative for *Wolbachia* infection were discarded along with their progeny. Infected G_1 females were sib mated, blood fed, isolated and allowed to oviposit. Following oviposition, G_1 females were PCR assayed for *Wolbachia* infection. G_1 females testing negative for *Wolbachia* infection were discarded along with their progeny. An introgressed line was generated by crossing *w*AlbB-infected females with UjuT males as previously described (Dobson et al., 2004). To determine CI levels, five virgin females were mated with five virgin males at G_3. Mated females were blood fed weekly using mice. Oviposition sites were available constantly to females, and oviposition paper was changed weekly. Hatch rates were scored 3 days after eggs were immersed into water. A majority of *Ae. albopictus* eggs hatch within a few hours of being submerged in deoxygenated water, Thus, delaying observations beyond 3 days would not affect estimates of egg hatch.

2.4. PCR amplification

Ovaries or testis of adults were dissected and homogenized in 100 ul STE with 0.4 mg/ml proteinase K to extract DNA as previously described (O'Neill et al., 1992). General *Wolbachia* primers (81F-681R) and primers specific for the *w*AlbA (328F-691 R) and *w*AlbB (183F–691R) infections were used as previously described (Zhou et al., 1998).

2.5. Fluorescence in situ hybridization (FISH)

Dissected ovaries and oocytes were fixed for 15 min in freshly prepared 4% formaldehyde in PBS and then washed in PBS with 0.1% Tween 20. Hybridization was conducted following the manufacturers instruction (GeneDetect, Bradenton, FL) with buffer containing 200 ng probes at 37 °C overnight. Two FITC 5′-end labeled 16s rDNA *Wolbachia* probes (synthesized by Sigma-Genosys Ltd., Haverhill, UK) were used with the sequence as following: [5′-ACCAGATAGACGCCTTCGGCC-3′] (Heddi et al., 1999) and [5′-CTTCTGTGAGTACCGTCATTATC-3′]. Following hybridization, samples were washed at 45 °C and mounted on a glass slide with Vecta shield mounting media (Vector Laboratories; Burlingame, CA). Samples were viewed with Olympus IX70 fluorescence microscope and photographed using Magnafire software (Optronics; Goleta, CA).

3. Results

Cytoplasm from superinfected *Ae. albopictus* embryos (Hou) were microinjected into uninfected embryos (HT1). In one experiment, ten of 77 embryos (G_0) survived microinjection (12% hatch rate). Two of the resulting adults were female. Since males are a dead end host for *Wolbachia* infection, males were not used to establish lines. Instead, the eight adult males were sacrificed for PCR *Wolbachia* detection assays (Fig. 1). Three males were PCR positive for both the *w*AlbA and *w*AlbB *Wolbachia* infection; two males were positive for

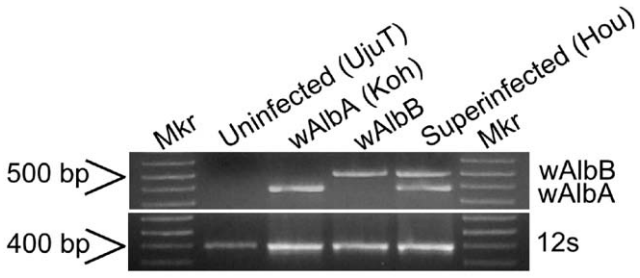

Fig. 1. Strain-specific amplification of *w*AlbA and *w*AlbB *Wolbachia* type. Template quality is verified by amplification of mitochondria DNA with 12S primer. Mkr: 1 kb plus molecular weight marker (Invitrogen Life Technologies).

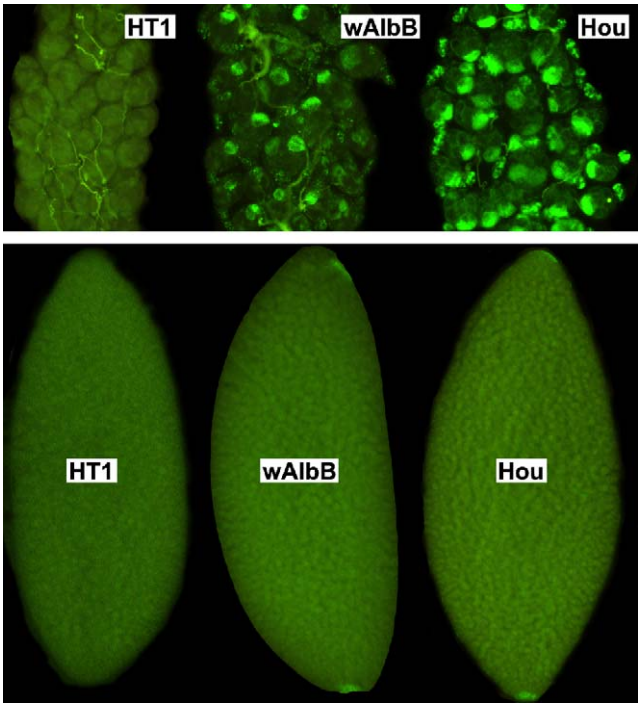

Fig. 2. Distribution of *Wolbachia* in *Ae. albopictus* ovaries (top) and oocytes (bottom). HT1 is an aposymbiotic (uninfected) strain; *w*AlbB is the transfected strain; and Hou is the naturally superinfected strain.

only the *w*AlbB infection; *Wolbachia* was not detected in the remaining three males.

PCR tests of infected G_0 females showed one G_0 female to be positive for both the *w*AlbA and *w*AlbB infection. *Wolbachia* was not detected in the second G_0 female. Twenty-one G_1 isofemale lines were established from the infected G_0 female. G_1 PCR assays demonstrated 11 females to be positive for the *w*AlbB infection only. One G_1 female was positive for the *w*AlbA infection only. *Wolbachia* was not detected in the remaining nine G_1 females.

Three *w*AlbB-infected isofemale lines were established. Eggs from the *w*AlbA-infected G_1 female failed to hatch, and thus this line was lost. To determine the stability of *Wolbachia* infection in the *w*AlbB-transfected lines, PCR was repeated in subsequent generations. Consistent PCR detection of the infection continued through the generation immediately prior to submission of this article (G_8).

To characterize the distribution of *Wolbachia* in the transfected line, ovaries were dissected from G_6 females and examined. Hou and *w*AlbB oocytes displayed a similar pattern of *Wolbachia* staining at both embryonic poles, which was absent from uninfected oocytes (Fig. 2). A reduced level of *Wolbachia* was observed in ovaries of *w*AlbB females compared to ovaries of superinfected Hou females.

Crosses to characterize the CI pattern of the transfected *w*AlbB strain resulted in a low egg hatch rate in crosses of *w*AlbB males with either uninfected or *w*AlbA-infected females (Table 1). Crosses of the *w*AlbB males with superinfected females are compatible. Crosses of the *w*AlbB females with uninfected or *w*AlbB-infected males were compatible, although relatively low egg hatch rate (38.0%) was observed in the latter crosses (Table 1). CI persists over the lifetime of *w*AlbB females. Egg hatch was observed to remain consistent in egg batches collected from the same females over a 4-week period (Fig. 3A, B). The CI level

Table 1
Crosses of the transfected *w*AlbB line (G_3)

Expected CI type	Cross[a]	Percent egg hatch[b]	Number of eggs/oviposition[b]	Oviposition number
Bidirectional CI	*w*AlbB × Koh	3.6±3.8	155±55	13
	Koh × *w*AlbB	2.4±4.4	162±64	15
Unidirectional CI	HT1 × *w*AlbB	0.0±0.0	170±45	14
	*w*AlbB × Hou	3.0±2.1	150±52	6
Compatible	Hou × *w*AlbB	73.8±12.1	192±60	15
	*w*AlbB × *w*AlbB	38.0±21.3	139±48	6
	HT1 × HT1	90.9±2.7	166±139	3
	Koh × Koh	88.0±2.8	177±85	4
	Hou × Hou	82.5±2.2	158±57	4

[a]Female × male; HT1 is an aposymbiotic (uninfected) strain; *w*AlbB is the transfected strain; Koh is a *w*AlbA-infected strain; and Hou is the naturally superinfected strain.
[b]Average ± standard deviation.

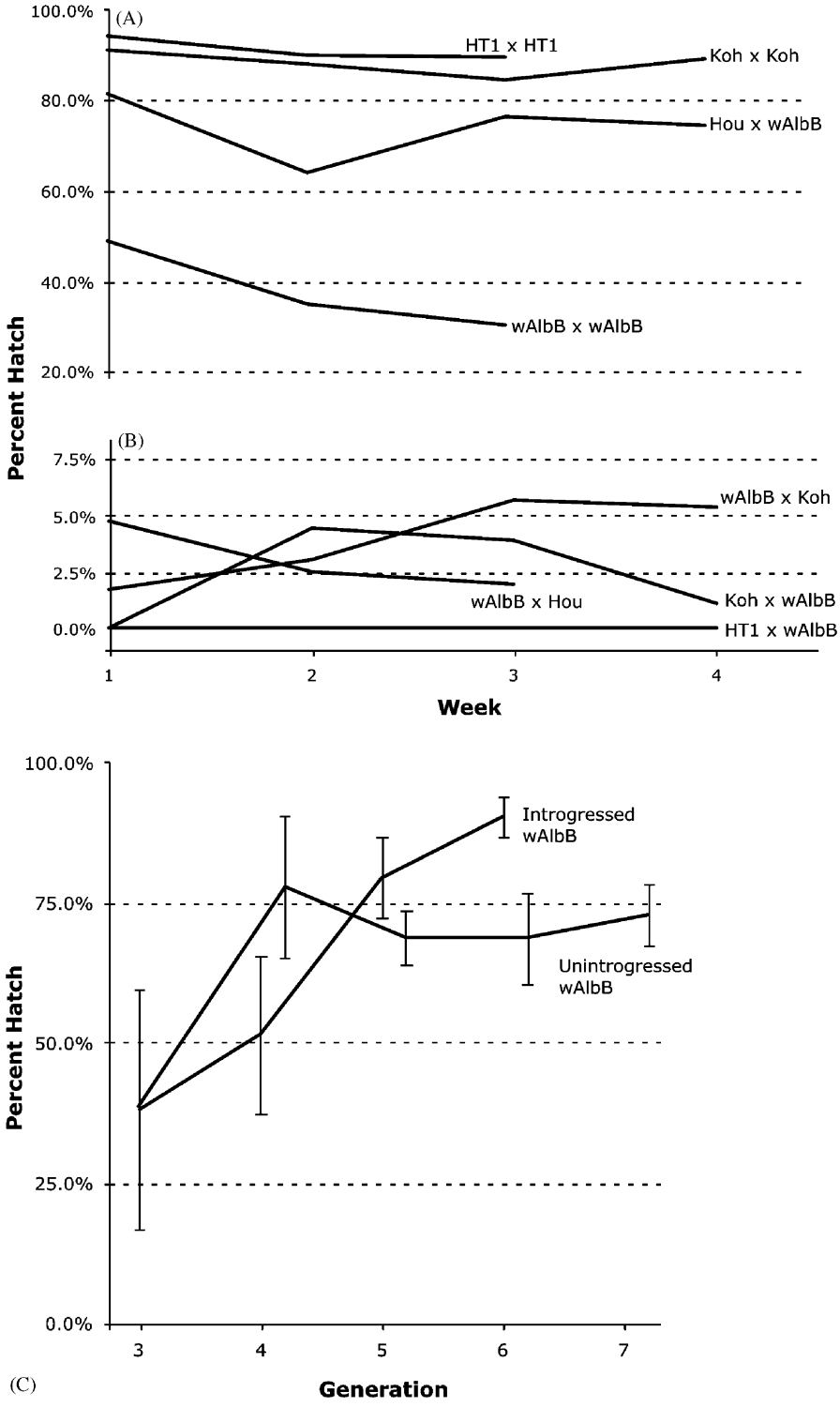

Fig. 3. Egg hatch rate of (A) compatible G_3 crosses, (B) incompatible G_3 crosses, and (C) unintrogressed and introgressed *w*AlbB lines. Egg hatch was measured either weekly (A, B) or once per generation (C). Bars show standard deviation. Crosses are female × male. HT1 is an aposymbiotic (uninfected) strain; *w*AlbB is the transfected strain; Koh is a *w*AlbA infected strain; and Hou is the naturally superinfected strain.

was re-examined at G_7, resulting in similar results as G_3. Greater than 86% hatch resulted in crosses of *w*AlbB males with superinfected Hou females. No egg hatch was observed in crosses of *w*AlbB males and uninfected HT1 females.

To reduce potential inbreeding effects, one *w*AlbB line was introgressed with UjuT for three generations. As shown in Fig. 3C, the hatch rate in the introgressed strain increased to greater than 93%. The hatch rate observed in the unintrogressed *w*AlbB line also

increased (72.8% in G_7; Fig. 3C). Introgression did not affect CI. No egg hatch was observed in crosses of introgressed *w*AlbB males and uninfected HT1 females.

Maternal inheritance rate was examined in the *w*AlbB line by screening 20 G_6 females using the PCR assay. *Wolbachia* infection was observed in all of the tested females. To examine for paternal transmission, rare progeny from incompatible crosses were reared to adult and then PCR assayed for infection type. Superinfection was not detected in the three progeny resulting from *w*AlbB × *w*AlbA and two progeny from *w*AlbA × *w*AlbB (female × male). The infection type in each of the progeny was consistent with expectations for maternal inheritance only (i.e., progeny infection type was the same as the maternal type).

4. Discussion

Wolbachia in *Ae. albopictus* is known to represent a true superinfection (i.e., co-infection with two *Wolbachia* types) and not multiple copies of diagnostic genetic loci in a single *Wolbachia* type (Sinkins et al., 1995), based upon observations of the *w*AlbA single infection in mosquito lines and the *w*AlbB single infection in vitro (O'Neill et al., 1997b). However, the *w*AlbB single infection has not been observed naturally. Surveys show that >99.4% of natural *Ae. albopictus* populations are superinfected (Kittayapong et al., 2002a, b). Furthermore, prior efforts to segregate the *w*AlbA and *w*AlbB infections using antibiotics were unsuccessful (Dobson and Rattanadechakul, 2001).

Based upon the genetic divergence of the *w*AlbA and *w*AlbB infections and prior crossing experiments, bidirectional incompatibility has been predicted for crosses between individuals single-infected with *w*AlbA and *w*AlbB (Sinkins et al., 1995; Dobson et al., 2004). Here, crosses of the transfected *w*AlbB line were used to directly test predictions. Consistent with expectations for differing modification and rescue mechanisms, less than 4% egg hatch rate resulted in crosses between *w*AlbB males with either *w*AlbA or uninfected females (Table 1). Crossing results demonstrate that the *w*AlbB infection is capable of inducing and rescuing the CI modification independent of the *w*AlbA infection. Crosses of *w*AlbB females with either *w*AlbA or superinfected males demonstrate that the *w*AlbB infection is unable to rescue the *w*AlbA modification. Although prior characterization of *Wolbachia* infections in other insects shows that CI levels can be affected by host age (Singh et al., 1976; Reynolds et al., 2003), the *w*AlbB infection in females is able to rescue modified sperm until female death (Fig. 3).

Despite the observation that ovaries from the *w*AlbB line appeared to have lower infection levels relative to Hou ovaries (Fig. 2), the *w*AlbB infection was observed to be stably maintained in the transfected lines. PCR assays at G_6 suggest maternal inheritance in excess of 95%, consistent with prior characterization of naturally infected lines (Kittayapong et al., 2002a, b). *Wolbachia* specific staining showed a similar infection level and *Wolbachia* distribution in *w*AlbB and Hou oocytes (Fig. 2).

Paternal transmission of *Wolbachia* infection provides a potential route for the evolution of superinfections. With paternal and maternal transmission, survivors of crosses between mates with different *Wolbachia* types would result in superinfected progeny. To examine for paternal transmission, the rare offspring from incompatible crosses between *w*AlbA and *w*AlbB strains were PCR tested. In each case, the *Wolbachia* infection was identical to the maternal infection type. Although this result is inconsistent with the hypothesized role of paternal transmission in superinfection evolution, we have examined few offspring and can only exclude high rates of paternal transmission. Future efforts should include repeating this experiment on a larger scale. In addition to the evolutionary significance, the results will also be important to proposed applied strategies. Paternal transmission resulting in superinfections would complicate strategies attempting to use *Wolbachia* to suppress insect populations, since superinfected field individuals would be compatible with released males.

Low hatch rate (38.0%) was observed in compatible crosses of *w*AlbB individuals. Hypotheses to explain this observation include inbreeding effects associated with the establishment of isofemale lines (i.e., increased homozygosity of deleterious loci) and high mortality associated with the artificially generated single *w*AlbB infection type. The observed increase in egg hatch with introgression (Fig. 3C) is consistent with predictions for an inbreeding effect. An increase in egg hatch was also observed in subsequent generations of a non-introgressed *w*AlbB line, reaching a plateau at approximately 70% (Fig. 3C). Thus, the *w*AlbB single infection does not appear to be associated with increased mortality.

Although superinfection was detected in G_0 individuals surviving microinjection, only single infections were observed in G_1. The presence of superinfection in G_0 but not thereafter suggests that maternal transmission between G_0 and G_1 represents a bottleneck for transfected *Wolbachia* and may result from artificially low infection levels in microinjected embryos or somatic G_0 infections that are not maternally inherited. Superinfection segregation following microinjection transfection has also been reported in Drosophila (Poinsot and Mercot, 2001; Riegler et al., 2004). It is useful to note that subsequent to G_1, maternal transmission loss was not observed. This is similar to prior transfection experiments in *Drosophila simulans* (Xi and Dobson, 2005) and suggests that future transfection studies may

be simplified by focusing PCR screening on G_0 and G_1 females.

Segregation of the superinfection in the transfection experiment was biased toward *w*AlbB infection. Only one *w*AlbA line was observed in the G_1 lines, relative to 11 *w*AlbB G_1 lines. This is similar to prior research generating a *Wolbachia*-infected cell line from superinfected *Ae. albopictus*, which resulted in an in vitro *w*AlbB single infection (O'Neill et al., 1997b). The observed *w*AlbB bias may reflect higher *w*AlbB infection levels relative to *w*AlbA in superinfected females (Dutton and Sinkins, 2004). Given the previously described *w*AlbB-bias in superinfected *Ae. albopictus*, it is somewhat surprising that a *w*AlbA-infected G_1 line was observed. Unfortunately, the possibility that this line represented a PCR artifact or somatic infection could not be tested since the female failed to produce hatching G_2 eggs and the line was lost. Egg hatch failure of the *w*AlbA line may have resulted from the experimental protocol. G_1 progeny from the infected isofemale line were sib mated. Given that a majority of PCR tested G_1 individuals were *w*AlbB infected, it is likely that the mate of the *w*AlbA female would be incompatible. This provides rationale for modifying the protocol presented here for future transfection experiments, such that virgin G_1 females are mated with uninfected males.

Here, we have demonstrated a technique for *Wolbachia* transfection in *Ae. albopictus*. An ability to generate artificial *Wolbachia* infections and new CI crossing types represents an important advance toward implementation of proposed *Wolbachia*-based strategies for suppression and replacement of medically important mosquito vector populations. While the experiments described here demonstrate a successful transfection protocol, the artificial *w*AlbB infection will not be useful for the suppression or replacement of superinfected *Ae. albopictus* field populations. Releases of *w*AlbB males would not be incompatible with superinfected females of field populations and therefore would not result in CI or suppression. Similarly, *w*AlbB-infected females would not be useful for population replacement strategies. Released *w*AlbB females would be incompatible with superinfected field males, and thus the *w*AlbB single infection in females would be quickly eliminated. Therefore, future experiments should repeat the transfection protocol described here with the variation of using donor tissue infected with different *Wolbachia* types that do not naturally occur in *Ae. albopictus*. For suppression strategies, injection of aposymbiotic *Ae. albopictus* could be used to generate strains that are bidirectionally incompatible with the superinfected field population. Injection of superinfected *Ae. albopictus* could be used to generate a triple-infected *Ae. albopictus* strain that is unidirectionally incompatible with superinfected field population. The latter would be similar to prior transfection experiments with Drosophila (Rousset et al., 1999). To reduce complications associated with generation of artificial associations, closely related *Wolbachia*-infected Aedes mosquitoes may be initially selected as donors (Sherron and Rai, 1983; Meek and Macdonald, 1984; Dean and Dobson, 2004). However, prior transfers between divergent hosts have been successful, including the transfer of *Wolbachia* from *Ae. albopictus* to *Drosophila simulans* (Braig et al., 1994). Additional experiments could repeat the transfection protocol reported here, but using *Ae. aegypti* (Yellow fever mosquito) as the recipient. *Ae. aegypti* populations are naturally uninfected. If successful, the latter would generate a strain useful for population replacement strategies with *Ae. aegypti* populations, which are widely recognized to be important vectors of dengue, yellow fever, filaria and additional medically important pathogens.

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