Mutualistic Wolbachia Infection in *Aedes albopictus*: Accelerating Cytoplasmic Drive

Stephen L. Dobson,[1] Eric J. Marsland and Wanchai Rattanadechakul

Department of Entomology, University of Kentucky, Lexington, Kentucky 40546

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ABSTRACT

Maternally inherited rickettsial symbionts of the genus Wolbachia occur commonly in arthropods, often behaving as reproductive parasites by manipulating host reproduction to enhance the vertical transmission of infections. One manipulation is cytoplasmic incompatibility (CI), which causes a significant reduction in brood hatch and promotes the spread of the maternally inherited Wolbachia infection into the host population (*i.e.*, cytoplasmic drive). Here, we have examined a Wolbachia superinfection in the mosquito *Aedes albopictus* and found the infection to be associated with both cytoplasmic incompatibility and increased host fecundity. Relative to uninfected females, infected females live longer, produce more eggs, and have higher hatching rates in compatible crosses. A model describing Wolbachia infection dynamics predicts that increased fecundity will accelerate cytoplasmic drive rates. To test this hypothesis, we used population cages to examine the rate at which Wolbachia invades an uninfected *Ae. albopictus* population. The observed cytoplasmic drive rates were consistent with model predictions for a CI-inducing Wolbachia infection that increases host fecundity. We discuss the relevance of these results to both the evolution of Wolbachia symbioses and proposed applied strategies for the use of Wolbachia infections to drive desired transgenes through natural populations (*i.e.*, population replacement strategies).

IN describing the evolutionary trajectories of obligate vertically inherited endosymbionts and their hosts, classical mutualism refers to the selection of symbionts for increased host reproductive success. Since the success of both host and vertically inherited symbionts relies upon the host gametes, improved host reproduction is expected to benefit both host and symbiont (Fine 1975; Ewald 1987). An alternative evolutionary strategy known as reproductive parasitism can occur with symbionts that are inherited exclusively through one host sex. With reproductive parasitism, symbionts increase the fitness of one host sex at the expense of the other sex. Maternally inherited Wolbachia bacteria that induce cytoplasmic incompatibility provide one example of reproductive parasitism (O'Neill *et al.* 1997; Werren 1997; Stouthamer *et al.* 1999).

Cytoplasmic incompatibility (CI) is characterized by the disruption of early fertilization events and arrested development in diploid host organisms. Wolbachia infection in the male host imprints the gamete ("modification") such that fertilization is followed by an improper functioning of the paternal pronucleus and karyogamy failure (Reed and Werren 1995; Dobson and Tanouye 1996; Presgraves 2000). However, if the male fertilizes a female harboring a similar infection type, Wolbachia can "rescue" the modified sperm, and normal karyogamy occurs (Bourtzis *et al.* 1998).

[1]*Corresponding author:* Department of Entomology, University of Kentucky, S225 Ag. Sci. Center, N. Lexington, KY 40546-0091.
E-mail: sdobson@uky.edu

In populations that include both Wolbachia-infected and uninfected hosts, CI provides infected female hosts with a reproductive advantage relative to uninfected females. Specifically, infected females can mate successfully with both male types while uninfected females can mate successfully only with uninfected males (Figure 1). The advantage afforded to infected females occurs at the expense of infected male hosts, which can mate successfully only with females that harbor similar Wolbachia infection types. Since male hosts are an evolutionary "dead end" for maternally inherited Wolbachia symbionts, selection on Wolbachia occurs exclusively through female hosts. Thus, decreases in male host fitness can be selected if this corresponds with an increased fitness of infected female hosts.

In addition to male host costs, CI permits the spread and maintenance of Wolbachia bacteria in natural host populations despite female fecundity costs associated with infections (Hoffmann and Turelli 1988; Hoffmann *et al.* 1990). Although models predict the selection of Wolbachia variants that increase female fecundity (Fine 1978; Stevens and Wade 1990; Turelli 1994), previously characterized CI-inducing Wolbachia infections include reproductive parasites that are associated with host fitness costs (Hoffmann and Turelli 1988; Hoffmann *et al.* 1990; Nigro and Prout 1990) and infections that do not appear to influence host fitness (Hoffmann *et al.* 1994, 1996; Giordano *et al.* 1995; Bourtzis *et al.* 1996; Bordenstein and Werren 2000). This observation has led to a hypothesized tradeoff in which increased host fecundity associated with infections corresponds with decreases in incompatibility lev-

els or lower maternal transmission rates (reviewed in Hoffmann and Turelli 1997).

Here, we have characterized a Wolbachia superinfection occurring in *Aedes albopictus* (Asian tiger mosquito). Our results provide the first clear evidence of a CI-inducing Wolbachia infection that increases female fecundity. This observation demonstrates that high maternal inheritance rates and CI levels do not necessarily come at the expense of female fecundity and further blurs the definition of Wolbachia as a mutualist or parasite (O'Neill 1995). We discuss these results in relation to the evolution of Wolbachia symbioses and applied population replacement strategies.

MATERIALS AND METHODS

Mosquito strains: The single-infected Koh Samui (Koh; Thailand, pre-1970) and superinfected Houston (Hou; Texas, 1986) strains of *Ae. albopictus* were generously provided by Scott O'Neill (Yale University). UjuTet (UjuT) is an uninfected strain artificially generated by tetracycline treatment (Otsuka and Takaoka 1997) and was generously provided by Yasushi Otsuka (Oita Medical University). The aposymbiotic HT1 strain was generated via tetracycline treatment of the Hou strain as previously described (Dobson and Rattanadechakul 2001). The HT1 individuals were maintained for six generations following tetracycline treatment prior to use in crossing experiments. For rearing and all experiments, mosquitoes were maintained using standard conditions at 28 ± 2° and 75 ± 10% relative humidity with an 18:6 hr light:dark (L:D) photoperiod (Gerberg *et al.* 1994).

Population cages: Three replicate population cages ("female release cages") were initiated with 100 UjuT adults (1:1 sex ratio) to which five Hou females were added ("P generation"). An additional control cage was identical, with the exception that no superinfected females were added. Two additional cages were established to test for paternal and horizontal transmission. The latter cages were established and maintained similar to the control cage, but superinfected males were added in each generation ("male release cages"). All population cages employed discrete generations by establishing new cages for each generation using 2- to 3-day-old adults resulting from the previous generation. Females in cages were blood fed when 10 days old. A constant supply of 10% sucrose was provided in all cages. Oviposition cups were introduced into cages for a 24-hr period when females were 2 weeks old. Collected eggs were dried and reared as previously described (Gerberg *et al.* 1994).

To determine infection frequency within the cages, 10-day-old females (10) and 5-day-old males (4) were removed in each generation of each cage for PCR assays and test crosses, respectively. For test crosses, males were removed from cages and mated with virgin females of known infection type. Each male used in test crosses was sequentially crossed with uninfected, single-infected, and superinfected females (4 females of each type). Superinfected males are expected to be incompatible with both the single- and uninfected females (Figure 1). Males infected with only the *w*AlbA infection should be incompatible with only the uninfected females. Uninfected males should be compatible with all female types. Test crosses with Hou females were conducted to demonstrate male fertility, since Hou females are expected to be compatible with all males. Females used in test crosses were 10 days old and blood fed at the time of mating. Following a 24-hr period for matings with each female infection type, females were isolated and permitted to oviposit. Due to equipment failure, a majority of F_3 test crosses died prior to female oviposition. Therefore, test cross data from the F_3 generation was not included.

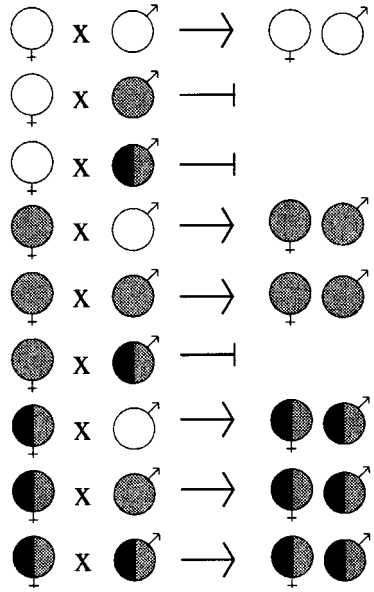

Figure 1.—Diagram of the expected brood and infection type resulting from crosses of uninfected (unshaded), single-infected (shaded), and superinfected (shaded/solid) hosts. Cytoplasmic incompatibility resulting in reduced brood hatch is expected to occur only when the male harbors an infection type that is not present in his mate. Due to maternal transmission, the infection type in offspring is expected to be similar to that of the mother.

Hou and HT1 crosses: Experimental units consisted of 2-day-old virgin females (10) and males (20 mosquitoes total). All four possible crosses using the Hou and HT1 strains were examined. Four cage replications were set up for each of the crossing types. For all crosses, a constant supply of 10% sucrose was provided to adults. Females were provided a mouse weekly for blood feeding. An oviposition container was constantly available to females and changed weekly for egg collection. Eggs were matured and dried using standard procedures (Gerberg *et al.* 1994). Following drying, eggs were hatched by submerging in a deoxygenated liverpowder solution. Following 2 days in hatching water, eggs were counted and scored as either hatched or unhatched. Egg papers were collected and counted weekly until females in the cage were dead. Numbers of surviving males and females were recorded weekly. Repeated measures ANOVA and Bonferroni mean separation were used for statistical comparisons of untransformed data.

PCR amplification: Infection type in mosquito strains was determined using diagnostic primers *w*AlbA (primers 328F and 691R) and *w*AlbB (primers 183F and 691R; Zhou *et al.* 1998). For samples failing to amplify using Wolbachia-specific primers, 12S primers were used to amplify mitochondria DNA as a positive control for template DNA quality (O'Neill *et al.* 1992). Ovaries or testes from individual mosquitoes were isolated and homogenized in 100 μl STE [0.1 M NaCl, 10 mM Tris HCl, and 1 mM EDTA (pH 8.0)]. Proteinase K was added to a final concentration of 0.4 mg/ml, and this mixture was incubated at 56° for 1 hr. Following heat inactivation at 95° for 15 min, 1 μl of these samples was amplified in 50 mM KCl, 20 mM Tris HCl (pH 8.4), 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 μM primers, and 1 unit Taq DNA polymerase in a total volume of 20 μl. Samples were denatured for 3 min at 94°

and then cycled 35 times at 94°, 55°, and 72° (1 min each), which was followed by a 10-min extension at 72° using a PTC-200 Thermal Cycler (MJ Research, Watertown, MA). A total of 10 μl of each amplification was separated on 1% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination.

RESULTS AND DISCUSSION

Infection dynamics model: Prior crossing experiments reported a significantly higher realized fecundity (R_0) of Hou females relative to UjuT females (Dobson *et al.* 2001). We hypothesized that an increased fecundity associated with a CI-inducing Wolbachia infection would increase cytoplasmic drive rates. Alternatively, if the previously observed fecundity advantage was due to host genetic differences, then the genetically inherited fecundity advantage would be predicted to quickly become unlinked from the cytoplasmically inherited Wolbachia infection (Curtis 1992). The latter would result in the independent spread of the genetic fecundity advantage trait and Wolbachia, and the cytoplasmic drive for infections would return to rates expected for Wolbachia infections that do not increase host fecundity.

To simulate population replacement with a mutualistic, CI-inducing Wolbachia infection, a simple modification was made to a previously developed model that defines parameters important in the spread of Wolbachia (Hoffmann *et al.* 1990; Turelli *et al.* 1992). The descriptive ability of this prior model is supported by the quantitative agreement between its predictions and the observed dynamics and apparent equilibria of *Drosophila simulans* field populations (Hoffmann *et al.* 1986; Turelli and Hoffmann 1995). However, a modification was required to describe infections affording a host fecundity advantage, since the previous model is based upon infections in *D. simulans* and assumes that Wolbachia infections are associated with reduced host fecundity. A simple modification of the previous model that permits simulation of Wolbachia mutualists is to have α represent the fecundity of uninfected females relative to infected females and to assume that $\alpha \leq 1$. Using methods and assumptions similar to the Hoffmann/Turelli model, Table 1 illustrates the consequences. After simplification, we obtain

$$p_{t+1} = \frac{p_t(1 - \mu)}{\alpha(1 - p_t)[p_t H + (1 - p_t)] + p_t^2\mu(H - 1) + p_t}, \quad (1)$$

in which p denotes Wolbachia infection frequency at time t, μ is the fraction of uninfected eggs produced by infected females, and H is the relative hatch rates from incompatible crosses. If Wolbachia is assumed to have no effect on host fecundity ($\alpha = 1$), the predictions of Equation 1 become identical to those of the Hoffmann/Turelli model. Importantly, the model demonstrates that an infection associated with increased host fecundity ($\alpha > 1$) does not negate the previously described minimum infection frequency threshold required for Wolbachia invasion into the host population (Hoffmann *et al.* 1990; Turelli *et al.* 1992). A mutualistic Wolbachia infection will not necessarily invade a host population if the maternal transmission rates (μ) or relative hatch rates from incompatible crosses (H) are low.

TABLE 1

Derivation of recursions for Wolbachia infection frequencies

Adult mating (p_t)		Resulting progeny (p_{t+1})	
Type[a]	Frequency	Infected (I)	Uninfected (U)
I × I	p^2	$1 - \mu$	μH
I × U	$p(1 - p)$	$1 - \mu$	μ
U × I	$p(1 - p)$	—	$H\alpha$
U × U	$(1 - p)^2$	—	α

Where p is Wolbachia infection frequency at time t, μ is the fraction of uninfected eggs produced by infected females, H is the relative hatch rates from incompatible crosses, and α is the relative fecundity of uninfected females.

[a] Female × male.

Assuming that Wolbachia has no effect on host fecundity ($\alpha = 1$), 10% initial Wolbachia infection rate, perfect maternal transmission ($\mu = 0$), and complete incompatibility ($H = 0$), Equation 1 predicts 15 generations to reach ≥98% infection levels (Figure 2). Using similar assumptions, this is also the maximum population replacement rate possible using the Hoffmann/Turelli model. Changing the parameters to assume either imperfect maternal transmission or incomplete incompatibility (*i.e.*, $\mu > 0$ or $H > 0$), both Equation 1 and the Hoffmann/Turelli model predict a slowing of the cytoplasmic drive rates. Use of the Hoffmann/Turelli model to assume a fecundity cost associated with infection also results in the slowing of cytoplasmic drive rates. Thus, using simulations with either the Hoffmann/Turelli model or Equation 1, the only way to increase the population replacement rate for a 10% initial infection frequency to <15 generations is to assume an increased fecundity associated with infection (*i.e.*, $\alpha < 1$; Figure 2).

Population cage experiments: Wolbachia infection frequency in the population cages was monitored by both test crosses and PCR assays. In each of the cages receiving Hou females in the P generation (*i.e.*, female release cages), F_1 and F_2 males that were removed from cages and used in test crosses were observed to be compatible with the three female infection types (Figure 3). High egg hatch rates were observed in all broods (73.9 ± 27.8% egg hatch; $n = 174$). This observed compatibility of males with single- and uninfected females is consistent with the crossing pattern expected for uninfected males (Dobson *et al.* 2001; Figure 1). Thus for the F_1 and F_2 generations, all of the examined males from the female release cages appeared to be uninfected.

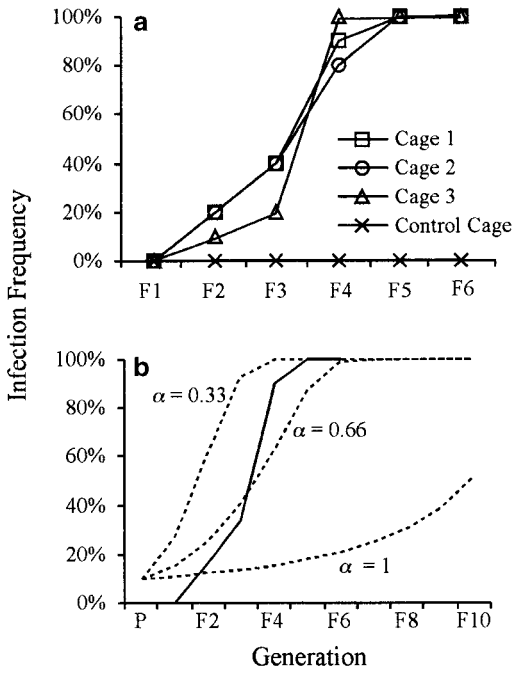

FIGURE 2.—Observed and predicted cytoplasmic drive rates for the replacement of an uninfected cytotype with a superinfected cytotype in *Ae. albopictus*. (a) Infection frequency observed in "female release" and control cages as determined by PCR assay. (b) Simulated (dashed lines) and observed (solid line) changes in Wolbachia infection frequency. The solid line representing the observed changes represents the infection frequency averaged for all female release population cages shown in a. All simulations were generated using the model described in the text and assuming $\mu = 0$ and $H = 0$.

By the F_4 generation, however, one male from each of the female release cages (Figure 3) was incompatible with both single- and uninfected females ($0.0 \pm 0.0\%$ egg hatch; $n = 14$) and compatible with superinfected females ($91.1 \pm 5.2\%$ egg hatch; $n = 7$). This crossing pattern is consistent with that expected for Wolbachia superinfection in these males (Figure 1). Test crosses of the remaining F_4 males from female release cages resulted in egg hatch with all three female infection types (Figure 3). Thus, test crosses of the F_4 generation suggested that one male from each female release cage was superinfected and that the remaining males were uninfected.

In the F_5 generation, all males sampled from female release cages and used in test crosses displayed a crossing pattern consistent with superinfection (Figure 3). All males failed to produce any hatching eggs in crosses with Koh and UjuT females ($0.0 \pm 0.0\%$ egg hatch; $n = 51$) and produced high egg hatch rates with Hou females ($79.4 \pm 9.6\%$ egg hatch; $n = 25$). Thus, test crosses of the F_5 generation demonstrated that all males from female release cages were superinfected.

Test crosses of males sampled from control cages resulted in high egg hatch rates throughout the study. This crossing pattern is as expected for uninfected males. One male from the control cage (F_4 generation) varied from expectations and failed to produce egg hatch when crossed with UjuT females (Figure 3). High egg hatch was observed in crosses of this male with both Koh and Hou females, demonstrating that this male was fertile. This crossing pattern is as expected for a single-infected male (*w*AlbA infection; Figure 1). However, since three of the four UjuT females mated with this male failed to produce eggs and since subsequent crossing tests and all PCR assays (discussed below) failed to detect Wolbachia infection in the control cage, we interpret this crossing pattern to result from fertilization failure in the cross between this male and UjuT females. These results demonstrate that individuals in the control cage remained uninfected throughout the study.

Wolbachia infection levels in the four population cages were also monitored by PCR assays. In the female release cages, PCR assays demonstrated an increase in infection frequency with each generation, resulting in 100% infection by the F_5 generation (Figure 2). In the control cage, PCR assays failed to detect Wolbachia infection throughout the study.

As shown in Figures 2 and 3, both PCR assays and crossing tests demonstrated that the uninfected cytotype was replaced by the superinfected cytotype by the F_5 generation in all three female release cages. As described above, the observed population replacement in <15 generations is consistent with predictions for a mutualistic, CI-inducing Wolbachia infection. Test crosses with Koh females demonstrated that both the *w*AlbA and *w*AlbB infections spread equivalently, as expected for two co-occurring, cytoplasmically inherited endosymbionts.

Paternal or horizontal transmission: Paternal or horizontal (*i.e.*, infectious) transmission of Wolbachia infections could provide an alternative explanation for increased rates of population replacement. The models above assume that no paternal or horizontal transmission occurs and are based upon observations of laboratory and field populations of infected species (HOFFMANN and TURELLI 1988; HOFFMANN *et al.* 1990; NIGRO and PROUT 1990; TURELLI *et al.* 1992; TURELLI and HOFFMANN 1995).

To examine for paternal or horizontal transmission, superinfected males were released into population cages in each of seven generations (*i.e.*, male release cages). Wolbachia infection was not detected in either of the male release cages. Thus, paternal (from males to offspring) and horizontal transmission (from males to females) was not observed to occur. As expected, the population density in these cages declined over successive generations due to cytoplasmically incompatible crosses (data not shown).

Crossing experiments using an aposymbiotic strain: As an additional test of the hypothesis that Wolbachia infection in *Ae. albopictus* is responsible for previously observed fecundity benefits (DOBSON *et al.* 2001), crosses

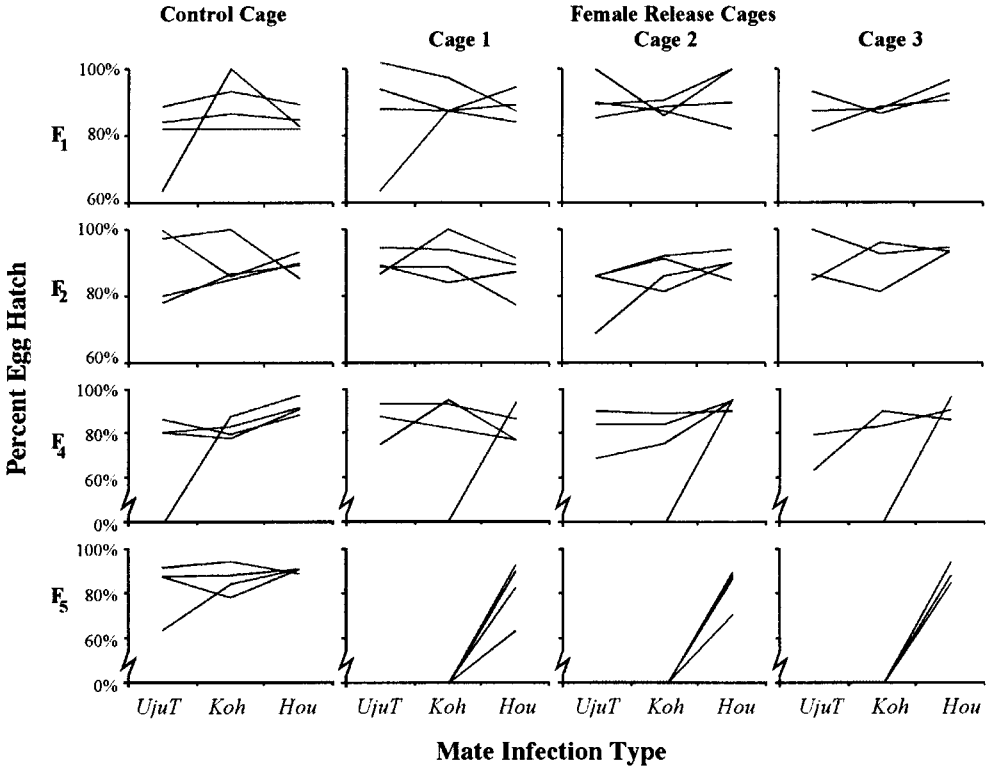

FIGURE 3.—Percentage of egg hatch resulting from test crosses conducted to estimate infection frequency and type occurring in female release and control population cages. Each line represents a single male that was removed from population cages and sequentially mated with UjuT, Koh, and Hou females.

were conducted using the superinfected Hou strain and the aposymbiotic HT1 strain (DOBSON and RATTANADECHAKUL 2001). Previous crosses of Wolbachia infections in *Ae. albopictus* suggested fecundity benefits associated with Wolbachia infection (DOBSON *et al.* 2001). However, this prior study was complicated by different host genetic backgrounds (including mitochondria differences). Thus similar to prior studies (STOLK and STOUTHAMER 1996; BORDENSTEIN and WERREN 2000), the fecundity disadvantage observed in uninfected hosts may have reflected differences in the Wolbachia infection type or host genetic background.

To examine CI levels and host fecundity effects associated with Wolbachia infection, oviposition rates, egg hatch rates, and adult longevity were monitored until all adults in the cage were dead. As shown in Table 2 and Figure 4, uninfected females were observed to have reduced longevity ($P < 0.001$) and decreased oviposition rates ($P < 0.0035$) relative to infected females. In addition, lower egg hatch rates ($P < 0.001$) were observed in the compatible HT1 × HT1 cross relative to compatible crosses of infected females (Hou × Hou and Hou × HT1; Table 2). In all crosses, egg hatch rates remained consistent over the lifetime of females (Figure 4). Combining the fecundity and female longevity, the realized fecundity (R_0) for compatible crosses of HT1 females (517.0 ± 57.6; $n = 8$) was significantly lower ($P < 0.0012$) than that observed for Hou females (628.1 ± 51.7; $n = 8$). Since individuals used in crosses had not been treated with tetracycline for six generations, it is unlikely that the observed differences reflect the direct effect of tetracycline treatment. These results are consistent with a previous study (DOBSON *et al.* 2001) and the hypothesis that Wolbachia infections are responsible for the fecundity advantage observed in infected strains.

TABLE 2

Average percentage of egg hatch

	Males	
Females	HT1	Hou
HT1	61.9 ± 10.7	0.02 ± 0.07
Hou	69.4 ± 15.0	83.5 ± 8.6

Average ± standard deviation (four cage replicates/cross type); significance, $F = 386.77$, d.f. = 3117, $P < 0.001$.

As expected due to CI, the infection type in males also had a significant effect on brood hatch rate. Relative to compatible crosses, significantly lower egg hatch ($P < 0.001$) resulted in incompatible crosses of uninfected females and infected males. Rare egg hatch did occur in incompatible crosses with 5 eggs hatching from a total of 20,440 eggs counted. This pattern of CI is consistent with previously reported CI levels (OTSUKA and TAKAOKA 1997; DOBSON *et al.* 2001).

These results provide the first clear evidence of a Wolbachia infection that both induces CI and increases female fecundity. Positive host fitness effects have been reported for CI-inducing infections in Drosophila, but these fitness effects have been shown to be transient

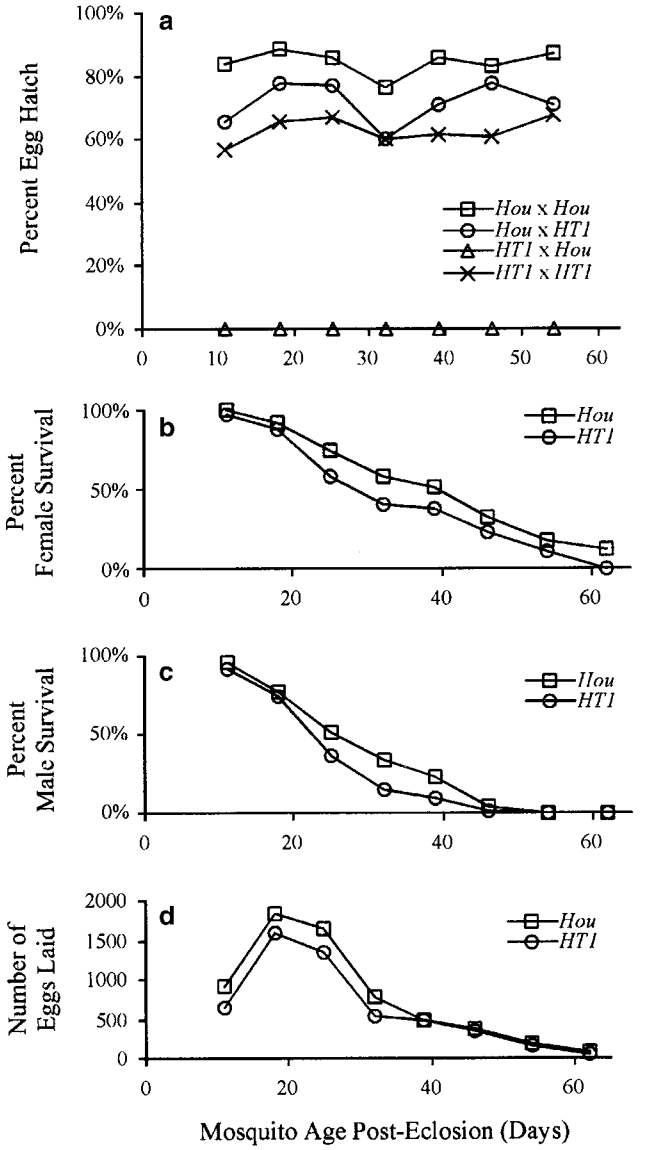

FIGURE 4.—Average percentages of egg hatch (*i.e.*, CI levels), adult longevity, and fecundity of superinfected (Hou) and aposymbiotic (HT1) *Ae. albopictus* strains. Crosses in a are female × male. Data points indicate averages of the four cage replications.

(POINSOT and MERCOT 1997). Contrasting reports of host fitness effects occur with Wolbachia infections in *Nasonia vitripennis* (STOLK and STOUTHAMER 1996; BORDENSTEIN and WERREN 2000). The overall host fitness effects of Wolbachia infection in Tribolium are complicated by both positive and negative fitness effects observed in males and females, respectively (WADE and CHANG 1995). Early studies with aposymbiotic strains of *Culex pipiens* suggest that Wolbachia infection may be mutualistic, but additional studies are required (AWAHMUKALAH and BROOKS 1985). Mutualistic Wolbachia infections have been reported in nematodes, but these infections have not been shown to induce CI (*i.e.*, classical mutualistic symbioses; GIRIN and BOULETREAU 1995; HARIRI *et al.* 1998; TAYLOR and HOERAUF 1999; VAVRE *et al.* 1999).

Although prior crossing studies (DOBSON *et al.* 2001) and crosses described here suggest a fecundity advantage of infected females between 1.2 and 1.6, comparisons of predicted and observed population replacement rates suggest a fecundity advantage between 1.5 and 3 (Figure 2). Furthermore, due to the use of only young (≤14-day-old adults) females in population cage experiments, the fecundity advantage afforded by Wolbachia infection over the lifetime of adult females would not be completely realized. This apparent discrepancy demonstrates the need for future studies to examine for additional host fitness effects caused by Wolbachia (*e.g.*, the potential effect of infection in other host developmental stages). Future experiments should also include defining the relative contribution of the *w*AlbA and *w*AlbB infections (SINKINS *et al.* 1995; ZHOU *et al.* 1998) to female fecundity and CI levels. Previously developed transinfection techniques (BRAIG *et al.* 1994) could be used to examine for host fecundity effects in alternative host species.

Evolution of mutualistic, CI-inducing Wolbachia infections: Unlike classical mutualistic endosymbionts that are expected to favor variants that increase the composite parameter $F(1 - \mu)$ (where F is the relative fecundity of infected females and $1 - \mu$ is the transmission efficiency), theory suggests that endosymbionts that induce CI will not inevitably evolve toward increasing $F(1 - \mu)$ (TURELLI 1994). For example, endosymbiotic variants with a decreased $F(1 - \mu)$ may spread due to an offsetting benefit resulting from sterilization of females with the alternative cytotype (TURELLI 1994). The latter evolutionary trajectory has been supported by the previous failure to identify CI-inducing Wolbachia infections that increase fecundity (reviewed in BORDENSTEIN and WERREN 2000).

Following invasion of the host population by a CI-inducing Wolbachia type (*i.e.*, population replacement), incompatible crosses are expected to decrease in frequency since uninfected hosts are rare or absent. With the reduced occurrence of CI in the host population, both nuclear and cytoplasmic selection will again favor variants with increased $F(1 - \mu)$. If one assumes fecundity costs associated with CI mechanisms, a "reversible/cyclical" evolution of Wolbachia symbioses is predicted, in which the population is invaded by "insensitive" Wolbachia variants that do not induce CI and that are not susceptible to the action of CI (HURST and MCVEAN 1996). The spread of the insensitive variants in turn permits the subsequent invasion and fixation of neutral Wolbachia variants (*i.e.*, that neither induce nor rescue CI) or the uninfected cytotype. Alternatively, if one assumes little or no costs associated with the CI mechanisms, selection may act to preserve the CI mechanisms for "resistance" to alternative CI-inducing parasites (TURELLI 1994). The latter evolutionary trajectory would

be predicted to select for increasingly benign or mutualistic Wolbachia variants that retain the ability to induce CI. Our report of a mutualistic, CI-inducing Wolbachia infection described here provides support for the latter evolutionary trajectory.

Concluding remarks: Here we report the first clear evidence of a Wolbachia infection that both induces CI and increases female host fecundity. As predicted by model simulations, we observed an increase in cytoplasmic drive rates in population cage studies that corresponds to increased fecundity associated with Wolbachia infection. Crossing experiments with genetically similar infected and aposymbiotic lines confirm earlier crossing results (Dobson *et al.* 2001) and demonstrate an increased fecundity associated with Wolbachia infection. Specifically, infected females were observed to have increased longevity, greater oviposition rates, and higher egg hatch rates resulting in compatible crosses. The results of both experiments are consistent with the expectations for a mutualistic, CI-inducing Wolbachia infection. The mechanism of the fecundity enhancement remains an important question for future studies and may be due to Wolbachia or compensatory evolution in the host. It is interesting to note that association between endosymbionts and the endoplasmic reticulum has been suggested as an early step in mutual obligatory symbiosis (Smith 1979). Previous ultrastructural characterization of Wolbachia infections in Aedes mosquitoes describes an association between Wolbachia and the endoplasmic reticulum (Wright and Barr 1980). Mutualism would also help to explain the difficulty in removing Wolbachia infections from *Ae. albopictus* relative to other invertebrate hosts (Dobson and Rattanadechakul 2001).

In addition to the evolutionary significance discussed above, the description of a mutualistic Wolbachia infection that induces CI is also relevant to applied research focused on employing Wolbachia infections to modify important pest species. The increased cytoplasmic drive rates would be expected to reduce the number of released individuals required for applied population replacement strategies and accelerate subsequent cytoplasmic drive rates (Curtis 1992; Sinkins and O'Neill 2000).

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