Heredity (2004) 93, 135–142

www.nature.com/hdy

Fitness advantage and cytoplasmic incompatibility in *Wolbachia* single- and superinfected *Aedes albopictus*

SL Dobson, W Rattanadechakul and EJ Marsland
Department of Entomology, University of Kentucky, Lexington, KY 40546, USA

Wolbachia are obligate, maternally inherited, intracellular bacteria that infect numerous insects and other invertebrates. *Wolbachia* infections have evolved multiple mechanisms to manipulate host reproduction and facilitate invasion of naive host populations. One such mechanism is cytoplasmic incompatibility (CI) that occurs in many insect species, including *Aedes albopictus* (Asian tiger mosquito). The multiple *Wolbachia* infections that occur naturally in *A. albopictus* make this mosquito a useful system in which to study CI. Here, experiments employ mosquito strains that have been introgressed to provide genetically similar strains that harbor differing *Wolbachia* infection types. Cytoplasmic incompatibility levels, host longevity, egg hatch rates, and fecundity are examined. Crossing results demonstrate a pattern of additive unidirectional cytoplasmic incompatibility. Furthermore, relative to uninfected females, infected females are at a reproductive advantage due to both cytoplasmic incompatibility and a fitness increase associated with *Wolbachia* infection. In contrast, no fitness difference was observed in comparisons of single- and superinfected females. We discuss the observed results in regard to the evolution of the *Wolbachia*/*A. albopictus* symbiosis and the observed pattern of *Wolbachia* infection in natural populations.
Heredity (2004) **93,** 135–142, advance online publication, 5 May 2004; doi:10.1038/sj.hdy.6800458

Keywords: cytoplasmic incompatibility; cytoplasmic drive; population replacement; reproductive parasite; mutualism

Introduction

Wolbachia are obligate intracellular rickettsia-like bacteria that occur in numerous invertebrates, with infection rate estimates in excess of 20% of insect species (Werren *et al*, 1995a; Jeyaprakash and Hoy, 2000; Werren and Windsor, 2000; Jiggins *et al*, 2001). The success of *Wolbachia* can be attributed in large part to its ability to manipulate the reproduction of its host to promote infection spread into the host population. *Wolbachia* infections provide multiple examples of mechanisms that promote infection spread, ranging along the continuum from mutualism (Hoerauf *et al*, 1999; Langworthy *et al*, 2000) to reproductive parasitism. Examples of the latter include cytoplasmic incompatibility (Hoffmann and Turelli, 1997), male killing (Hurst *et al*, 1999), parthenogenesis (Stouthamer *et al*, 1993), and feminization (Rousset *et al*, 1992). Although variable in strategy, the multiple mechanisms by which *Wolbachia* manipulates host reproduction are similar in that they provide infected female hosts with a reproductive advantage relative to uninfected females.

Cytoplasmic incompatibility (CI) results in karyogamy failure and arrested development of early embryos in diploid insects (Tram and Sullivan, 2002). Unidirectional CI occurs in matings between *Wolbachia*-infected males and uninfected females (Figure 1). The reciprocal cross and matings between individuals that harbor similar *Wolbachia* infections are compatible. Thus, in host populations that include both infected and uninfected individuals, CI provides a reproductive advantage to infected females since they can mate successfully with all male types. In contrast, uninfected females are incompatible with infected males, reducing reproductive success. The advantage afforded to females by CI comes at the expense of infected males, which are incompatible with uninfected females. However, this is of no cost to *Wolbachia*, since males are an evolutionary dead end for the maternally inherited infections. As demonstrated both theoretically and empirically, the reproductive advantage afforded by CI to infected females can result in population replacement, with the infected cytotype driving into the host population and replacing the uninfected cytotype (reviewed in Hoffmann and Turelli, 1997).

Wolbachia infections in *Aedes albopictus* (Asian tiger mosquito) provide a useful system in which to study CI and population replacement. Naturally occurring populations of *A. albopictus* can be single-infected with the *w*AlbA *Wolbachia* type or superinfected with the *w*AlbA and *w*AlbB *Wolbachia* types (Kambhampati *et al*, 1993; Sinkins *et al*, 1995b; O'Neill *et al*, 1997; Otsuka and Takaoka, 1997; Dobson *et al*, 2001). The *w*AlbA and *w*AlbB types are within the A and B *Wolbachia* clades, respectively (Zhou *et al*, 1998). Prior experiments demonstrate that the *w*AlbA infection is unidirectionally incompatible with uninfected hosts, and that the *w*AlbA/*w*AlbB superinfection has an additive effect, such

Correspondence: SL Dobson. Current address: Department of Entomology; University of Kentucky, Lexington, KY 40546, USA.
E-mail: sdobson@uky.edu
Received 23 September 2003; accepted 27 February 2004; published online 5 May 2004

Female	Male AB	A	B	O
AB	+	+	+	+
A	-	+	-	+
B	-	-	+	+
O	-	-	-	+

Figure 1 Generalized crossing pattern illustrating unidirectional and bidirectional cytoplasmic incompatibility (−) and compatibility (+) that results in crosses of uninfected (O), single-infected (A or B), and superinfected (AB) individuals. Note that incompatibility is observed when the male host harbors an infection type that is not present in the host female mate.

that the superinfection is unidirectionally incompatible with both single and uninfected hosts (see Figure 1; Dobson *et al*, 2001). This pattern of additive unidirectional CI in combination with the observed geographic distribution of single and superinfections in naturally occurring *A. albopictus* populations (Sinkins *et al*, 1995b; Perrot-Minnot *et al*, 1996) has led to the hypothesis that superinfection in *A. albopictus* originated via sequential population replacement events: that the superinfection (*w*AlbA + *w*AlbB) replaced a population that had been previously invaded by the *w*AlbA single-infection.

Here, we have examined CI levels, longevity, egg hatch, and fecundity in strains of *A. albopictus* (Asian tiger mosquito) that are uninfected, single-infected, and superinfected. Determination of *Wolbachia* effects can be complicated if the compared strains differ in both *Wolbachia* infection type and host genetic backgrounds (Dobson *et al*, 2001). Thus, we compare the differing infection types within a homogeneous host genetic background that was generated via introgression. The results demonstrate that, relative to uninfected females, infected females are at a reproductive advantage due to both cytoplasmic incompatibility and a fitness increase associated with *Wolbachia* infection. No fitness difference was observed in comparisons of single and superinfected females. A pattern of additive unidirectional cytoplasmic incompatibility was observed, which is consistent with prior reports (Kambhampati *et al*, 1993; Sinkins *et al*, 1995b; Otsuka and Takaoka, 1997; Dobson *et al*, 2001, 2002). We discuss the results in regard to the proposed evolution of *Wolbachia* superinfection in *A. albopictus* by sequential population replacement. As host genotype can affect *Wolbachia* infection dynamics, we compare the experimental results with introgressed strains with additional *A. albopictus* strains that harbor similar infection types but that differ in host genotype.

Materials and methods

Mosquito stocks and culture

Mosquito strains and infection types are outlined in Table 1. Mosquitoes were maintained using standard conditions (Gerberg *et al*, 1994) at 28±2°C and 75±10% RH with an 18 h light cycle. Eggs were hatched in deoxygenated water and reared at low density in water augmented with liver powder. For adult maintenance and experimental crosses, a constant supply of 10% sucrose was provided to adults. Females were provided weekly with a mouse for blood feeding and eggs were collected weekly.

Introgressions and experimental crosses

Characterization of *Wolbachia* effects can be complicated by differing host genetic backgrounds (Dobson *et al*, 2001). In a previous study, genetically homogeneous *A. albopictus* strains that differed in their infection type were generated via tetracycline treatment (Dobson and Rattanadechakul, 2001; Dobson *et al*, 2002). However, tetracycline treatment failed to generate a single-infected strain. Here, we have used introgressions to generate genetically homogeneous strains that are uninfected, single-infected (*w*AlbA) or superinfected (*w*AlbA + *w*AlbB). Introgression crosses were conducted using inbred *Hou*, *Koh*, and *UjuT* mosquito strains (Table 1). The *UjuT* nuclear genome was introgressed into single- and superinfected cytoplasm types by repeated backcrossing of *UjuT* males with virgin *Koh* or *Hou* females, respectively. In the next generation, the resulting hybrid daughters were mated with *UjuT* males. The introgression crosses were repeated for a total of seven generations. In theory, each backcross generation will replace half of the maternal nuclear genome with the paternal nuclear genome. The percentage nuclear substitution in females is calculated as (0.5^n), where n is the number of backcross generations. Thus, after seven generations, the amount of the original maternal genome remaining will theoretically be only 7.8×10^{-3} percent of the genotype. Although the original genotype is largely replaced by the *UjuT* genome in these introgressed lines, the maternally inherited *Wolbachia* infection should remain consistent during the introgressions. The resulting strains were designated *IK7* and *IH7* for the single- and superinfected introgressed lines, respectively (Table 1).

Following introgressions, crossing experiments designed to characterize *Wolbachia* effects were conducted using the *UjuT*, *IK7*, and *IH7* strains. Experimental crosses consisted of 10 2-day-old virgin females and males (20 mosquitoes total) placed in cages. 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 dried over a 2-day period and then allowed to mature for 5 days at 28±2°C (80±5% RH). Following drying and maturation, eggs were hatched by submerging in a deoxygenated water/liver powder solution. Larvae used for maintenance and crossing experiments were reared at low density in an excess of a yeast/yeast food suspension. Numbers of surviving males and females were recorded weekly. Egg papers were collected and counted until females in the cage were dead. Four replicate cages were monitored simultaneously for each of the nine crossing types.

PCR assay

Infection type in mosquito strains was initially confirmed using diagnostic primers that amplify a region of the gene encoding the *Wolbachia* outer surface protein: *wAlbA* (primers 328F and 691R) and *wAlbB* (183F and 691R primers; Zhou *et al*, 1998). For samples failing

Table 1 *Aedes albopictus* strains used in introgressions and experiments

Strain designation	*Genotype*	*Wolbachia type*	*Notes*	*Reference(s)*
UjuT	U	Uninfected	Artificially generated; aposymbiotic	Otsuka and Takaoka (1997)
Koh	K	*w*AlbA	Naturally occurring; field collected	Kambhampati *et al* (1993); Sinkins *et al* (1995a, b)
Hou	H	*w*AlbA+*w*AlbB	Naturally occurring; field collected	Kambhampati *et al* (1993); Sinkins *et al* (1995a, b)
HT1	H	Uninfected	Artificially generated; aposymbiotic	Dobson *et al* (2002); Dobson and Rattanadechakul (2001)
IK7	U	*w*AlbA	Artificially generated; introgressed	This paper
IH7	U	*w*AlbA+*w*AlbB	Artificially generated; introgressed	This paper

to amplify using *Wolbachia*-specific primers (eg, *UjuT* strain), 12S primers were used to amplify mitochondria DNA as a positive control for template DNA quality (O'Neill *et al*, 1992). For PCR amplifications, 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°C for 1 h. Following heat inactivation at 95°C 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 mM primers, and 1 U Taq DNA polymerase in a total volume of 20 µl. Samples were denatured for 3 min at 94°C, cycled 35 times at 94, 55, and 72°C (1 min each), followed by a 10 min extension at 72°C using a PTC-200 Thermal Cycler (MJ Research). A volume of 10 µl of each amplification was separated on 1% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination.

Results

Molecular confirmation of *Wolbachia* type in introgressed strains

PCR amplification with primers diagnostic for the *w*AlbA and *w*AlbB infections (Zhou *et al*, 1998; Dobson *et al*, 2001) confirmed the *Wolbachia* infection type following introgression crosses. Both the *w*AlbA and *w*AlbB amplification products were observed in *IH7* individuals, indicating superinfection. Only the *w*AlbA amplification product was observed in *IK7* individuals. No amplification product was observed in *UjuT* individuals. This pattern of amplification products is similar to that observed in the original *A. albopictus* strains (Dobson *et al*, 2001) and demonstrates that the *Wolbachia* infection type remained constant throughout introgression crosses.

Cytoplasmic incompatibility

As shown in Table 2, cytoplasmic incompatibility and reduced egg hatch were observed in crosses only when the male harbored a *Wolbachia* infection type that was not present in the female mate. Superinfected *IH7* females were compatible with all male infection types. Single-infected *IK7* females were incompatible only with superinfected males. Uninfected *UjuT* females were compatible with uninfected males only. Thus, crosses of the introgressed strains demonstrated an additive pattern of unidirectional cytoplasmic incompatibility. Differences in egg hatch rates (ie, CI levels) were significant by ANOVA and Bonferroni mean separation on arcsin(sqrrt(x)) transformed data. As illustrated in Figure 2, the egg hatch rate remained relatively constant over time.

Table 2 Percent egg hatch resulting from crosses of introgressed strains (mean ± standard error)

Female type	*Male type*		
	IH7	*IK7*	*UjuT*
IH7	90.2 ± 1.0^{A}	87.7 ± 2.0^{A}	89.7 ± 1.1^{A}
IK7	0.0 ± 0.0^{C}	86.1 ± 1.2^{A}	88.9 ± 1.9^{A}
UjuT	0.0 ± 0.0^{C}	0.2 ± 0.04^{D}	73.6 ± 3.9^{B}

Superscripted letters indicate significant differences ($P < 0.0001$, df = 8, F = 133.0).

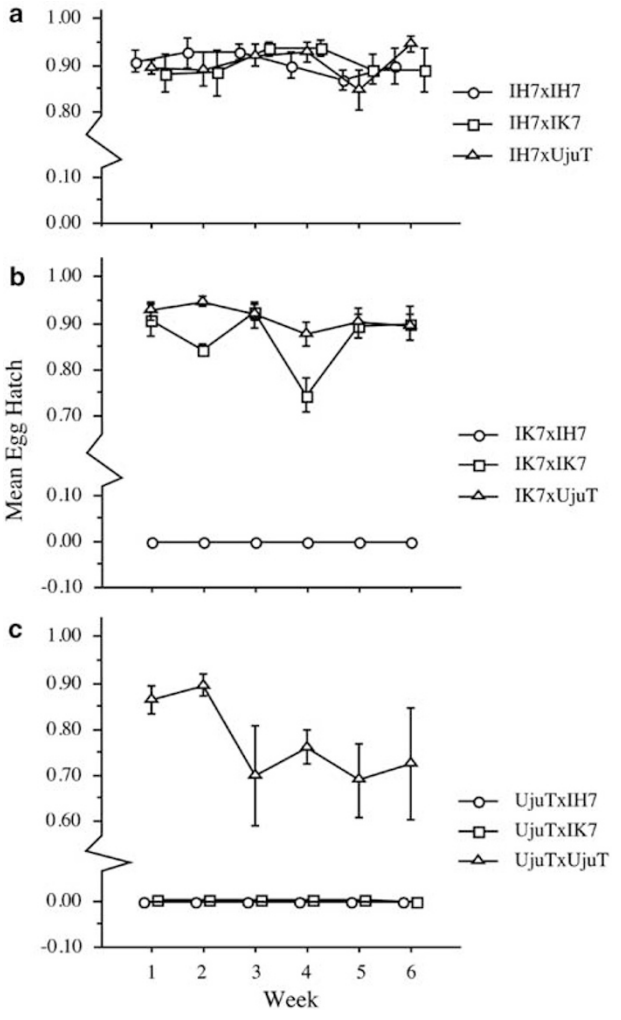

Figure 2 Mean proportion egg hatch rate (±standard error) over time. Crosses are shown as female × male. Week number indicates time post eclosion. In (**a**), the symbols are offset for clarity.

Comparisons within the six compatible cross types demonstrated significantly lower egg hatch resulting from the *UjuT* × *UjuT* cross relative to the remaining five compatible crossing types ($P<0.0001$; Table 2). Egg hatch in compatible crosses of infected females did not differ significantly. In a comparison of the three incompatible cross types, egg hatch was higher in crosses of single-infected males relative to incompatible crosses of superinfected males ($P<0.0001$; Table 2). No difference in egg hatch was observed between incompatible crosses of superinfected males.

Host longevity

Differences in host longevity were observed in comparisons of the different *Wolbachia* infection types. Comparisons were made using two methods. Survivor curves for individual hosts were compared using the Kaplan–Meier method and log-rank test. In a second approach, repeated-measures analysis was used to compare the proportion of surviving individuals in each cage over time. In the latter test, cages were the experimental units and an arcsine transformation of the square root of the proportion was used to ensure near normality of distribution and to stabilize the variance (Gill, 1978). Both analyses yielded the same conclusions. Here, we present the results of the survivor curve analysis.

Comparisons of females with similar infection types demonstrated that the type of male mate did not affect female longevity (*IH7* females, $\chi^2=4.259$, df = 2, $P>0.119$; *IK7* females, $\chi^2=3.141$, df = 2, $P>0.208$; *UjuT* females, $\chi^2=0.215$, df = 2, $P>0.898$). However, significant differences in female longevity were observed in comparisons of females that were infected with different *Wolbachia* types. As shown in Figure 3a, superinfected females were longer lived relative to uninfected females ($\chi^2=24.634$, df = 1, $P<0.0001$). Single-infected females were also significantly longer lived than uninfected females ($\chi^2=19.294$, df = 1, $P<0.0001$). No difference was observed in comparisons of single- and superinfected female longevity ($\chi^2=0.437$, df = 1, $P>0.5$). *Wolbachia* infection type in males was not observed to affect male longevity (Figure 3b; *IH7*, $\chi^2=4.714$, df = 2, $P>0.095$; *IK7*, $\chi^2=3.737$, df = 2, $P>0.154$; *UjuT*, $\chi^2=1.609$, df = 2, $P>0.447$).

Wolbachia effect on host fecundity

Repeated-measures ANOVA was used to compare fecundity. Female oviposition rates were estimated as the number of eggs from a cage divided by the number of surviving females within the cage. Cages and not individual mosquitoes were the experimental units. Comparisons of females with similar infection types demonstrated that the type of male mate did not affect female fecundity (*IH7* females, F = 0.178, df = 2, $P>0.84$; *IK7* females, F = 0.328, df = 2, $P>0.72$; *UjuT* females, F = 0.551, df = 2, $P>0.59$). In examining the effect of female *Wolbachia* infection type on fecundity (Figure 3c), uninfected females produced fewer eggs than single-infected females (F = 2.989, df = 2, $P<0.0538$) and superinfected females ($P<0.0321$). No difference was observed in fecundity comparisons of single- and superinfected females ($P>0.8086$). Similar results were observed in lifetime fecundity, comparing the different *Wolbachia* infection types.

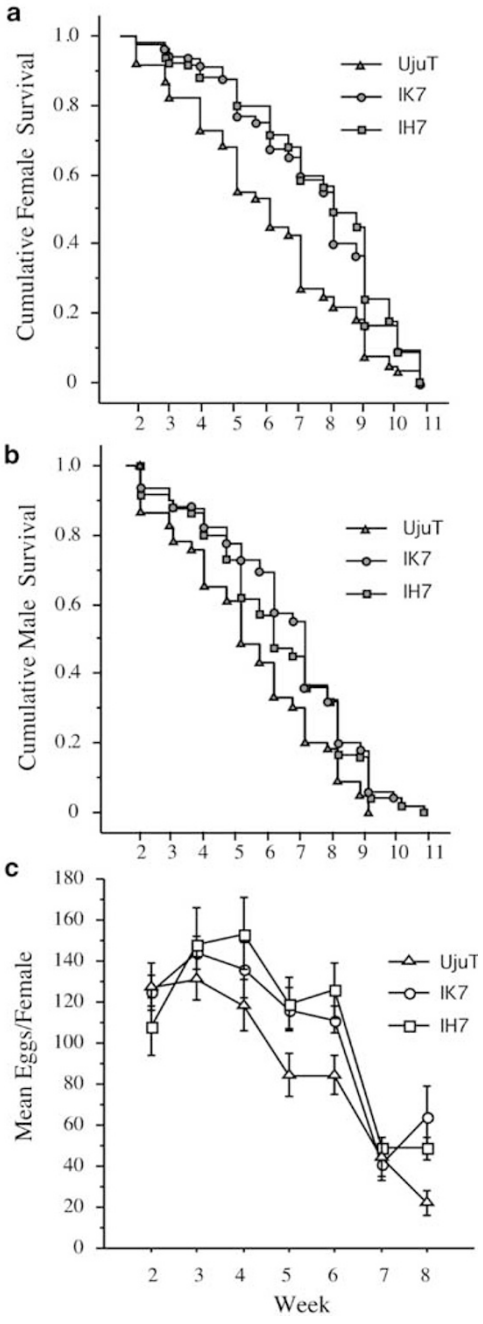

Figure 3 Comparison of (**a**) female longevity, (**b**) male longevity, and (**c**) fecundity by *Wolbachia* infection type. Week number indicates time post eclosion.

The compatibility type of the cross was not observed to affect fecundity. No difference was observed in comparisons of cytoplasmically incompatible versus compatible crosses of *UjuT* females (F = 0.005, df = 1, $P>0.94$). Similarly, no difference was observed in comparisons of cytoplasmically incompatible versus compatible crosses of *IK7* females (F = 0.078, df = 1, $P>0.78$).

Wolbachia infections within differing host genotypes

To better define the effect of host genotype on egg hatch, longevity, and fecundity, introgression cross results ('U' genetic background) were compared with results obtained in 'H' genotype crosses (Table 1). As *Wolbachia*

infection and compatibility type can affect egg hatch, longevity, and fecundity, comparisons were made between individuals with identical infection types.

To examine host genotype effect on compatible cross egg hatch rates, two cross pairs were compared. In both cross pairs, higher egg hatch was observed in 'U' genotype mosquitoes. Comparison of a superinfected cross pair (*Hou* × *Hou* versus *IH7* × *IH7*) demonstrated higher egg hatch ($F = 16.467$, $df = 1$, $P < 0.001$; Figure 4) in the 'U' genotype relative to the 'H' genotype. Higher egg hatch was again observed in the 'U' genotype ($F = 7.243$, $df = 1$, $P < 0.0092$; Figure 4) in comparisons of the uninfected (*UjuT* × *UjuT*; *HT1* × *HT1*) cross pair.

In an examination of genotype effect on fecundity, 'H' genotype females were observed to be more fecund than 'U' genotype females in a comparison of both uninfected (*UjuT* × *UjuT*; *HT1* × *HT1* cross pair; $F = 48.774$, $df = 1$, $P < 0.0004$; Figure 5a) and superinfected crosses (*Hou* × *Hou* *vs* *IH7* × *IH7*; $F = 4.740$, $df = 1$, $P < 0.0724$; Figure 5b).

In uninfected crosses, 'U' genotype individuals were longer lived relative to 'H' genotype individuals. A comparison of female longevity between superinfected females that were of the H or U genotype (ie, the *Hou* and *IH7* strains, respectively) demonstrated that U genotype females lived longer ($F = 9.614$, $df = 1$, $P < 0.0019$; Figure 6a). Similarly, U genotype females were again observed to be longer lived in a comparison of uninfected females ($F = 3.859$, $df = 1$, $P < 0.0495$; Figure 6b). A comparison of male longevity between males that were of the H or U genotype demonstrated that males with the U genotype lived longer regardless of whether superinfected ($F = 3.821$, $df = 1$, $P < 0.0506$; Figure 6c) or uninfected ($F = 13.420$, $df = 1$, $P < 0.0002$; Figure 6d).

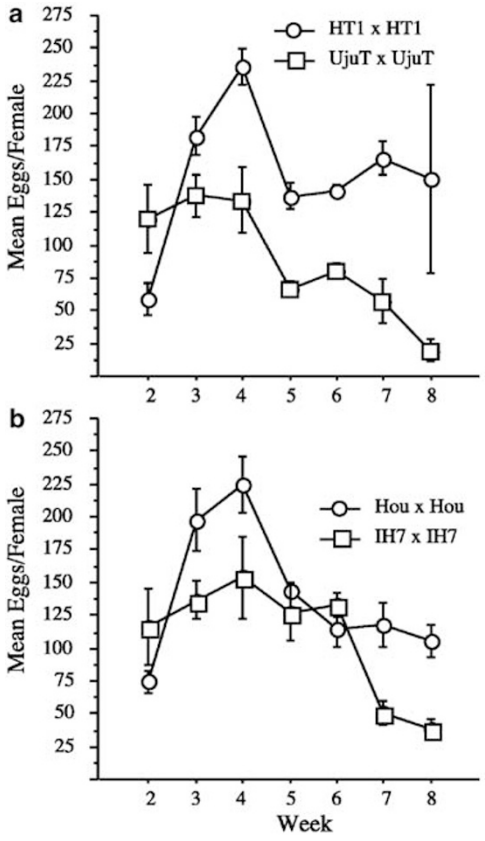

Figure 5 Fecundity comparison of cross pairs in which the host strains differ in genotype but are similarly (**a**) uninfected or (**b**) superinfected. Bars indicate standard error. Week number indicates time post eclosion.

Discussion

To improve our understanding of *Wolbachia* infection dynamics in *A. albopictus*, we have examined *Wolbachia* single- and superinfections within a uniform host genetic background generated via introgression crosses. The results demonstrate an increased host fitness associated with *Wolbachia* infection in *A. albopictus*. Specifically, single- and superinfected females are longer lived, have higher egg hatch in compatible crosses, and are more fecund relative to uninfected females. In contrast, no fitness differences were observed in comparisons of single- and superinfected mosquitoes. Crosses of the introgressed strains demonstrate an additive pattern of unidirectional cytoplasmic incompatibility consistent with previous reports (Kambhampati *et al*, 1993; Sinkins *et al*, 1995b; Otsuka and Takaoka, 1997; Dobson *et al*, 2001, 2002). Similar to a prior study (Dobson *et al*, 2001), egg hatch in incompatible crosses of single-infected males was higher than incompatible crosses of superinfected males.

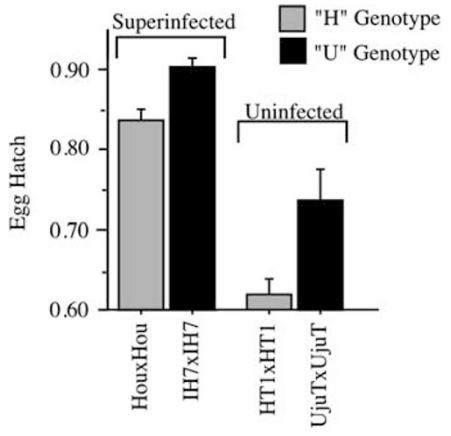

Figure 4 Comparison of host genotype effects on mean proportion egg hatch rate (±standard error), comparing cross pairs of similar infection type. See Table 1 for genotype designation.

In contrast, male *Wolbachia* infection type was not observed to affect male longevity. As males are an evolutionary dead end for *Wolbachia* infections, there is no direct selection on *Wolbachia* to affect male fitness. Indirectly, *Wolbachia* will be selected to affect male fitness if the male effect corresponds with a benefit to infections in females (reproductive parasitism; Bandi *et al*, 2001). Comparisons of introgression crosses also demonstrate that male infection type does not significantly affect egg hatch in compatible crosses, female mate longevity or fecundity. It is important to note that the comparisons described here are limited and do not examine for fitness effects induced by *Wolbachia* infections in immature hosts and other life stages (e.g., male mating competitiveness).

To examine for host genotype effects on *Wolbachia* infections, we compared the crossing results of similar

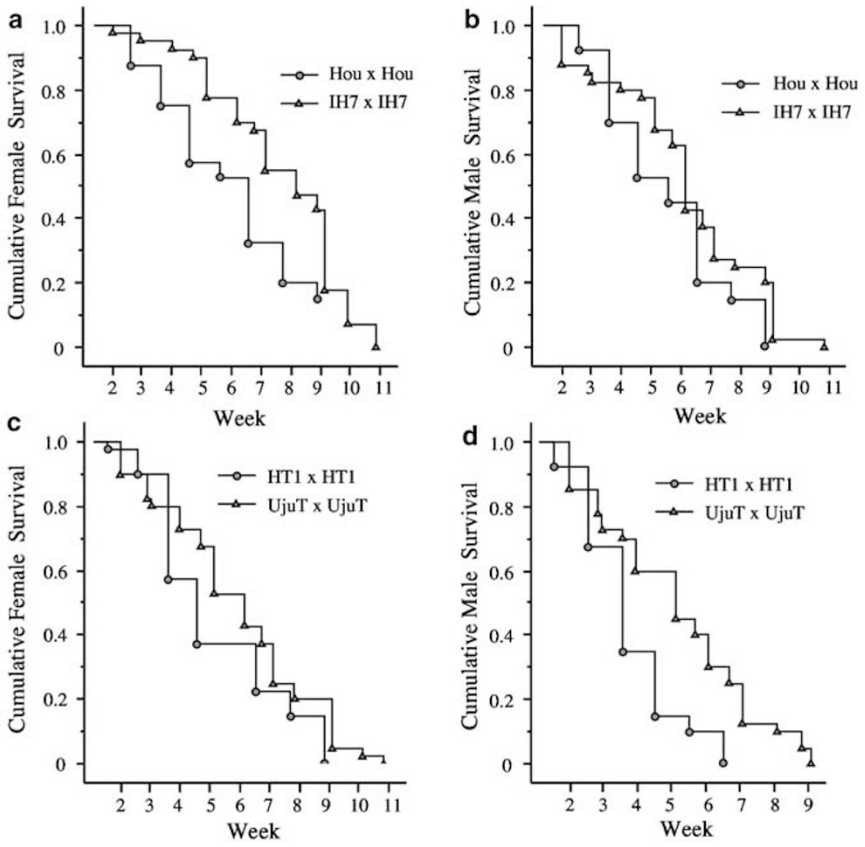

Figure 6 Comparison of host genotype effect on (**a** and **c**) female and (**b** and **d**) male longevity using cross pairs in which the host strains differ in genotype but are similarly (**a** and **c**) superinfected or (**b** and **d**) uninfected. Week number indicates time post eclosion.

Wolbachia infection types within differing genotypes. In general, *Wolbachia* effects on cytoplasmic incompatibility and host fitness are consistent regardless of host genotype, but differences have been observed in the egg hatch, longevity, and fecundity (Dobson *et al*, 2001, 2002). This supports the hypothesis that variable host genotype and *Wolbachia* infection type can complicate the interpretation of crossing results and demonstrate the importance of uniform host genotype in comparisons of *Wolbachia* infection effects. Given that the *UjuT* and *HT1* strains were cleared of infection in 1997 and 2001, respectively (Otsuka and Takaoka, 1997; Dobson and Rattanadechakul, 2001), the differences between the aposymbiotic *UjuT* and *HT1* strains may reflect differential adaptation of the host mosquitoes to the absence of *Wolbachia*. Importantly, although introgressions reduced host genetic variability, introgressions did not reduce mitochondrial variability. Thus, mitochondrial variation may contribute to the observed fecundity differences. However, *A. albopictus* displays low mtDNA variation across a wide geographic range (Kambhampati and Rai, 1991; Birungi and Munstermann, 2002). Furthermore, a similar fecundity difference was observed in prior comparisons of superinfected and aposymbiotic strains of *A. albopictus* that did not differ in their mitochondria type (Dobson *et al*, 2002).

Based upon the observed pattern of additive unidirectional CI, superinfected *A. albopictus* females are at a reproductive advantage relative to both uninfected and single-infected females. Single-infected females are at a reproductive advantage relative to uninfected females (Figure 1). The *Wolbachia* infection in *A. albopictus* is also associated with an increase in fecundity, female longevity, and egg hatch. Here, we observe no difference in female longevity or fecundity in comparisons of single- and superinfected females. Thus, the reproductive advantage of superinfected females relative to single-infected females is due to cytoplasmic incompatibility only. Therefore, different infection dynamics would be predicted for invasions of *Wolbachia* single- and superinfections into *A. albopictus* populations.

As previously demonstrated (reviewed in Hoffmann and Turelli, 1997), the reproductive advantage afforded to infected females by cytoplasmic incompatibility can permit the *Wolbachia* infection to spread, replacing the cytotype of the host population ('population replacement'). Theoretical predictions are that population replacement will not always result following the introduction of *Wolbachia* infection into a naive host population (Figure 7a). Due to fecundity costs associated with *Wolbachia* and the failure of females to transmit the infection to offspring, a minimum threshold infection frequency is required for *Wolbachia* to spread. *Wolbachia* occurring at infection frequencies below this minimum threshold are expected to be lost from the host population.

As previously described (Dobson *et al*, 2002), the combination of CI and host fitness increase can reduce the threshold infection frequency required for *Wolbachia* invasion and accelerate the rate of *Wolbachia* invasion

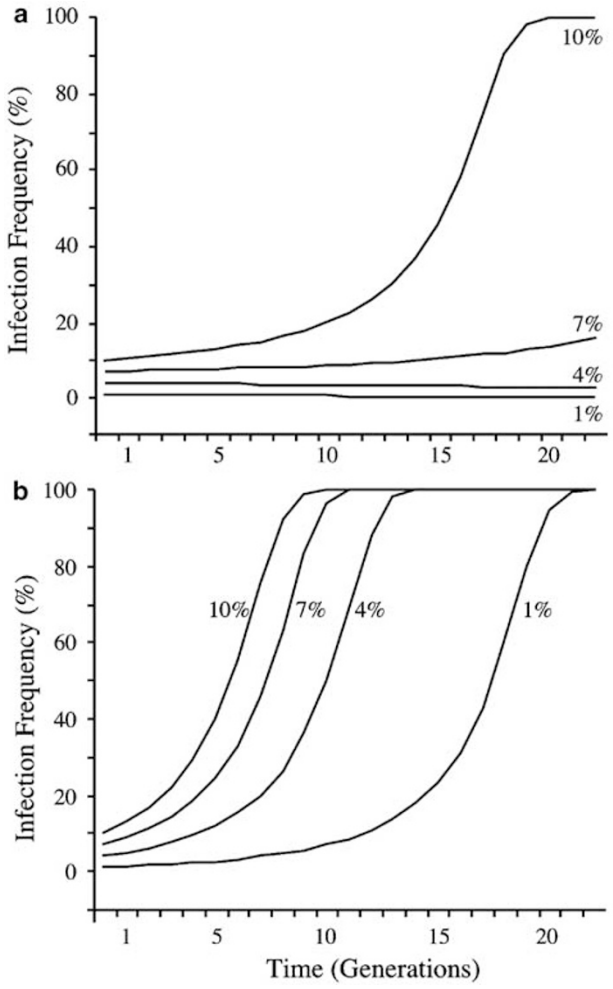

Figure 7 Model predictions of population replacement by (**a**) *Wolbachia* infections that cause CI and do not affect host fecundity and (**b**) *Wolbachia* infections that both cause CI and increase host fecundity. In both (**a**) and (**b**), plotted lines illustrate predicted changes in *Wolbachia* infection frequency over time following a 1, 4, 7, and 10% initial infection frequency. As illustrated in (**a**), the 1 and 4% initial infection frequencies are below the infection threshold required for population replacement, resulting in an eventual loss of the *Wolbachia* infection. In contrast, identical introductions illustrated in (**b**) result in population replacement. Simulations employ a previously described model (Dobson *et al*, 2002) and assume a 1% egg hatch resulting from CI crosses and 95% maternal transmission of *Wolbachia* infection. (**a**) assumes no fecundity difference between the infected and uninfected hosts. (**b**) assumes 20% lower fecundity in uninfected hosts.

(ie, cytoplasmic drive rate). Therefore, similar dynamics would be expected for the invasion of an uninfected population by either the single- or superinfections. In both population replacement events, the invasion would be driven by both CI and a fitness increase associated with *Wolbachia* infection (Figure 7b). In contrast, a higher threshold infection frequency and slower replacement rate is expected for the invasion of a single-infected population by the *Wolbachia* superinfection, since it is driven only by CI (Figure 7a).

The threshold infection frequency required for *Wolbachia* invasion is relevant to the evolution of *Wolbachia* symbioses and the reported geographic distribution of *Wolbachia* infections. Specifically, the threshold frequency is relevant to the mechanism by which *Wolbachia* is introduced into a naive host population. The introduction mechanism must occur at a rate that is sufficient to establish infection in a number of host individuals that surpasses the required threshold *Wolbachia* infection frequency and permits infection invasion. One mechanism that has been proposed for the introduction of *Wolbachia* infection into a naive host species is interspecific horizontal transmission. However, based on phylogenetic analyses of *Wolbachia* infections in insects, naturally occurring interspecific *Wolbachia* transmission events are predicted to be rare (O'Neill *et al*, 1992; Rousset *et al*, 1992; Werren *et al*, 1995b; Zhou *et al*, 1998; Vavre *et al*, 1999). Thus, a reduction of the required threshold by a *Wolbachia*-associated fitness increase could facilitate the initial invasion of *Wolbachia* into a naive *A. albopictus* population following rare interspecific transmission events. Alternatively, the fitness increase associated with *Wolbachia* infection in *A. albopictus* reported here may have evolved subsequent to the initial symbiotic association and *Wolbachia* invasion.

The higher threshold required for superinfection invasion of a single-infected population may be related to the geographic distribution of *Wolbachia* infections in *A. albopictus*. Superinfection has been described in naturally occurring *A. albopictus* populations from Japan, India, America, Malaysia, and Thailand (Kambhampati *et al*, 1993; Otsuka and Takaoka, 1997; Kittayapong *et al*, 2000). In contrast, naturally occurring *w*AlbA single infections have only been described in island populations (Koh Samui and Mauritius; Kambhampati *et al*, 1993; Sinkins *et al*, 1995b; Kittayapong *et al*, 2000; Dobson *et al*, 2001) that are geographically isolated from superinfected populations. Thus, immigration rates of superinfected mosquitoes from the mainland to Koh Samui and Mauritus may have been insufficient to surpass the required threshold. However, mosquito immigration rates are expected to increase with higher human trafficking between the mainland and islands. It should be noted that additional factors including host population structure and *Wolbachia* maternal inheritance rates are also important in determining the required infection threshold.

Future experiments should include population cage experiments to empirically examine model-predicted population replacement events and examining for additional *Wolbachia* effects on *A. albopictus*, including effects in other life stages and effects on mating competition. Experiments examining for potential routes of horizontal transmission of *Wolbachia* infection into *A. albopictus* will also help to elucidate the evolutionary trajectory that has resulted in the observed patterns and distribution of *Wolbachia/A. albopictus* symbiosis.

Acknowledgements

We thank Charles Fox, Sylvain Charlat, and Claudia Rauter for their comments and suggestions on early versions of the manuscript. We also thank Helena Truszczynska for her aid with statistical analyses. This research was supported in part by the United States Department of Agriculture NRICGP grant #9902683 and grant AI051533 from the National Institutes of Health. This is publication 02-08-143 of the University of Kentucky Agricultural Experiment Station.

References

Bandi C, Dunn AM, Hurst GDD, Rigaud T (2001). Inherited microorganisms, sex-specific virulence and reproductive parasitism. *Trends Parasitol* **17**: 88–94.

Birungi J, Munstermann LE (2002). Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: evidence for an independent invasion into Brazil and United States. *Ann Entomol Soc Am* **95**: 125–132.

Dobson SL, Marsland EJ, Rattanadechakul W (2001). *Wolbachia*-induced cytoplasmic incompatibility in single- and super-infected *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* **38**: 382–387.

Dobson SL, Marsland EJ, Rattanadechakul W (2002). Mutualistic *Wolbachia* infection in *Aedes albopictus*: accelerating cytoplasmic drive. *Genetics* **160**: 1087–1094.

Dobson SL, Rattanadechakul W (2001). A novel technique for removing *Wolbachia* infections from *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* **38**: 844–849.

Gerberg EJ, Barnard DR, Ward RA (1994). Manual for mosquito rearing and experimental techniques. American Mosquito Control Association, bulletin #5 (revised).

Gill JL (1978). *Design and Analysis of Experiments in Animal and Medical Sciences*. Iowa State University Press: Ames.

Hoerauf A, Nissen-Pahle K, Schmetz C, Henkle-Duhrsen K, Blaxter ML, Buttner DW, Gallin MY, Al-Qaoud KM, Lucius R, Fleischer B (1999). Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *J Clin Invest* **103**: 11–18.

Hoffmann AA, Turelli M (1997). Cytoplasmic incompatibility in insects. In: O'Neill SL, Hoffmann AA, Werren JH (eds) *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford University Press: Oxford, pp 42–80.

Hurst GDD, Jiggins FM, von der Schulenburg JHG, Bertrand D, West SA, Goriacheva II, Zakharov IA, Werren JH, Stouthamer R, Majerus MEN (1999). Male-killing *Wolbachia* in two species of insect. *Proc R Soc Lond (Biol)* **266**: 735–740.

Jeyaprakash A, Hoy MA (2000). Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. *Insect Mol Biol* **9**: 393–405.

Jiggins FM, Bentley JK, Majerus MEN, Hurst GDD (2001). How many species are infected with Wolbachia? Cryptic sex ratio distorters revealed to be common by intensive sampling. *Proc R Soc Lond (Biol)* **268**: 1123–1126.

Kambhampati S, Rai KS (1991). Mitochondrial DNA variation within and among populations of the mosquito *Aedes albopictus*. *Genome* **34**: 288–292.

Kambhampati S, Rai KS, Burgun SJ (1993). Unidirectional cytoplasmic incompatibility in the mosquito, *Aedes albopictus*. *Evolution* **47**: 673–677.

Kittayapong P, Baisley KJ, Baimai V, O'Neill SL (2000). Distribution and diversity of *Wolbachia* infections in southeast Asian mosquitoes (Diptera: Culicidae). *J Med Entomol* **37**: 340–345.

Langworthy NG, Renz A, Mackenstedt U, Henkle Duhrsen K, Bronsvoort MBD, Tanya VN, Donnelly MJ, Trees AJ (2000). Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. *Proc R Soc Lond (Biol)* **267**: 1063–1069.

O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM (1992). 16S *rRNA* phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc Natl Acad Sci USA* **89**: 2699–2702.

O'Neill SL, Pettigrew M, Sinkins SP, Braig HR, Andreadis TG, Tesh RB (1997). *In vitro* cultivation of *Wolbachia pipientis* in an *Aedes albopictus* cell line. *Insect Mol Biol* **6**: 33–39.

Otsuka Y, Takaoka H (1997). Elimination of *Wolbachia pipientis* from *Aedes albopictus*. *Med Ent Zool* **48**: 257–260.

Perrot-Minnot M, Guo LR, Werren JH (1996). Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis*: effects on compatibility. *Genetics* **143**: 961–972.

Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M (1992). *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc R Soc Lond (Biol)* **250**: 91–98.

Sinkins SP, Braig HR, O'Neill SL (1995a). *Wolbachia pipientis*: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus*. *Exp Parasit* **81**: 284–291.

Sinkins SP, Braig HR, O'Neill SL (1995b). *Wolbachia* superinfections and the expression of cytoplasmic incompatibility. *Proc R Soc Lond (Biol)* **261**: 325–330.

Stouthamer R, Breeuwer JA, Luck RF, Werren JH (1993). Molecular identification of microorganisms associated with parthenogenesis. *Nature* **361**: 66–68.

Tram U, Sullivan W (2002). Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science* **296**: 1124–1126.

Vavre F, Fleury F, Lepetit D, Fouillet P, Bouletreau M (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. *Mol Biol Evol* **16**: 1711–1723.

Werren JH, Windsor D, Guo LR (1995a). Distribution of *Wolbachia* among neotropical arthropods. *Proc R Soc Lond (Biol)* **262**: 197–204.

Werren JH, Windsor DM (2000). *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proc R Soc Lond (Biol)* **267**: 1277–1285.

Werren JH, Zhang W, Guo LR (1995b). Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc R Soc Lond (Biol)* **261**: 55–63.

Zhou W, Rousset F, O'Neill SL (1998). Phylogeny and PCR based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc R Soc Lond (Biol)* **265**: 509–515.