Wolbachia Effects on *Aedes albopictus* (Diptera: Culicidae) Immature Survivorship and Development

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ABSTRACT *Wolbachia* bacteria manipulate the reproduction of mosquito hosts via a form of sterility known as cytoplasmic incompatibility (CI), promoting the spread of infections into host populations. The rate at which an infection invades is affected by host fitness costs associated with the *Wolbachia* infection. Here, we examine for an effect of *Wolbachia* infection on the immature fitness of the Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae). In two experiments, we examine for a *Wolbachia* effect on immature survivorship and developmental rate, adult size, and an effect of larval nutrition on CI level. The highest survivorship can be observed in uninfected larvae, primarily because of reduced survivorship of *Wolbachia*-infected males. Although differences in the developmental rates are observed between the examined strains, the differences cannot be readily attributed to *Wolbachia*. An effect of *Wolbachia* on adult size is not observed. Poor male nutrition is associated with reduced fecundity and egg hatch of mates. The latter is hypothesized to explain the reduced egg hatch observed in CI crosses of malnourished males relative to well fed males. We discuss the results in relation to previously identified differences in adult fitness, naturally occurring invasions of *Wolbachia*, applied strategies of population replacement, and the need for additional modeling effort.

KEY WORDS *Aedes albopictus*, *Wolbachia*, cytoplasmic incompatibility, immature survivorship, population replacement

Obligate, intracellular *Wolbachia* bacteria occur commonly in invertebrates, including medically important insects and filaria (Kittayapong et al. 2000, Werren and Windsor 2000, Taylor 2002). The evolutionary success of *Wolbachia* can be attributed in part to the mechanisms by which it manipulates host reproduction, promoting *Wolbachia* invasion and maintenance within the host population (O'Neill et al. 1997). One mechanism is known as cytoplasmic incompatibility (CI) and has been observed in many mosquito species, including the Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae).

CI can be observed in matings between mosquitoes that harbor different *Wolbachia* infection types. Embryos resulting from CI crosses display karyogamy failure and early developmental arrest (Tram and Sullivan 2002). In mosquito populations that include both infected and uninfected individuals, the infected females can mate successfully with all males in the population. In contrast, uninfected females in the population are at a reproductive disadvantage because their broods fail to hatch if they mate with a *Wolbachia*-infected male. The reproductive advantage afforded by CI to infected females can promote the spread of the maternally inherited *Wolbachia* infection into the host population. The resulting *Wolbachia*-driven replacement of the uninfected host cytotype with the infected cytotype has been termed "population replacement."

Multiple models have been developed to describe the infection dynamics of CI-inducing *Wolbachia* infections (Hoffmann and Turelli 1997, Dobson et al. 2002a, Telschow et al. 2002). Key parameters predicted to impact *Wolbachia* infection dynamics include the fidelity of *Wolbachia* maternal transmission, CI level (egg hatch resulting from an incompatible cross), and *Wolbachia* effects on host fitness. Early models made a simplifying assumption to consider only *Wolbachia* effects on host fecundity as an indication of *Wolbachia* host fitness effects. Subsequent models have extended the definition of *Wolbachia* host fitness effects to include impacts on host survivorship (Brownstein et al. 2003, Rasgon and Scott 2003). The latter models demonstrate that a reduction of host survivorship by *Wolbachia* acts similar to *Wolbachia*-associated fecundity costs: increasing the threshold infection frequency required to initiate a population replacement event, slowing the rate at which *Wolbachia* invades and reducing the equilibrium infection frequency after *Wolbachia* invasion.

A focus of previous empirical studies has been to define *Wolbachia* maternal inheritance rates, CI levels, and *Wolbachia* effects on adult fitness in *Ae.*

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albopictus hosts (Dobson et al. 2001, 2002b, 2004; Kittayapong et al. 2002a,b,c 2002b). In contrast, *Wolbachia* effects on immature *Ae. albopictus* fitness have not been well characterized. Here, two related experiments have been conducted to improve current understanding of *Wolbachia* fitness effects. The first experiment compares the developmental rate and survivorship of infected and uninfected strains. A second experiment compares developmental time, adult size, and CI levels resulting from males that were either malnourished or well fed as larvae. The results suggest a fitness cost associated with *Wolbachia* infection in *Ae. albopictus* immatures. We discuss the results in relation to naturally occurring *Wolbachia* infections in *Ae. albopictus*, applied strategies that would use *Wolbachia* as a vehicle to drive transgenes into mosquito populations, and the need for additional modeling effort.

Materials and Methods

Mosquito Strains and General Maintenance. *Ae. albopictus* strains used in this experiment are the UjuTet (UT), introgressed Koh Samui (IK), and introgressed Houston (IH) strains. The UT strain is aposymbiotic (uninfected) after tetracycline treatment (Otsuka and Takaoka 1997). The IK strain is single-infected with the *w*AlbA *Wolbachia* type. Individuals of the IH strain are superinfected with both the *w*AlbA and *w*AlbB *Wolbachia* types. All three strains are predicted to have a similar nuclear background after seven generations of introgression crosses (Dobson et al. 2004). General maintenance of mosquitoes was as described by Gerberg et al. (1994). In brief, eggs were hatched in deoxygenated water and reared at low density in water augmented with lyophilized bovine liver powder (MP Biomedicals, Irvine, CA). For adult maintenance and experimental crosses, oviposition cups and a supply of 10% sucrose were constantly available to adults. Females were provided weekly with a mouse for blood feeding. Eggs were collected weekly by exchanging the old oviposition cup with a new cup. Maintenance cages and rearing pans was at 28 ± 2°C and 75 ± 10% RH with a photoperiod of 16:8 (L:D) h.

Measurement of Immature Development and Survivorship. Eggs were hatched synchronously by submerging egg papers in deoxygenated water. Replicate larval rearing pans were established with 200 first instars (<2 h posthatch) and 600 mg of liver powder in 600 ml of water. Five replicate pans for each of the three strains were maintained at 25 ± 0.5°C and a photoperiod of 16:8 (L:D) h. Rearing pans were augmented with an additional 300 mg of liver powder 5 d after pan initiation. Rearing pans were monitored for pupae at 6-h intervals beginning at day 5. Pupae were removed from rearing pans and placed individually in labeled test tubes. Test tubes were maintained and monitored for adult eclosion identically to rearing pans. After eclosion, adult sex was determined.

Nutritional Stress Effect and CI Crosses. In a second experiment, the aforementioned feeding regime was altered to generate food-stressed individuals (ST treatment). Rearing pans were identical to the aforementioned description, with the exception that they received 10 mg of liver powder on the day of hatching. Pans were augmented with an additional 10 mg of liver powder on day 7 and again on day 14. Nonfood-stressed individuals (WF; well fed) were reared as described for the immature developmental experiments.

As expected because of variable larval competitiveness and stochastic events, a range of eclosion times was observed with individuals from the malnourished treatment. Late-eclosing adults were selected for crossing experiments, based upon previous studies showing that they represent the greatest level of food stress (Armbruster and Hutchinson 2002, Dieng et al. 2002). Pupae were isolated in test tubes for eclosion to ensure virginity. Adult size was estimated by measuring wing length. Measurement was made between the axillary incision to the apical margin, excluding fringe setae (Tun-Lin et al. 2000).

The cross design consisted of four females mated with one male. Females were well fed in all crosses. Males were either well fed or starved. Females and males were <24 h posteclosion and were provided with a constant supply of 10% sucrose. This design permits testing incompatibility levels for individual males by crossing the same male with groups of females that are infected with different *Wolbachia* types. The sequence of the female groups was such that the matings progressed from incompatible to compatible crosses. The latter was used to confirm male fertility. Four UT females were initially mated with IH males. After allowing 2 d for mating, the UT females were replaced with four IK females. Two days later, the IK females were replaced with four IH females. In crosses of IK males, four UT females were initially mated with the IK male. After allowing 2 d for mating, the UT females were replaced with four IK females. After mating, females were blood fed by using anesthetized mice and provided with an oviposition cup. After 7 d, oviposition cups were removed from cages and allowed to mature for 5 d. The eggs were hatched in deoxygenated water, and the numbers of hatched and unhatched eggs were recorded using a dissecting microscope.

Statistical Procedures. All statistical comparisons were conducted using StatView version 5.0.1 (SAS Institute, Cary, NC). Normality of the experimental data was determined by the Kolmogorov–Smirnov test. Nonparametric tests (Mann–Whitney *U* [MWU], one-way Kruskal–Wallis, and Fisher exact tests) were used to analyze data sets that were not normally distributed. Multiple MWU tests were corrected using the sequential Bonferroni technique as described previously (Rice 1989). Analysis of variance (ANOVA) and post hoc analysis were used to analyze normally distributed data sets. The latter included brood size (i.e., fecundity), egg hatch rates, and comparisons of eclosion and pupation times.

Table 1. Survivorship and sex ratio comparisons of *Ae. albopictus* strains

Strain	No. pupae	Pupal mortality	No. females	No. males	No. adults	Sex ratios[a]
UT	198.4 ± 1.34	6.6 ± 2.97	91.0 ± 4.58	100.8 ± 5.59	191.8 ± 2.28	0.47 ± 0.03
IK	178.4 ± 5.94	9.8 ± 6.18	71.8 ± 10.99	96.8 ± 7.73	168.6 ± 4.98	0.42 ± 0.06
IH	189.4 ± 1.14	7.6 ± 4.88	90.2 ± 6.87	91.6 ± 3.51	181.8 ± 5.63	0.50 ± 0.03

Source of variation	df	χ^2	KW UT-IK-IH (*P* value)	MW UT-IK (*P* value)	MW UT-IH (*P* value)	MW IK-IH (*P* value)
No. pupae	2	12.500	0.0019	0.0090*	0.0090*	0.0090*
Pupal mortality	2	0.815	0.6653	0.4647	0.6761	0.4647
No. females	2	8.495	0.0143	0.0163*	0.4647	0.0122*
No. males	2	5.465	0.0651	0.6015	0.0122*	0.2101
No. adults	2	12.020	0.0025	0.0090*	0.0163*	0.0090*
Sex ratios	2	3.605	0.1649	0.2101	0.4647	0.0758

KW, Kruskal–Wallis; MW, Mann–Whitney *U*. Data are average ± SD.
[a] Number of females/total number of adults.
Asterisk (*) indicates significance after Bonferroni correction for multiple comparisons.

Results

Experiment 1: *Wolbachia* Effects on Immature Survivorship and Development. Survivorship comparisons reveal significant differences between the three strains (Table 1). The highest survivorship to pupation and eclosion are observed in the uninfected UT strain, followed by the superinfected IH, and then the single-infected IK strain. Male mortality accounts for the survivorship difference between the IH and UT strains. No difference is observed between the IH and UT strains in the number of successfully eclosing females. The low survivorship observed in the single-infected IK strain is due to the low number of surviving females. The number of successfully eclosing IK males did not differ significantly from the other two strains. Adult sex ratio was not observed to vary between the three strains.

No difference was observed in comparisons of female pupation or eclosion times (Table 2). In contrast, male pupation ($F = 8.332$, df = 2, $P < 0.0003$; ANOVA) and eclosion ($F = 5.587$, df = 2, $P < 0.0038$; ANOVA) differed significantly between the infection types. Post hoc analysis revealed that the difference was due to IK males, which developed slower than IH and UT males ($P < 0.0058$). Significant differences were not observed between IH and UT male pupation and eclosion.

Table 2. Developmental time comparisons of *Ae. albopictus* strains

Pupation		Eclosion	
Female		Female	
IH	153.9 ± 15.1a; *n* = 452	IH	225.0 ± 16.3d; *n* = 452
IK	155.3 ± 14.2a; *n* = 360	IK	225.6 ± 15.2d; *n* = 360
UT	155.7 ± 12.1a; *n* = 455	UT	227.2 ± 14.7d; *n* = 455
Male		Male	
IH	140.0 ± 12.4b; *n* = 458	IH	208.4 ± 13.5e; *n* = 458
IK	144.0 ± 14.7c; *n* = 482	IK	211.1 ± 16.0f; *n* = 482
UT	141.8 ± 8.4b; *n* = 505	UT	208.7 ± 10.3e; *n* = 505

Average hours ± SD; *n* is number of individuals. Letters indicate significant differences (see description in text)

Experiment 2: *Wolbachia* and Immature Nutrition Effects. Eclosion time differed significantly ($P < 0.0001$; MWU) between adults selected for the ST (602.4 ± 91.2 h; $n = 75$) and WF (182.4 ± 16.8 h; $n = 92$) treatments. As expected based upon previous reports (Briegel and Timmermann 2001, Dutton and Sinkins 2004), adult *Ae. albopictus* females are consistently larger than males (Table 3). Comparing within the WF treatment group, adult size differed significantly ($P < 0.0001$; MWU) between females (2.66 ± 0.07 mm; $n = 15$) and males (2.06 ± 0.09 mm; $n = 46$). Similarly, comparison within the ST treatment revealed a size difference ($P < 0.0001$; MWU) between females (1.77 ± 0.08 mm; $n = 10$) and males (1.47 ± 0.11 mm; $n = 65$). As expected, immature nutrition affected adult size (Table 3). Significant size difference was observed between males receiving different nutrition levels ($P < 0.0001$; MWU) and between females receiving different nutrition levels ($P < 0.0001$; MWU). In contrast, *Wolbachia* infection type was not observed to affect adult size. Size was not observed to differ between the infection types in comparisons within the female and male groups that received similar nutrition (Table 3).

Table 3. Wing size comparison of *Ae. albopictus* strains reared at two nutritional levels

Females	Males
Starved (ST)	
IK 1.80 ± 0.1; *n* = 5	IK 1.48 ± 0.12; *n* = 33
IH 1.74 ± 0.06; *n* = 5	IH 1.47 ± 0.12; *n* = 32
P > 0.3075; MW	*P* > 0.6555; MW
Well Fed (WF)	
UT 2.70 ± 0.07; *n* = 5	UT 2.08 ± 0.08; *n* = 15
IK 2.64 ± 0.09; *n* = 5	IK 2.05 ± 0.08; *n* = 13
IH 2.64 ± 0.06; *n* = 5	IH 2.06 ± 0.1; *n* = 18
P > 0.4242; KW	*P* > 0.5272; KW

Average ± SD; number of crosses; *P* values are for comparisons of infection types within a group of the same sex and nutrition treatment (i.e., comparison of different *Wolbachia* infection types); KW, Kruskal–Wallis comparison MW, Mann–Whitney *U* comparison.

Table 4. Frequency of broods with egg hatch among CI crosses of *Ae. albopictus* strains reared at two nutritional levels

	With hatch	Without hatch
UT × IK		
ST	1	33
WF	2	8
	$P > 0.0599$	
UT × IH		
ST	0	32
WF	0	10
	$P = 1.000$	
IK × IH		
ST	0	19
WF	1	9
	$P > 0.1607$	

Cells indicate the number of crosses with or without egg hatch; Fisher exact *P* values are for comparisons between the starved (ST) and well fed (WF) male treatments within a cross type; Crosses are shown as female × male; females are well fed in all crosses.

Similar to previous reports (Dobson et al. 2002b, 2004), egg hatch was rare in CI crosses (Table 4), occurring in only four incompatible crosses: three UT × IK crosses (18, 7, and 1.4% egg hatch; female × male) and one UT × IH cross (1% hatch). The egg hatch occurring in the latter four crosses is significantly lower ($P < 0.0001$; MWU) than that observed in the compatible crosses.

Larval nutrition was not observed to affect *Wolbachia*-induced CI when the three incompatible cross types were considered separately (Table 4). Logistic regression was used to test for effects of cross type and nutrition on the frequency of nonhatching broods. Broods were treated as binary data: either unhatching or with some egg hatch. Egg hatch was observed to occur more frequently ($\chi^2 = 3.89$, $P < 0.0487$) in crosses of WF males (hatch observed in 10% of crosses; $n = 30$ crosses) relative to crosses of ST males (hatch observed in 1.2% of crosses; $n = 85$). There was no evidence for an effect of CI cross type and no nutrition × CI type interaction. A similar effect was observed among the compatible crosses (Table 5). Egg hatch was lower ($F = 13.106$, df = 1, $P < 0.0006$; ANOVA) in crosses of ST males (76.2 ± 16.2%; $n = 40$) relative to crosses of WF males (87.3 ± 6.1%; $n = 31$). Examination of the compatible crosses did not reveal an effect of *Wolbachia* infection type on the resulting egg hatch (Table 5).

Table 5. Egg hatch and fecundity among compatible crosses of *Ae. albopictus* strains reared at two nutritional levels

Cross type[a]	% egg hatch[b]	Fecundity[c]	No. of crosses
WF males			
IH × IH	83.6 ± 5.9	137.3 ± 34.4	8
IK × IK	86.1 ± 7.8	91.1 ± 23.2	8
UT × UT	89.9 ± 3.9	128.7 ± 42.3	15
ST males			
IH × IH	78.3 ± 11.8	143.2 ± 50.7	19
IK × IK	74.3 ± 19.5	104.7 ± 37.3	21

[a] Crosses are shown as female × male; females are well fed in all crosses.
[b] Average percentage of egg hatch ± SD.
[c] Average number of eggs ± SD.

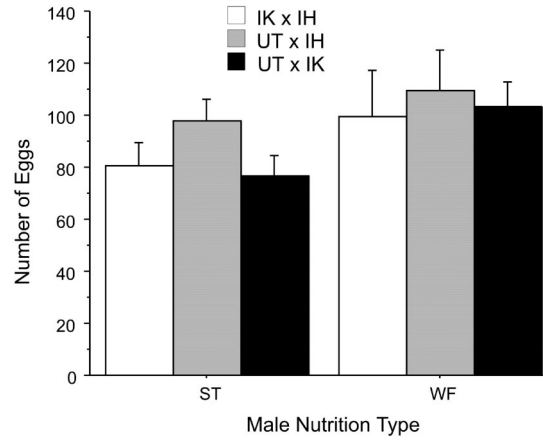

Fig. 1. Average fecundity resulting from incompatible matings of starved (ST) and well fed (WF) *Ae. albopictus* males. Crosses are shown as female × male. Standard error bars are shown.

Wolbachia infection type was not observed to affect fecundity in comparisons of the three incompatible cross types (Fig. 1). Similar brood sizes are observed in each of the three incompatible cross types ($P > 0.39$; ANOVA). Among the compatible matings, fecundity was observed to differ between the strains ($F = 7.169$, df = 2, $P < 0.0015$; ANOVA) (Table 5). Bonferroni and Fisher post hoc analysis demonstrated fecundity differences in comparisons of IK relative to IH ($P < 0.0004$) and between the UT and IK strains ($P < 0.0354$), but no fecundity difference were noted between the UT and IH crosses ($P > 0.3$).

A possible effect of male nutrition on the brood size of mates was observed. More eggs ($F = 3.977$, df = 1, $P < 0.0486$; ANOVA) were observed in broods from CI matings of WF males (Fig. 1; 104.1 ± 44.9, $n = 30$) (Fig. 1) relative to crosses of ST males (85.7 ± 44.2; $n = 85$). In contrast, male nutrition was not observed to affect fecundity among the compatible crosses (Table 5).

Discussion

The results provide evidence for a negative effect of *Wolbachia* infection on immature *Ae. albopictus* survivorship. Uninfected larvae are observed to have the highest immature survivorship under the optimal rearing conditions used in this study. Immature survivorship differences between the UT and IH strains are due primarily to higher male mortality in the superinfected strain, which is consistent with evolutionary theory. Because *Wolbachia* is transmitted exclusively through female hosts, selection against *Wolbachia*-associated host fitness costs is predicted to be stronger in females relative to males. Model theory suggests that in extreme examples, this sex-specific pattern of selection can lead to examples of male killing *Wolbachia* infections as observed in some insects (Hurst and Majerus 1993).

Increased mortality of IH male immatures provides a possible mechanism for fitness differences previously reported between UT and IH adult females (Dobson et al. 2004). Container breeding *Ae. albopictus* immatures compete with conspecifics for limited resources. Increased competition can result in reduced resources available to immature females, which can negatively affect adult female fitness (Blackmore and Lord 2000). A *Wolbachia*-induced reduction in immature male survivorship could benefit females by reducing conspecific resource competition. Additional experiments are required to test the latter hypothesis.

Previous experiments demonstrate that egg hatch resulting from CI crosses can be affected by environmental factors, including male age (Singh et al. 1976, Turelli and Hoffmann 1995), temperature (Trpis et al. 1981; Hoffmann et al. 1986, 1990; Stevens 1989; Feder et al. 1999), the presence of antibiotics (Stevens 1989), and crowding and nutrition (Sinkins et al. 1995a, Clancy and Hoffmann 1998). In the results presented here, a correlation between immature male nutrition and egg hatch resulting from incompatible crosses is observed. Specifically, higher egg hatch was observed in incompatible crosses of WF males relative to similar crosses of ST males. Interestingly, this result is the opposite of that observed in a previous *Drosophila* study in which stress resulted in decreased CI levels (i.e., increased egg hatch; Sinkins et al. 1995b, Clancy and Hoffmann 1998). The observation of complete CI (no egg hatch) in crosses of UT females with either WF and ST IH males is consistent with a previous report (Dutton and Sinkins 2004).

The observation that reduced egg hatch results from immature male malnutrition in both compatible and CI crosses suggests that male malnutrition is not directly affecting the CI mechanism. Alternatively, a non-*Wolbachia* effect of immature male starvation may be transmitted to mates, resulting in a reduced egg hatch. Thus, the observed differences in egg hatch would simply represent an additive effect of both male malnutrition and CI. A similar effect of male nutrition on mate fecundity (Fig. 1) may explain the differences observed in brood size comparisons within the incompatible crosses. A potential mechanism for the preceding model is the transfer of compounds from males to females during mating. Nuptial feeding occurs commonly in insects (Vahed 1998). Although we were unable to identify previous reports of nuptial feeding in mosquitoes, multiple examples can be observed within Diptera. In *Drosophila*, amino acids are transferred to females in ejaculates. *Drosophila* females receiving larger ejaculates are more fecund (Markow et al. 1990). In mosquitoes, male nutrition has been shown to affect the transfer of accessory gland products during copulation, which can affect female blood-seeking behavior, oviposition, and egg hatch (Adlakha and Pillai 1975, 1976; Klowden 1993; Lee et al. 2000, 2001).

A comparison of the compatible crosses failed to detect an effect of *Wolbachia* type on egg hatch. This is in contrast with previous results showing increased egg hatch among compatible crosses of *Wolbachia*-infected females relative to uninfected females (Dobson et al. 2002b, 2004). However, the experiment described here differs from the previous studies, which examined egg hatch over the adult lifetime. Here, only the first oviposition was examined. The reduced UT egg hatch that is observed in the previous reports is primarily due to reduced egg hatch occurring after the second oviposition (see Fig. 2c in Dobson et al. 2004).

An immature fitness cost associated with *Wolbachia* infection in *Ae. albopictus* is similar to that reported in *Trichogramma kaykai* Pinto & Stouthamer (Huigens et al. 2004). However, unlike *T. kaykai* in which *Wolbachia* infection also is associated with slower immature development (Hohmann and Luck 2000), no clear effect of *Wolbachia* infection on immature *Ae. albopictus* developmental rate was observed. Specifically, no difference was observed between the immature developmental rates of the superinfected IH and uninfected UT strains.

Differences in fecundity were observed among the compatible crosses in comparisons of the different strains receiving similar nutrition levels. However, the differences cannot be readily attributed to the *Wolbachia* infection type. Specifically, no difference was observed in a comparison of fecundity resulting from superinfected IH crosses with that resulting from uninfected UT crosses.

In general, the IK strain seems weaker than the IH or UT strains. The IK strain displays significantly lower immature survivorship, slower development, and reduced fecundity. However, the lower fitness cannot easily be explained as simply because of the presence of the *w*AlbA *Wolbachia* infection without complicating assumptions. Specifically, because the *w*AlbA infection is present in both the IK and IH strains, the latter interpretation would require the assumption of an interaction between the two co-occurring infection types in IH or between *w*AlbA and the IH host genetic background (Fry and Rand 2002) that offsets the fecundity deficit. Alternative hypotheses for the reduced vigor observed in IK include mitochondrial or nuclear differences, or both, between the strains (Birungi and Munstermann 2002, Armbruster et al. 2003, Ballard and James 2004) that persist despite introgression crosses.

Previous studies demonstrate that environmental factors can influence *Wolbachia* host fitness effects (Olsen et al. 2001). Here, a *Wolbachia* effect on adult size was examined under two types of immature rearing conditions that represent opposite extremes: optimal conditions and conditions of food stress. Under both condition types, *Wolbachia* was not observed to affect the size of resulting adults.

Effects of *Wolbachia* infection on *Ae. albopictus* survivorship are relevant to understanding the evolution of naturally superinfected populations and to proposed strategies that would use *Wolbachia* to replace medically important vector populations with strains that are refractory to pathogen transmission (Sinkins and O'Neill 2000, Dobson 2003). Previous studies demonstrate that under optimal laboratory rearing

conditions, *Wolbachia*-infected *Ae. albopictus* female adults live longer and are more fecund relative to uninfected females, increasing the rate of *Wolbachia* invasion (Dobson et al. 2002b, 2004). Although previous models describing *Wolbachia* infection dynamics in mosquitoes allow for differences in survivorship, they have been used to examine *Wolbachia* effects on adult survivorship only (Brownstein et al. 2003, Rasgon et al. 2003). The results presented here demonstrate that models examining *Wolbachia* infection dynamics will need to incorporate *Wolbachia* effects on immature survivorship.

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