Wolbachia Effects on Host Fitness and the Influence of Male Aging on Cytoplasmic Incompatibility in *Aedes polynesiensis* (Diptera: Culicidae)

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ABSTRACT The endosymbiotic bacteria *Wolbachia* manipulate host reproduction by inducing a form of sterility known as cytoplasmic incompatibility (CI), promoting the invasion of infection into natural host populations. CI has received attention for use in applied strategies to control insect vectors of disease. Thus, to understand both naturally occurring *Wolbachia* invasions and evaluate potential applied strategies, it is important to understand *Wolbachia* interactions with its host, including impacts on fitness and the CI level. In this study, we examined for an effect of *Wolbachia* on survivorship, developmental time, sex ratio, longevity, fecundity, and egg hatch of *Aedes polynesiensis* Marks, which is the primary vector of *Wuchereria bancrofti* in the South Pacific. In this study, we have compared strains of *A. polynesiensis* that are naturally and artificially infected with *Wolbachia* and additional strains that are aposymbiotic (*Wolbachia* removed to generate an uninfected strain). Artificially infected strains were observed to have increased larval mortality and decreased adult longevity when compared with aposymbiotic strains. Naturally infected strains were observed to have decreased larval mortality, pupal mortality, increased adult longevity, and a larger adult size when compared with aposymbiotic strains. Artificially infected males that were 4 wk old were able to induce high rates of CI, similar to young males. We discuss the results in relation to the natural spread of *Wolbachia* and *Wolbachia*-based applied strategies to modify *A. polynesiensis* populations.

KEY WORDS *Wolbachia*, cytoplasmic incompatibility, *Aedes polynesiensis*, population replacement, incompatible insect technique

Maternally inherited *Wolbachia* bacteria are widespread in insects with estimated infection rates of up to 70% (Saridaki and Bourtzis 2010, Hilgenboecker et al. 2008, Werren et al. 2008). The evolutionary success of *Wolbachia* bacteria is attributed in part to its ability to manipulate the reproduction of its host, promoting the spread of infection (Werren et al. 2008). Cytoplasmic incompatibility (CI) is the most common and well-studied insect reproductive manipulation, and is defined by the disruption of karyogamy and failure of embryonic development (Werren 1997). CI prevents infected males from mating successfully with females that lack an infection or are infected with a different *Wolbachia* type. If a population contains infected and uninfected individuals, infected females can have a reproductive advantage, because they can mate successfully with both infected and uninfected males. CI can also be bidirectional, when two different *Wolbachia* infection types occur separately within individuals of the same host population (Werren et al. 2008). In the latter populations, a female can successfully reproduce only when mated with males infected with the same *Wolbachia* type.

The mosquito *Aedes polynesiensis* is the primary vector of *Wuchereria bancrofti*, the filarial nematode that causes lymphatic filariasis (LF) in the South Pacific. LF is a leading cause of disability in South Pacific regions, where 96% of the 1.7 million population are at risk of LF infection (Ottesen 2000). *A. polynesiensis* is known to harbor a natural clade-A *Wolbachia* infection, which is fixed in natural populations (Dean and Dobson 2004). In a previous study, an artificially infected *A. polynesiensis* strain was generated that displays high maternal inheritance and strong bidirectional CI when mated with a naturally infected strain (Brelsfoard et al. 2008). Prior reports have not examined for potential host fitness effects associated with natural and artificial infection types in *A. polynesiensis.*

In this study, we examined for an effect on host fitness associated with different *Wolbachia* infection types. To control for the host genetic background, infected strains are compared with aposymbiotic strains (i.e., treated with antibiotics to remove *Wolbachia* infections). Effects on immature survivorship, developmental time, adult longevity, fecundity, egg hatch, adult size, and sex ratio are examined. In addi-

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Table 1. *A. polynesiensis* strains used in experiments

Strain designation	*Wolbachia* type	Mitochondrial type	Notes	Reference(s)
CP	B-clade; *w*Con group	*Aedes riversi*	Artificially generated; introgressed	Brelsfoard et al. 2008
CPT	Uninfected	*Aedes riversi*	Artificially generated from CP; aposymbiotic	This paper
AP	A-clade; *w*Mel group	*Aedes polynesiensis*	Naturally occurring; field collected	Brelsfoard et al. 2008; Dean and Dobson 2004
APT	Uninfected	*Aedes polynesiensis*	Artificially generated from AP; aposymbiotic	Brelsfoard et al. 2008; Dean and Dobson 2004

tion, the influence of male aging on CI rates in incompatible crosses of laboratory strains is examined. Results are discussed within the context of *Wolbachia*-based applied strategies that could be used to modify natural populations of *A. polynesiensis* to aid in the control of lymphatic filariasis in the South Pacific.

Materials and Methods

Mosquito Stocks and Egg Hatch. The mosquito strains AP, APT, are as previously described (Dean and Dobson 2004). The CP strain is as previously described (Brelsfoard et al. 2008), and was generated by interspecific crosses with *Aedes riversi.* As a result, CP and CPT share the same mitochondrial type with that of *A. riversi.* Eggs were hatched in a 6 g/L suspension of liver powder (MP Biomedicals LLC, Solon, OH) in water, which was allowed to age ≥1 d to allow for bacterial growth and the deoxygenation of the water. All larvae for experiments were reared in 21 × 21 × 7.5-cm disposable plastic pans (Pactive, Lake Forest, IL) containing 500 ml of water and liver powder. Eggs and immature and adult mosquitoes were maintained at 29 ± 0.6°C, 73 ± 2% RH, and a photoperiod of 16:8 light:dark. Eggs were allowed to mature for 7–10 d on a damp oviposition substrate. Adults were provided a constant supply of 10% sucrose. Adults in all experiments were blood fed using mice for 20 min (Institutional Animal Care and Use Committee 00905A2005).

***Wolbachia* Infection Status.** Ten female and five male individuals of the aposymbiotic, tetracycline-treated strains were tested for infection using the general *Wolbachia* primers, 81 F/691R (Zhou et al. 1998). For the aposymbiotic strains that failed to amplify with general *Wolbachia* primers, 12s mitochondrial DNA primers, 12sA1/12sB1 were used to test DNA template quality (O'Neill et al. 1992). Infection status was confirmed using polymerase chain reaction (PCR). A- and B-type infections were confirmed using the *wsp* primers, 136 F/691R and 81 F/522R, respectively (Zhou et al. 1998) (Table 1). DNA was extracted by emulsifying whole adult mosquitoes in a 1.5-ml Eppendorf tube containing 100 μl of buffer containing 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, and 50 mM NaCl, at pH 8.2, using a Mini-beadbeater (Biospec Products, Bartlesville, OK). After homogenization, samples were incubated at 100°C for 5 min and centrifuged at 16,000 × *g* for 5 min. PCR was conducted, as described previously (Dobson et al. 2004).

Tetracycline Treatment. Adult mosquitoes were provided a 10% sucrose solution containing 1 mg/ml tetracycline, as described by Dobson and Rattanadechakul (2001). Tetracycline treatment was repeated for three generations. The fourth generation was tested for *Wolbachia* infection using PCR, as described above. To minimize the opportunity for a direct effect of tetracycline treatment, aposymbiotic strains were maintained in the absence of tetracycline for three generations before experiments.

Fitness Comparison of Infected and Aposymbiotic Strains. Four hours after submersion of eggs, first-instar larvae were transferred to disposable plastic pans using a 14.6-cm Pasteur Pipette (BD Biosciences, Franklin Lakes, NJ). Larvae were provided 200 mg of liver powder immediately after being transferred to pans. Two days after the initial feeding, the larvae were provided an additional 200 mg of liver powder. Larvae were monitored for pupation at 8-h intervals. Pupae were transferred to 13 × 100-mm culture tubes (Fisher, Pittsburgh, PA) containing 3 ml of distilled water. Pupae were monitored for adult emergence every 8 h. After adult emergence, adults were separated by sex and emergence time. Five newly emerged adult females and five adult males from the same 6-h emergence window were released into a cage. Females were blood fed weekly for 6 wk. Egg papers were removed from cages daily. Adult mortality was recorded daily. Eggs were matured 7 d and hatched. Hatch rates were scored by counting hatched and unhatched eggs using a dissection scope (Leica MZ75, Bannockburn, IL). Proportion of larvae and pupae surviving and egg hatch data were arcsine square root transformed before statistical analysis.

Female adult size was estimated by measuring wing length (Landry et al. 1988). To determine wing length, wings were photographed at ×10 magnification, using a dissection microscope (MZFLIII, Leica, Bannockburn, IL) with an attached camera (LH037290, Olympus, Center Valley, PA). Wing length was measured using the computer program Image J (Barboriak 2005). A picture of a 1-mm-stage micrometer at the same magnification was used to calibrate the number of pixels as a function of length in ImageJ. Measurements were between the axillary incision to the apical margin of each wing, excluding the fringe setae (Tun-Lin et al. 2000).

Male Age Effects on CI. Experimental crosses consisted of 10 CP males released into cages. Approximately 2 d postaddition of the CP males, 10 virgin AP females were added to the cages and were provided

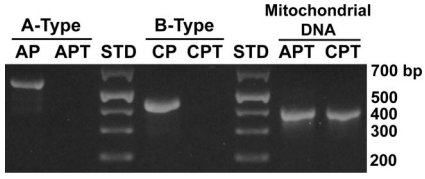

Fig. 1. PCR with *Wolbachia* type-specific primers show that the AP and CP strains of *A. polynesiensis* are infected with A- and B-type *Wolbachia*, respectively. PCR with general *Wolbachia* primers show that APT and CPT strains do not contain *Wolbachia* infections. PCR with 12s mitochondrial primers provide controls to demonstrate that a negative PCR result for *Wolbachia* is not a false negative because of a deficient DNA isolation. STD = molecular weight marker: 1-kb DNA Ladder Plus (Fermentas, Hanover, MD).

the opportunity to mate for approximately 5 d. Females were then blood fed. Two days postblood feeding, gravid females were removed from the cages and transferred to an individual container lined with oviposition paper (Anchor Paper, St. Paul, MN). Males remained in the original cage. After removing females, a new cohort of virgin females was added to the cage containing males at a 1:1, female:male ratio. This cycle was repeated once per week for 4 wk. Compatible crosses (e.g., CP × CP and AP × AP) were performed, as a control for CP and AP fertility. Five replicate cages for the incompatible crosses and two replicate cages for the compatible crosses were monitored simultaneously. A subset of females that did not feed in each gonotrophic cycle was checked for insemination by dissecting their spermathecae. All females checked were positive for sperm, suggesting the eggs that did not hatch were the result of CI.

Results

Generation of Aposymbiotic Strains. Absence of amplification product with *Wolbachia* primers demonstrates the CPT and APT strains to be uninfected with *Wolbachia* (Fig. 1). As a control for template quality, amplification with 12s primers demonstrated the presence of mitochondrial DNA in preparations of the aposymbiotic strains. PCR assays show AP and CP strains to be infected with A- and B-type *Wolbachia* infections, respectively (Fig. 1).

Fitness Comparison of Infected Strains With Aposymbiotic Strains. Larval mortality differed significantly by *Wolbachia* infection type (analysis of variance [ANOVA], $F_{3,12} = 32.5$, $P < 0.001$). *Wolbachia* infection type was also observed to affect pupal mortality (ANOVA, $F_{3,12} = 4.0$, $P < 0.04$). Posthoc multiple comparisons were used to further examine for effects of *Wolbachia* infection type on larval and pupal mortality of the different strains (Table 2). The infected CP strain had increased larval mortality compared with CPT, and uninfected APT had increased larval mortality compared with infected AP.

Differences in pupation and eclosion times were observed in comparisons of different sexes and *Wolbachia* infection type (multivariate analysis of variance [MANOVA], Wilks' $\Lambda = 0.78$, $F_{14,2030} = 19.3$, $P < 0.0001$). Multivariate effects of *Wolbachia* infection type (Wilks' $\Lambda = 0.92$, $F_{6,2030} = 14.4$, $P < 0.0001$) and sex (Wilks' $\Lambda = 0.14$, $F_{2,1015} = 71.5$, $P < 0.0001$) were significant, with differences in both pupation and eclosion times. The interaction term of sex and *Wolbachia* infection type was also significant (Wilks' $\Lambda = 0.97$, $F_{6,2030} = 5.1$, $P < 0.0001$). An ANOVA of the observed sex ratio (arcsine sqrt transformed) of *Wolbachia* infection status was significant ($F_{3,12} = 6.2$, $P = 0.009$). The AP strain was significantly different from the expected 1:1 sex ratio (χ^2, $P < 0.05$), with a male bias (Table 2). *Wolbachia* infection type was also associated with the percentage of females and males (arcsine sqrt transformed) that survived to adulthood (ANOVA; females, $F_{3,12} = 6.5$, $P < 0.008$; males, $F_{3,12} = 8.3$, $P < 0.003$) (Table 2).

Differences in wing length were observed between the strains with different *Wolbachia* infections and sex. An ANOVA on wing length was significant ($F_{7,394} = 595.4$, $P < 0.0001$), with significant multivariate effects of *Wolbachia* infection type ($F_{3,394} = 458.6$, $P < 0.001$) and sex ($F_{1,394} = 2957.3$, $P < 0.0001$). The interaction term of *Wolbachia* infection type and sex was not significant ($F_{3,394} = 0.84$, $P = 0.47$). As shown in Fig. 2, AP males and females had larger wing lengths in comparisons with APT, whereas CP and CPT wing lengths did not differ.

Table 2. Survivorship, sex ratio, and developmental time of *A. polynesiensis* strains

		CP	CPT	AP	APT
% larval mortality	–	41.5 ± 2.0; 4a	14.0 ± 5.0; 4b	10.0 ± 1.0; 4b	39.3 ± 1.6; 4a
% pupal mortality	–	5.0 ± 1.3; 4a	16.8 ± 10.1; 4ab	7.8 ± 1.0; 4a	24.7 ± 1.9; 4b
% survivorship through adult[a]	Female	24 ± 1.0; 4a	39 ± 5.0; 4b	31 ± 1.0; 4ab	22 ± 2.0; 4a
% survivorship through adult[a]	Male	35 ± 3.0; 4a	34 ± 7.0; 4ab	52 ± 2.0; 4b	23 ± 2.0; 4a
Sex ratio (percent female)	–	49.0 ± 3.1; 4a	54.5 ± 3.0; 4a	38.0 ± 1.3; 4b	49.0 ± 3.7; 4a
Time to pupation (h)	Female	158.4 ± 2.0; 113	155.7 ± 1.6; 155	157.6 ± 1.9; 124	154.5 ± 1.7; 89
	Male	149.4 ± 2.0; 109a	147.1 ± 1.6; 136a	142.3 ± 0.63; 207b	143.4 ± 1.1; 93b
Time to eclosion (h)	Female	207.3 ± 2.1; 107	205.1 ± 1.6; 155	207.0 ± 1.9; 124	201.4 ± 1.7; 89
	Male	197.2 ± 2.3; 113a	195.5 ± 1.7; 136a	187.8 ± 0.78; 207b	185.0 ± 1.4; 93b

Different superscripted letters represent significant differences between strains across each row using Tukey-Kramer honestly significant difference tests ($P < 0.05$). Values are expressed as mean ± SE; replicates/sample size.

[a] Data are based on an assumption of a 1:1 sex ratio when larvae were placed into rearing pans. Immature data are based on replicate larval pans.

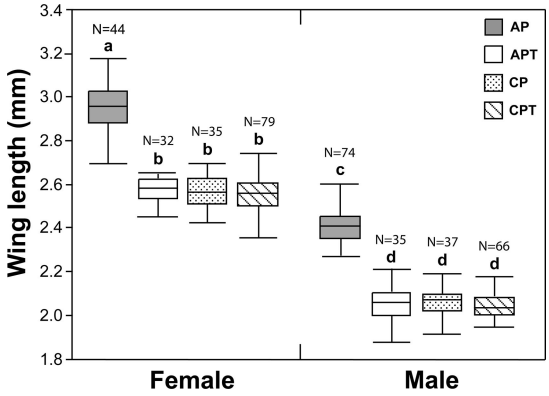

Fig. 2. Box plot of *A. polynesiensis* wing size from adults with a similar genetic background, but different *Wolbachia* infection types. The boxes represent the 25th and 75th percentiles separated by the median. The error bars represent a 95% confidence interval. Different letters indicate significant differences using Tukey-Kramer honestly significant difference tests ($P < 0.05$). Sample size is indicated above each of the boxes.

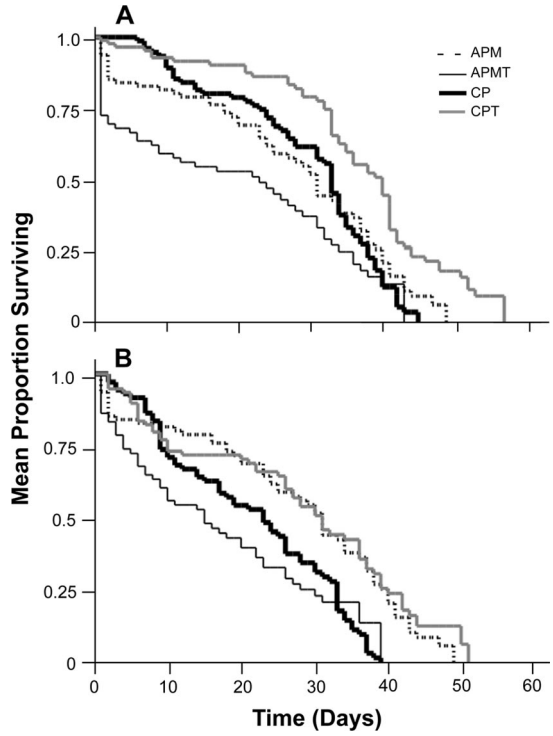

Fig. 3. The effect of *Wolbachia* infection type on *A. polynesiensis* female (A) and male (B) longevity.

In the conditions tested in this study, *A. polynesiensis* strains were observed to have different longevity. Comparisons of survivorship curves using the Kaplan-Meier and log rank tests demonstrated that the artificially infected CP strain was shorter lived compared with aposymbiotic CPT strain (females, $\chi^2 = 19.1$, df = 1, $P = 0.0001$; males, $\chi^2 = 15.5$, df = 1, $P < 0.0001$). The naturally infected AP strain was longer lived than aposymbiotic APT strain (females, $\chi^2 = 5.72$, df = 1, $P = 0.0168$; males, $\chi^2 = 16.7$, df = 1, $P < 0.0001$) (Fig. 3).

No effect of *Wolbachia* infection status on lifetime fecundity between strains was observed over multiple gonotrophic cycles (repeated measures ANOVA; Wilks' $\Lambda = 0.38$, $F_{12,24} = 0.88$, $P = 0.58$) (total eggs counted across all gonotrophic cycles: CP 4981, CPT 4072, AP 6523, and APT 1469) (Fig. 4A). No difference of egg hatch rates between strains was observed over multiple gonotrophic cycles (repeated measures ANOVA; Wilks' $\Lambda = 0.55$, $F_{12,19} = 18.8$, $P = 0.95$) (egg hatch across all gonotrophic cycles: CP 67%, CPT 80%, AP 83%, APT 89%) (Fig. 4B). No effect of *Wolbachia* infection type on the predicted number of L1 larvae was observed over multiple gonotrophic cycles (repeated measures ANOVA; Wilks' $\Lambda = 0.31$, $F_{12,16} = 0.74$, $P = 0.69$) (Fig. 4C).

Male Age Effects on CI. No loss of penetrance of CI was observed when CP males were allowed to mate with virgin AP females each week for 4 wk (χ^2, $P > 0.05$) (Fig. 5). However, there were a few hatching eggs resulting from the incompatible crosses (week 2, 40/3,214, hatched/unhatched eggs; week 3, 13/1,237, hatched/unhatched eggs) (Fig. 5). Hatching eggs were observed in control crosses up to the third week. By week 4, all adults had died. According to a repeated measures ANOVA, a significant difference in egg hatch rates over multiple gonotrophic cycles was observed for CP × CP ($F_{2,5} = 5.9$, $P = 0.048$), but not for the AP × AP control cross ($F_{2,3} = 2.3$, $P = 0.25$). Control crosses suggest males and females used in the experimental incompatible crosses were viable and fertile because both crosses produced hatching eggs throughout the duration of the experiment.

Discussion

Results suggest variable fitness effects associated with natural and artificial *Wolbachia* infections in *A. polynesiensis.* To minimize genetic differences, all strains used were inbred lines maintained in the laboratory for >30 generations, and uninfected lines were generated directly from infected lines. In this study, we focused primarily on comparisons of CP versus CPT and AP versus APT. Comparisons of CP and AP are complicated by mitochondrial variability. Specifically, the CP and CPT retain the maternally inherited mitochondria type from *A. riversi*, whereas the AP and APT have mitochondria from *A. polynesiensis* (described in Brelsfoard et al. 2008). For this reason, comparisons focus on strains with similar mitotypes. The differences observed in comparisons of the latter pairs appear to be the result of the *Wolbachia* type. This is similar to previous experiments in which differences in fecundity in superinfected and uninfected *Aedes albopictus* were observed with and without the presence of potential mitochondrial variation (Dobson et al. 2002, 2004). To examine the *Wolbachia* types within a similar mitotype background, future experiments could examine strains generated via microinjection.

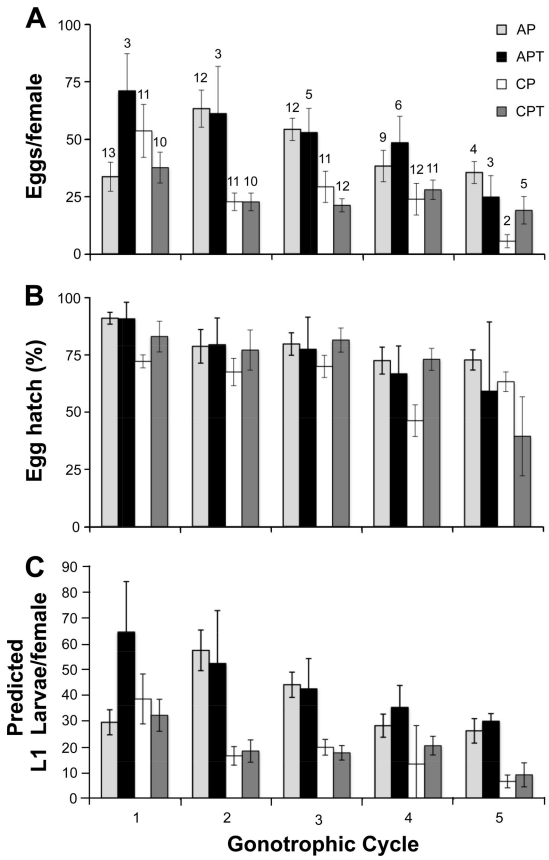

Fig. 4. Fecundity (A) and egg hatch (B) predicted L1 larvae (C) comparisons of *A. polynesiensis* strains that are similar in genotype, but differ in *Wolbachia* infection type. The predicted number of L1 larvae per female was calculated by multiplying fecundity by egg hatch per female for each cage replicate. Data are shown for five gonotrophic cycles. Data are displayed as mean ± SE. The number of cage replicates for each strain and gonotrophic cycle is represented above each column.

The natural *Wolbachia* infection in *A. polynesiensis* is phylogenetically divergent from the B-type infection introduced into the CP strain (Dean and Dobson 2004). Therefore, it was hypothesized that there may be a cost associated with the presence of the B-type *Wolbachia* infections and the *A. polynesiensis* host, resulting in lower fitness of the CP strain relative to the CPT strain. However, the fitness effects were varied in comparisons of CP and CPT. Data suggested a higher larval mortality, a decrease in female survivorship through adulthood, and a decrease in adult longevity in the CP strain when compared with CPT (Table 2 and Fig. 3). However, other parameters, such as pupal mortality, male survivorship through adult, sex ratio, time to pupation and time to eclosion, wing length, egg hatch, egg number, and predicted L1 larvae did not differ (Table 2; Figs. 2 and 4). In contrast, a higher overall fitness was observed with the *Wolbachia* infection in comparisons of the naturally infected AP strain with the aposymbiotic APT strain (Table 2; Figs. 2 and 3). In naturally infected *A. albopictus*, females lived longer, produced more eggs, and had higher hatch rates than their uninfected counterparts (Dobson et al. 2004). *Wolbachia*-infected strains of *Drosophila* and *Psocoptera* have also been shown to have a fitness benefit when compared with uninfected strains (Dean 2006, Dong et al. 2007). As observed in this study, naturally infected strains may be differentially adapted to the natural *Wolbachia* infection, whereas the recent introduction of the B-type infection is associated with several negative effects on host fitness when compared with its uninfected counterpart (e.g., reduced adult longevity, an increase in larval mortality, and female survivorship through adulthood).

The mechanism for the observed physiological difference is unknown. We hypothesize that one explanation could be that the artificial infection in CP may effect the host immune response, impacting survivorship and adult longevity. Negative correlations between immunity and other aspects of host fitness have been reported in the literature. In the terrestrial isopod *Armadillidium vulgare*, *Wolbachia* infection was associated with a decrease in hemocyte number, a more intense septicaemia in their hemolymph, and a decrease in survivorship (Braquart-Varnier et al. 2008).

Tetracycline treatment has been observed to influence life history traits, including fecundity and longevity (Ballard and Melvin 2007). We cannot exclude the possibility of a genetic bottleneck during the tetracycline treatment, resulting in genetic differentiation of the aposymbiotic lines. Furthermore, antibiotic treatment may have impacted another symbiont. However, we maintained tetracycline-treated lines for three generations after tetracycline treatment to allow recovery of environmental bacteria that may have been lost as a result of tetracycline treatments.

The CI phenotype is strongly expressed, regardless of males' age in the CP strain. Data show CP is a strong expresser of CI even at an age of 4 wk, suggesting that the modification of sperm from CP is stable over time (Fig. 5). Results are similar to previous studies wherein CI expression remained high in *Drosophila simulans* (Hoffmann et al. 1998), *Culex pipiens* (Duron et al. 2007), and *Aedes albopictus* (Kittayapong et al. 2002). The few hatching eggs are hypothesized to be the result of a loss of CI. However, none of these eggs that hatched eclosed to adults. Control crosses produced hatching eggs for three gonotrophic cycles, confirming CP males and AP females were fertile. The reduction of egg hatch in control crosses is hypothesized to be the result of sperm depletion in males.

Insect control strategies based upon *Wolbachia*-induced CI are of interest because of environmental and public health concerns of the use of insecticides and problems with insecticide resistance. Furthermore, recent work has demonstrated that *Wolbachia* can be transferred into a range of important disease vectors (Zabalou et al. 2004; Xi et al. 2005a, 2005b; Bourtzis 2008; McMeniman et al. 2009). *Wolbachia*-based strategies include use of the reproductive advantage af-

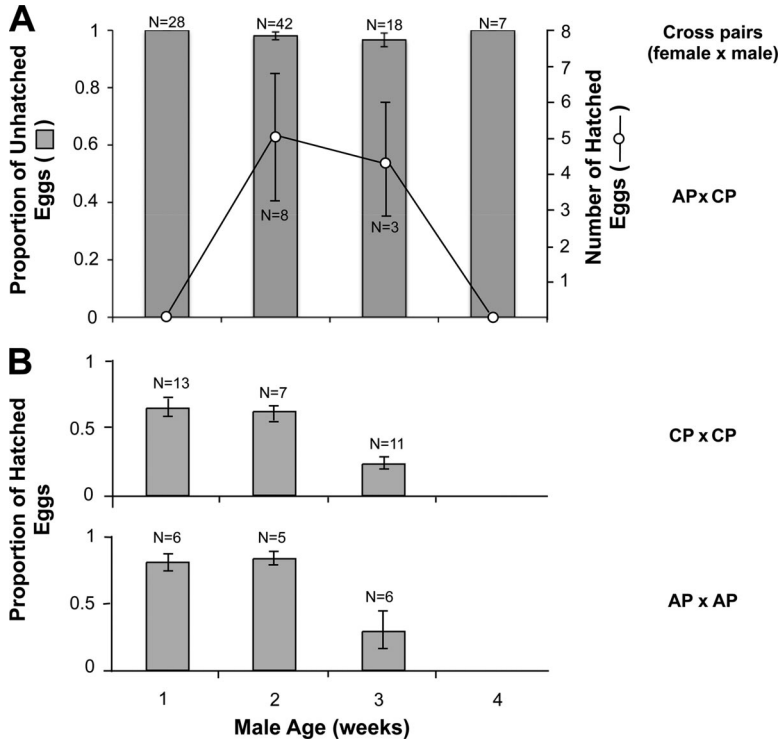

Fig. 5. The effect of CP male age on CI. (A) Mean ± SE proportion of unhatched eggs (left axis) from incompatible crosses with males of increasing age. The solid line represents the mean ± SE number of hatched eggs (right axis) from the females that produced hatching eggs. Below each data point are the number of females that produced hatching eggs. (B) Proportion of hatched eggs in control crosses for each week. Above each bar is the number of females that produced eggs for each week.

forded by *Wolbachia* to replace populations with individuals refractory to disease transmission, either genetically modified strains (Dobson 2003, Sinkins and Godfray 2004, Sinkins and Gould 2006, Bourtzis 2008, Brelsfoard and Dobson 2009) or by artificially induced infections that inhibit disease transmission (Kambris et al. 2009, 2010; Moreira et al. 2009; Bian et al. 2010). An alternative strategy is the incompatible insect technique (IIT), in which sterility is maintained by repeated releases of incompatible males analogous to the sterile insect technique (Laven 1967, Zabalou et al. 2004, Bourtzis 2008, Brelsfoard et al. 2008, Brelsfoard and Dobson 2009).

Understanding immature survivorship, fecundity, egg hatch, longevity, and CI rate is important to the development of *A. polynesiensis Wolbachia*-based IIT and replacement strategies. The observed high rates of CI in older males are relevant because high rates of CI/sterility would facilitate population suppression during an IIT strategy and would aid the spread of *Wolbachia* during a population replacement strategy. It is also critical for an IIT strategy that the infection type of the released strain does not become established.

A reduction of CP female fitness is a disadvantage for a replacement strategy. Based upon previously developed models, a reduction in fitness may slow the rate of *Wolbachia* infection invasion (Jansen et al. 2008, Rasgon 2008). In contrast, however, reduced female fitness could be an advantage for an IIT strategy. Specifically, if a female(s) were accidentally released, she would be less fit than the naturally occurring females, lowering the risk of unintentional population replacement. A third strategy would be to use an IIT strategy along with other forms of traditional vector control (e.g., pesticide applications and larval habitat removal) to suppress the population to low levels. After the suppression event, incompatible females could be released with males, giving those females an advantage because of the large numbers of compatible males to mate with and low levels of competition for resources and larval habitat with the wild-type *A. polynesiensis.* Given that *A. polynesiensis* populations are fixed for the A-type infection, sterility between matings of incompatible males and naturally occurring females should remain high.

The observed reduction in adult longevity of CP females could also be looked at as an advantage. *W. bancrofti* requires a time period from ingestion by the mosquito via a blood meal until it can be transmitted to a vertebrate host (i.e., the extrinsic incubation period). The extrinsic incubation period for *W. bancrofti* in *A. polynesiensis* is dependent upon ambient temperature, but ranges from ≈12 to 20 d (Lardeux and Cheffort 2001). Although in this study ≈50% of females were still alive at 20 d (Fig. 3), a reduction in CP

female longevity in more natural conditions could inhibit the completion of the extrinsic incubation period and limit the vector competence of the CP strain. Studies are needed to examine the vector competence of the CP strain and longevity in more natural conditions.

Results suggest that the *Wolbachia* and host interaction can be complicated, depending upon the *Wolbachia* type and host. It is understood that the fitness experiments were conducted in laboratory cages under optimal conditions, and that the *Wolbachia* infection dynamics in *A. polynesiensis* will need to be examined in more field-like conditions. The observed results provide data to be incorporated into population suppression and replacement models that will support the development of *Wolbachia*-based control strategies for the main vector of *W. bancrofti* in the South Pacific.

Acknowledgments

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