



**Stable Introduction of a Life-Shortening Wolbachia Infection into the Mosquito *Aedes aegypti***

Conor J. McMeniman, *et al.*

*Science* **323**, 141 (2009);

DOI: 10.1126/science.1165326

**The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of January 8, 2009 ):**

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/323/5910/141>

**Supporting Online Material** can be found at:

<http://www.sciencemag.org/cgi/content/full/323/5910/141/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/cgi/content/full/323/5910/141#related-content>

This article **cites 20 articles**, 6 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/323/5910/141#otherarticles>

This article appears in the following **subject collections**:

Microbiology

<http://www.sciencemag.org/cgi/collection/microbio>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

formed plaques on any of the seven serotypes of *L. monocytogenes* tested (table S4). Thus, the true overall host range of a phage may be much wider if it includes infection without plaque formation, which can be assessed only by gene transfer or phage DNA delivery. To distinguish whether the variation in transduction frequency was due to phage adsorption or host DNA restriction, we measured helper phage adsorption by *L. monocytogenes*. Serotypes 1/2a and 3b exhibited phage adsorption frequencies comparable to that of the staphylococcal control and were the strains most amenable to transduction, indicating there was a correlation between phage adsorption and transfer. This also implied that DNA restriction was not an important determinant of transduction frequency for these serotypes.

We then tested for the transfer of a virulence determinant unrelated to the SaPIs and used a detoxified derivative of phage  $\phi$ SLT (14) containing a tetracycline-resistance marker (*tetM*) inserted into the Panton-Valentine leukocidin (PVL) locus (15). Although  $\Phi$ SLT generates lysates with relatively low titers, its transfer to *L. monocytogenes* was demonstrated by selection for the *tetM* marker (table S5). In contrast to SaPI transfer, this transduction requires lysogenization because PVL is carried in the  $\phi$ SLT genome. Similarly to the four SaPI helper phages,  $\Phi$ SLT did not form plaques on *L. monocytogenes* (table S4), and converted strains did not liberate detectable plaque-forming phage particles upon mitomycin C induction. The potential for environmental  $\Phi$ SLT transduction to *L. monocytogenes* is disconcerting, considering that PVL has been implicated in staphylococcal diseases (15). All the staphylococcal phages we tested mediated genetic transfer to *L. monocytogenes*.

We predicted the occurrence of phage-mediated SaPI transfer in an environment in which *S. aureus* and *L. monocytogenes* occur together. These species are common causes of bovine mastitis (16–18), and we analyzed cow's milk as a medium for spontaneous prophage induction and SaPI transduction. When detoxified *S. aureus* derivatives of laboratory and clinical isolates were co-cultured with *L. monocytogenes* strains in raw milk, we detected spontaneous prophage induction and transfer of SaPII and SaPIbov1 to *L. monocytogenes* (SOM text and table S6).

Bovine mastitis costs the world's dairy industries billions in revenue each year; roughly 11% of total production is lost annually (19). Of the mastitis pathogens, *S. aureus* is of particular concern because of the low cure rate with antibiotic treatment and the rapid rise of antibiotic-resistant strains (20). A promising alternative to antibiotic treatment of *S. aureus* infections is phage therapy, which is currently the focus of several clinical trials for bovine mastitis (21–24). To determine whether this strategy could promote SaPI transfer, we co-cultured *S. aureus* strains carrying detoxified SaPIs with streptomycin-resistant derivatives of *L. monocytogenes* strains EGDe and SK1442 in raw milk, adding SaPI-less phage 80 $\alpha$  (propagated on RN450). As expected, high titers of

phage 80 $\alpha$  were efficient at clearing the *S. aureus* strains (table S7); however, the phage particles resulting from lysis also promoted the transfer of SaPII and SaPIbov1 to *L. monocytogenes* (Fig. 2).

Although superantigen-producing *L. monocytogenes* strains have not yet been reported, it is certainly true that environmental isolates of *S. aureus* carrying SaPIs are ubiquitous. Thus, the widespread use of anti-*aureus* phages in agriculture may accelerate the spread of staphylococcal toxins to *Listeria* or to any other bacteria to which the phages can adsorb (SOM text).

Phages form the framework for a living network of genetic information, interconnecting the microbes of the biosphere. This study hints that there is a pipeline of silent phage-mediated genetic information transfer among bacteria, indicating that phages are involved in far more numerous microbial connections than previously imagined.

#### References and Notes

1. A. D. Brabban, E. Hite, T. R. Callaway, *Foodborne Pathog. Dis.* **2**, 287 (2005).
2. P. Mazodier, J. Davies, *Annu. Rev. Genet.* **25**, 147 (1991).
3. B. F. Cheetham, M. E. Katz, *Mol. Microbiol.* **18**, 201 (1995).
4. J. A. Lindsay, A. Ruzin, H. F. Ross, N. Kurepina, R. P. Novick, *Mol. Microbiol.* **29**, 527 (1998).
5. A. Ruzin, J. Lindsay, R. P. Novick, *Mol. Microbiol.* **41**, 365 (2001).
6. A. Subedi, C. Ubeda, R. P. Adhikari, J. R. Penades, R. P. Novick, *Microbiology* **153**, 3235 (2007).
7. E. Maiques *et al.*, *J. Bacteriol.* **189**, 5608 (2007).
8. C. Ubeda *et al.*, *Mol. Microbiol.* **67**, 493 (2008).
9. K. Shimada, R. A. Weisberg, M. E. Gottesman, *J. Mol. Biol.* **63**, 483 (1972).

10. J. R. Fitzgerald *et al.*, *J. Bacteriol.* **183**, 63 (2001).
11. R. P. Novick, A. Subedi, *Chem. Immunol. Allergy* **93**, 42 (2007).
12. R. Novick, *Virology* **33**, 155 (1967).
13. T. Bae, T. Baba, K. Hiramatsu, O. Schneewind, *Mol. Microbiol.* **62**, 1035 (2006).
14. S. Narita *et al.*, *Gene* **268**, 195 (2001).
15. M. Labandeira-Rey *et al.*, *Science* **315**, 1130 (2007).
16. N. E. Jensen, F. M. Aarestrup, J. Jensen, H. C. Wegener, *Int. J. Food Microbiol.* **32**, 209 (1996).
17. J. Stephenson, *J. Am. Med. Assoc.* **298**, 1388 (2007).
18. J. L. Watts, *Vet. Microbiol.* **16**, 41 (1988).
19. H. Miles, W. Lesser, P. Sears, *J. Dairy Sci.* **75**, 596 (1992).
20. K. Kenny, R. F. Reiser, F. D. Bastida-Corcuera, N. L. Norcross, *J. Clin. Microbiol.* **31**, 706 (1993).
21. R. Stone, *Science* **298**, 728 (2002).
22. J. J. Gill *et al.*, *Antimicrob. Agents Chemother.* **50**, 2912 (2006).
23. S. O'Flaherty *et al.*, *Lett. Appl. Microbiol.* **41**, 482 (2005).
24. P. García, C. Madera, B. Martínez, A. Rodríguez, *Int. Dairy J.* **17**, 1232 (2007).
25. We thank D. Portnoy, R. Calendar, M. Bowden, and M. Wiedmann for gifts of strains and for helpful discussions. The expert technical assistance of A. Subedi is gratefully acknowledged. This work was supported by grants-in-aid from the Kimmel Center for Biology and Medicine, from New York University Medical Center, and by personal funds (R.P.N.).

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5910/139/DC1  
Materials and Methods  
SOM Text  
Figs. S1 to S4  
Tables S1 to S9  
References

18 August 2008; accepted 10 November 2008  
10.1126/science.1164783

## Stable Introduction of a Life-Shortening *Wolbachia* Infection into the Mosquito *Aedes aegypti*

Conor J. McMeniman,<sup>1</sup> Roxanna V. Lane,<sup>1</sup> Bodil N. Cass,<sup>1</sup> Amy W.C. Fong,<sup>1</sup> Manpreet Sidhu,<sup>1</sup> Yu-Feng Wang,<sup>1,2</sup> Scott L. O'Neill<sup>1\*</sup>

Most pathogens require a relatively long period of development in their mosquito vector before they can be transmitted to a new human host; hence, only older insects are of epidemiological importance. The successful transfer of a life-shortening strain of the inherited bacterial symbiont, *Wolbachia*, into the major mosquito vector of dengue, *Aedes aegypti*, halved adult life span under laboratory conditions. The association is stable, and the *Wolbachia* strain is maternally inherited at high frequency. It is capable of inducing complete cytoplasmic incompatibility, which should facilitate its invasion into natural field populations and its persistence over time. Our data suggest that targeting mosquito age with inherited *Wolbachia* infections may be a viable strategy to reduce the transmission of pathogens such as dengue viruses.

The control of dengue primarily targets *Aedes aegypti*, a domesticated mosquito that prefers to live in and around human habitation (1). With few exceptions, dengue management strategies have been complicated by the inability to completely eradicate *A. aegypti* from urban settings and the ineffective application of long-lasting vector-control programs (2). This has led

to a worldwide resurgence of dengue and has highlighted the urgent need for novel and sustainable disease-control strategies.

Most pathogens that are transmitted by mosquitoes share a common property; they have to undergo a significant period of development in their insect vector before they can be transmitted to a new host. After a female mosquito ingests an

infectious blood-meal, parasites or arboviruses, such as dengue, penetrate the mosquito's midgut and replicate in various tissues before infecting the salivary glands, where they are transmitted to a new host during subsequent blood-feeding. This time period from pathogen ingestion to potential infectivity is termed the extrinsic incubation period (EIP) and lasts ~2 weeks for both dengue (3, 4) and malaria (5).

A female mosquito must survive longer than its initial nonfeeding period (usually less than 2 days) plus the EIP to successfully contribute to pathogen transmission. Mosquito survival is therefore considered a critical component of a vector population's capacity for pathogen transmission (6). Interventions that aim to reduce the daily survivorship of adult mosquitoes, such as the spraying of residual insecticides in houses and insecticide-treated bed nets for malaria control, yield large reductions in pathogen transmission rates (7, 8), because of the sensitive relationship between mosquito survival and vectorial capacity (9, 10).

A strain of the obligate intracellular bacterium *Wolbachia pipientis*, *wMelPop*, has been described that reduces adult life span of its natural fruit fly host *Drosophila melanogaster* (11). It has been proposed that life-shortening *Wolbachia* strains, such as *wMelPop*, might be used to skew mosquito population age structure toward younger individuals, thereby reducing pathogen transmission without eradicating the mosquito population (12, 13). *Wolbachia* are maternally inherited bacteria that use mechanisms such as cytoplasmic incompatibility (CI), a type of embryonic lethality that results from crosses between infected males with uninfected females, to rapidly spread into insect populations (14). Evidence from other *Wolbachia*-insect associations, suggests that CI could allow *Wolbachia* strains, such as *wMelPop*, to invade mosquito populations even if they confer a fitness cost such as increased mortality (15). Current models predict that this strategy may result in significant reductions in pathogen transmission (16, 17). However, life-shortening *Wolbachia* strains do not occur in mosquitoes naturally.

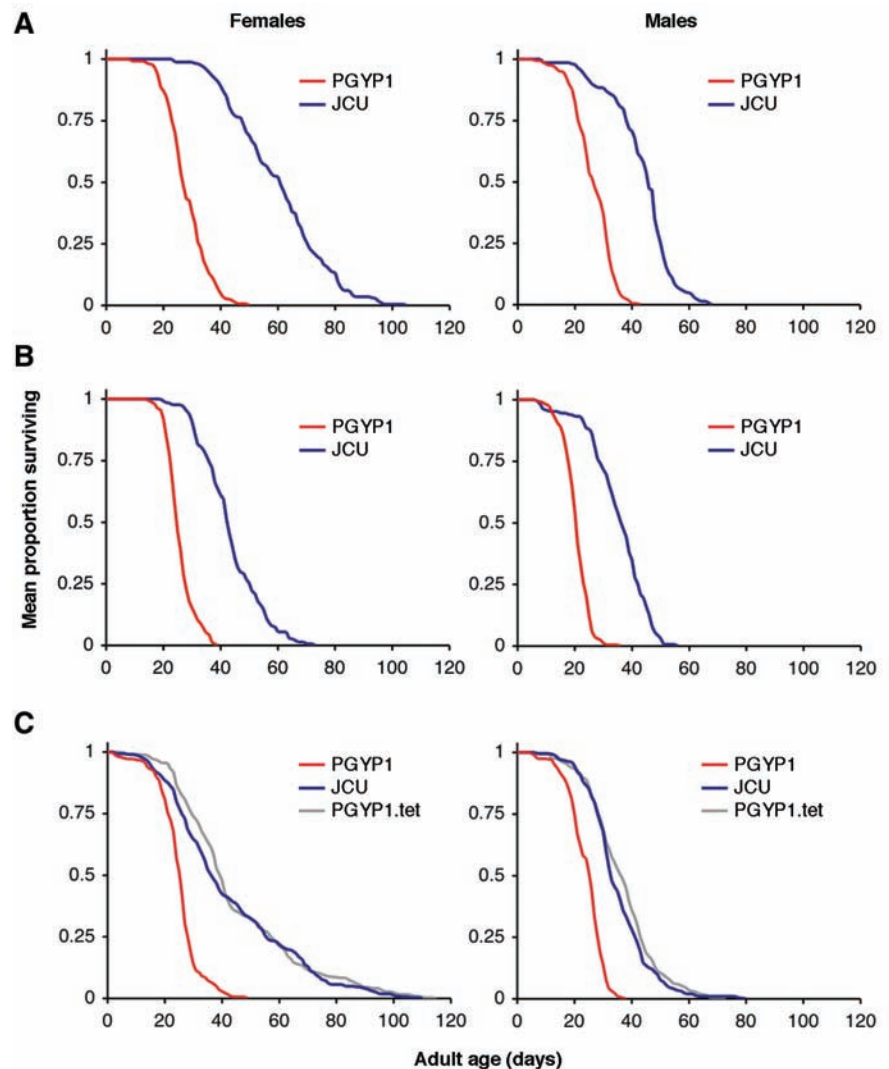
To facilitate the transfer of the life-shortening *Wolbachia* strain *wMelPop* that infects *D. melanogaster* (11) into the mosquito *A. aegypti*, we adapted the bacteria by continuous serial passage in mosquito cell culture for 3 years (18, 19). A consequence of this culturing was a reduction in growth rates and associated virulence when transferred back into *Drosophila* (19). We purified the mosquito cell line-adapted isolate of *wMelPop* and microinjected it into naturally uninfected *A. aegypti* embryos (JCU strain). Surviving adult females were isolated and blood-fed, and then, after egg laying, we assayed them for *Wolbachia*

infection using diagnostic PCR (18, 19). Eight independent isofemale lines carrying the *wMelPop* infection were generated. Six of these lines were lost from  $G_1$  to  $G_3$  (supporting online text), and the remaining two lines formed stable associations. These two lines, "PGYP1" and "PGYP2" were chosen for further characterization and, after a period of experimental selection, have remained persistently infected by *wMelPop* (100% infection frequency) until  $G_{33}$  and  $G_{30}$  respectively, when last assayed (fig. S1).

In *Drosophila* species, *wMelPop* shortens the life span of adult flies by up to 50% (11, 20). We performed several life-span assays in *A. aegypti* for a range of experimental conditions. As *wMelPop*-induced early death in *Drosophila* is temperature-

sensitive (11, 21), we compared the life span of the newly generated *wMelPop*-infected PGYP1 line to the naturally uninfected JCU strain at 25°C and 30°C (Fig. 1, A and B).

In contrast to *Drosophila*, where the life-shortening phenotype is weakly expressed at 25°C and strongly at 30°C, rapid mortality of PGYP1 mosquitoes ( $G_6$ ) relative to the uninfected parental JCU strain was observed at both temperatures. Under laboratory conditions at 25°C and 80% relative humidity (RH) (Fig. 1A), the median adult longevity for PGYP1 females of 27.0 days was significantly different from the JCU control of 61.0 days (log-rank statistic 11.67,  $P < 0.0001$ ). A similar trend was observed for males (Fig. 1A). At a higher temperature of 30°C and 80% RH



**Fig. 1.** Survival of *wMelPop*-infected PGYP1 *A. aegypti* (red lines) compared with the naturally uninfected JCU (blue lines) and tetracycline-cleared PGYP1.tet (gray lines) strains. Life-span assays were initially conducted at  $G_6$  post transinfection by the comparison of PGYP1 and JCU strains at 25°C (A) and 30°C (B). For each strain, six replicate groups of 50 mosquitoes (25 of each sex) were maintained in an incubator at their respective test temperature, and 80% RH. (C) Subsequently, after tetracycline treatment at  $G_{13}$  post transinfection, survival of PGYP1 was compared with that of PGYP1.tet and JCU strains in larger cages under insectary conditions. For this assay, three replicate 30 by 30 by 30 cm cages of 200 mosquitoes (100 of each sex) were maintained for each strain at  $25 \pm 1^\circ\text{C}$ , 70 to 90% RH, 12:12 hours light:dark. In all three experiments, mosquitoes were provided with 2% sucrose, and cages were checked daily for mortality.

<sup>1</sup>School of Integrative Biology, The University of Queensland, Brisbane 4072, Australia. <sup>2</sup>College of Life Sciences, Central China Normal University, Wuhan 430079, China.

\*To whom correspondence should be addressed. E-mail: scott.oneill@uq.edu.au

(Fig. 1B), the differential effect on median adult longevity was still apparent, although the life span of all the mosquitoes was reduced: females PGYP1, 25.0 days; JCU, 43.0 days (log-rank statistic 11.50,  $P < 0.0001$ ).

To examine the effect of the *wMelPop* infection under more biologically realistic conditions, we exposed a cohort of PGYP1 ( $G_9$ ) and JCU strains to a fluctuating temperature and humidity regime and provided female mosquitoes with daily access to a human blood meal (fig. S2). Under these conditions, the life span of PGYP1 females was reduced by more than half, relative to JCU females. Median longevity was significantly different between treatments: PGYP1, 21.0 days; JCU, 50.0 days (log rank statistic, 10.13,  $P < 0.0001$ ). A smaller difference in median survival times was observed for males from both strains (PGYP1, 9.0 days; JCU, 10.0 days), although overall PGYP1 males still died at a significantly faster rate than JCU males (log-rank statistic = 3.34,  $P = 0.0009$ ).

To exclude the possibility that observed reductions in life span resulted from genetic drift during the establishment of the PGYP1 strain, we generated an uninfected strain from PGYP1 (PGYP1.tet) by addition of the antibiotic tetracycline to the adult diet (22). After antibiotic curing of the *wMelPop* infection (18), no significant differences in the rate of mortality were observed between females or males of uninfected PGYP1.tet and JCU strains (e.g., females, log-rank statistic = 1.23,  $P = 0.2191$ ). Both females and males from the PGYP1 ( $G_{13}$ ) strain had a significantly reduced life span when compared with those from the PGYP1.tet strain (e.g., females, log-rank statistic = 13.70,  $P < 0.0001$ ), indicative of *wMelPop*-induced life-shortening (Fig. 1C). These results were confirmed by using identical assays with the PGYP2 ( $G_{15}$ ) strain as a biological replicate (fig. S3).

To test for CI, we made crosses between the PGYP1 and wild-type JCU and PGYP1.tet strains and measured egg hatch rates. Consistent with the induction of strong CI in *A. aegypti* (23), no eggs hatched from more than 2500 embryos obtained from crosses between male PGYP1 ( $G_9$ ) and uninfected JCU females (Fig. 2A). Similarly, only

2 eggs hatched from more than 1900 embryos obtained from crosses between male PGYP1 ( $G_{13}$ ) and the tetracycline-cleared PGYP1.tet females (Fig. 2B). In both assays, PGYP1 females were capable of rescuing CI, as indicated by the high egg hatch seen in PGYP1  $\times$  PGYP1 crosses.

In its natural *D. melanogaster* host, *wMelPop* infection induces CI that quickly diminishes with male age (24). This effect could slow the invasion of the strain into natural populations. Crosses between uninfected *A. aegypti* females and *wMelPop*-infected males up to 17 days old resulted in a complete absence of egg hatch from more than 9500 embryos (Table 1), which indicated that *wMelPop* infection induced CI that is insensitive to male age.

Overall, no significant differences in fecundity between PGYP1, PGYP1.tet, and JCU strains were observed at  $G_{13}$  after transinfection (fig. S4). An evaluation of CI and reproductive fitness in PGYP2 at  $G_{16}$  revealed that the *wMelPop* infection induced very strong CI but, unlike PGYP1, had a 19% fecundity cost when compared with its tetracycline-cleared counterpart (fig. S5). In *D. simulans*, fecundity costs associated with the *wMelPop* infection were initially high after transinfection, but subsequently attenuated, whereas the life-shortening effect remained stable (20). Further studies are required to determine whether this will be the case for PGYP2, and whether observed differences in reproductive fitness between PGYP1 and PGYP2 are related to *Wolbachia* or host genotypes.

High maternal inheritance of *Wolbachia* from infected females to their progeny is a key parameter for successful population invasion. The maternal transmission rate predicts stable prevalence of the

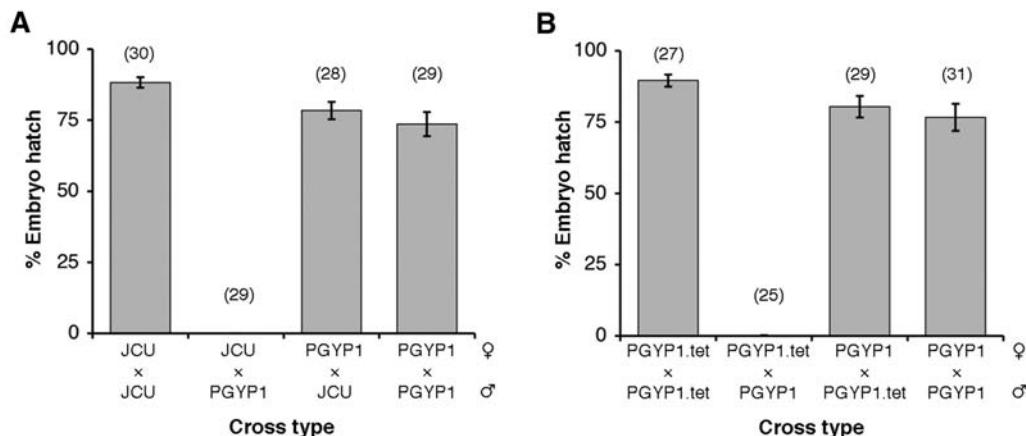
infection once it has invaded a population under the action of CI (14). To estimate maternal transmission rates of *wMelPop* over the life span of *A. aegypti*, we used the polymerase chain reaction to determine the proportion of *Wolbachia*-infected progeny derived from the first and third reproductive cycles of PGYP1 females ( $G_{17}$ ) mated with uninfected wild-type JCU males. Of the 515 larvae screened from 31 females (~17 larvae sampled per female) from the first reproductive cycle (females aged 9 days old),  $99.74 \pm 0.26\%$  were infected. This estimate of maternal inheritance was not significantly different from that obtained from the third reproductive cycle (females aged 23 days old) in which 527 larvae were screened from five cohorts of 20 females (~105 larvae sampled per cohort) and were  $99.45 \pm 0.37\%$  infected (Mann-Whitney rank sum test,  $P = 0.208$ ).

In other *Wolbachia*-insect associations, strong CI, high maternal inheritance, and low fecundity costs facilitate the initial spread and subsequent maintenance of the infection at high prevalence in populations (14). A comparison of results from this study, with simulations from recent theoretical models that examine the potential of life-shortening *Wolbachia* to modify mosquito population age structure (16, 17), suggest that *wMelPop* should be able to initiate a population invasion of *A. aegypti*. Given the relation between mosquito survival and vectorial capacity (9, 10), if the longevity of adult *A. aegypti* can be approximately halved under field conditions, as observed in our laboratory experiments, then the introduction of life-shortening *Wolbachia* strains would be predicted to reduce pathogen transmission and the incidence of human disease. Nevertheless, field cage trials conducted

**Table 1.** Effect of male age on CI. Percent embryo hatch  $\pm$  SEM and number of replicate crosses (in parentheses) are shown for incompatible crosses between uninfected PGYP1.tet females and aged PGYP1 males, as well as control crosses with aged PGYP1.tet males (minimum 2700 embryos total counted per cross).

Cross (female $\times$ male)	Percent embryo hatch for male age of		
	3 days	10 days	17 days
PGYP1.tet $\times$ PGYP1	0.00 $\pm$ 0.00 (32)	0.00 $\pm$ 0.00 (35)	0.00 $\pm$ 0.00 (35)
PGYP1.tet $\times$ PGYP1.tet	86.86 $\pm$ 3.42 (34)	83.67 $\pm$ 2.07 (33)	88.30 $\pm$ 3.10 (32)

**Fig. 2.** *Wolbachia*-mediated CI resulting from crosses of the *wMelPop*-infected PGYP1 *A. aegypti* strain with (A) the naturally uninfected JCU and (B) tetracycline-cleared PGYP1.tet strains. Female parents are listed first in each cross. Results are mean percent embryo hatch  $\pm$  SEM (minimum 1400 embryos total counted per cross), and number of replicates for each of the four cross types are shown in parentheses. Crosses were conducted as described (18).



under seminatural conditions are needed to gain a quantitative estimate of the potential efficacy of this strategy.

Vertically inherited parasites like *Wolbachia* are predicted to evolve toward reduced virulence over time (25). Unlike chemical insecticides, biological agents that induce mortality in late life, such as *wMelPop* or entomopathogenic fungi are expected to impose relatively weak selection pressures for the evolution of resistance (26). This is because the majority of individuals in the population are able to initiate several reproductive cycles before death, minimizing costs to reproductive output. Moreover, since the initial description of *wMelPop* in *D. melanogaster* over 10 years ago, no signs of resistance to life-shortening have emerged in laboratory stocks.

Hence, the ability of *Wolbachia* to spread into *A. aegypti* populations and persist over time may provide an inexpensive approach to dengue control, particularly in urban areas that are less amenable to conventional vector control strategies. Given the ability of *wMelPop* to induce life-shortening and CI in a range of insect hosts, this strategy may be broadly applicable to reduce pathogen transmission by other insect disease vectors of medical or agricultural importance.

#### References and Notes

- D. J. Gubler, in *Dengue and Dengue Hemorrhagic Fever*, D. J. Gubler and G. Kuno, Eds. (CAB International, New York, 1997), pp. 1–22.
- A. C. Morrison, E. Zielinski-Gutierrez, T. W. Scott, R. Rosenberg, *PLoS Med.* **5**, e68 (2008).
- J. F. Siler, M. W. Hall, A. P. Hitchens, *Philipp. J. Sci.* **29**, 1 (1926).
- D. M. Watts, D. S. Burke, B. A. Harrison, R. E. Whitmire, A. Nisalak, *Am. J. Trop. Med. Hyg.* **36**, 143 (1987).
- H. M. Gilles, D. A. Warrell, *Essential Malariaology* (Arnold, London, ed. 4, 2002).
- C. Dye, *Annu. Rev. Entomol.* **37**, 1 (1992).
- M. L. Mabaso, B. Sharp, C. Lengeler, *Trop. Med. Int. Health* **9**, 846 (2004).
- J. R. Schellenberg *et al.*, *Lancet* **357**, 1241 (2001).
- C. Garrett-Jones, *Nature* **204**, 1173 (1964).
- G. MacDonald, *The Epidemiology and Control of Malaria* (Oxford Univ. Press, London, 1957).
- K. T. Min, S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10792 (1997).
- P. E. Cook, C. J. McMeniman, S. L. O'Neill, *Adv. Exp. Med. Biol.* **627**, 126 (2008).
- S. P. Sinkins, S. L. O'Neill, in *Insect Transgenesis: Methods and Applications*, A. M. Handler and A. A. James, Eds. (CRC Press, London, 2000), pp. 271–287.
- A. A. Hoffmann, M. Turelli, in *Influenial Passengers: Inherited Microorganisms and Arthropod Reproduction*, S. L. O'Neill, A. A. Hoffmann, and J. H. Werren, Eds. (Oxford Univ. Press, Oxford, 1997), pp. 42–80.
- S. P. Sinkins, *Insect Biochem. Mol. Biol.* **34**, 723 (2004).
- J. S. Brownstein, E. Hett, S. L. O'Neill, *J. Invertebr. Pathol.* **84**, 24 (2003).
- J. L. Rasgon, L. M. Styer, T. W. Scott, *J. Med. Entomol.* **40**, 125 (2003).
- Materials and methods are available as supporting material on Science Online.
- C. J. McMeniman *et al.*, *Appl. Environ. Microbiol.*, **74**, 6963 (2008).
- E. A. McGraw, D. J. Merritt, J. N. Droller, S. L. O'Neill, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2918 (2002).
- K. T. Reynolds, L. J. Thomson, A. A. Hoffmann, *Genetics* **164**, 1027 (2003).
- S. L. Dobson, W. Rattanadechakul, *J. Med. Entomol.* **38**, 844 (2001).
- Z. Xi, C. C. Khoo, S. L. Dobson, *Science* **310**, 326 (2005).
- K. T. Reynolds, A. A. Hoffmann, *Genet. Res.* **80**, 79 (2002).
- M. Lipsitch, E. R. Moxon, *Trends Microbiol.* **5**, 31 (1997).
- M. B. Thomas, A. F. Read, *Nat. Rev. Microbiol.* **5**, 377 (2007).
- We thank S. Dobson, Z. Xi, and Y. Fu for their advice on microinjection of mosquito eggs; C. Williams and S. Ritchie for supplying the JCU mosquito strain; and E. McGraw and members of the O'Neill laboratory for helpful comments. This research was supported by a grant from the Foundation for the NIH through the Grand Challenges in Global Health Initiative of the Bill and Melinda Gates Foundation.

#### Supporting Online Material

[www.sciencemag.org/cgi/content/full/323/5910/141/DC1](http://www.sciencemag.org/cgi/content/full/323/5910/141/DC1)

Materials and Methods

SOM Text

Figs. S1 to S5

References

10.1126/science.1165326