time of scrapie exceeded the natural life span of these mice.

All clinically unaffected *tga20* indicator mice were killed at ≥200 dpi. Histopathological and immunoblot analyses confirmed scrapie in all clinically diagnosed *tga20* mice and excluded it from all others (Fig. 2, A to C, and fig. S5C). Phosphotungstate-mediated concentration of PrP^{Sc} from 1000 μg of protein did not reveal PrP^{Sc} in brains of clinically healthy urine-inoculated *tga20* mice (fig. S5B). Thus, two pathogenetically distinct chronic inflammatory conditions of the kidney, in concert with prion infection, result in prionuria well before the onset of clinically overt prion disease.

Whereas RIPLTα and NZBW mice suffer from combined interstitial lymphofollicular inflammation and glomerulonephritis, MFG-$E8^{-/-}$, NZW, and NZB mice display glomerulonephritis but lack lymphofollicular foci (figs. S1 and S2). Hence, prionuria necessitates intrarenal organized inflammatory foci (*6*) and is not elicited by isolated glomerulonephritis (Fisher's exact test, $P = 0.031$). Urinary proteins from presymptomatic and terminal RIPLTα mice induced similar attack rates, suggesting similar urinary prion infectivity titers in presymptomatic and scrapie-sick mice. The consistent lack of infectivity in urine from noninoculated mice and prion-sick wild-type mice makes it unlikely that infectivity found in urine of nephritic mice represents a contaminant.

Scrapie-infected hamsters and Creutzfeldt-Jakob disease (CJD) patients were reported to excrete urinary PrP^{Sc} ($UPrP^{Sc}$) (*11*). However, these findings were not reproduced (*12*) and were deemed artifactual (*13*, *14*). We attempted to detect $UPrP^{Sc}$ in presymptomatic and terminally sick RIPLTα, MFG-$E8^{-/-}$, *tga20*, C57BL/6, and 129Sv × C57BL/6 mice, as well as in presymptomatic NZW, NZB, and NZBW mice. Overnight dialysis did not affect the quantitative recovery of spiked PrP^{Sc} from urine (fig. S4, A and B); the detection threshold was ≥100 ng of terminal brain homogenate per milliliter of urine (Fig. 3, B and D), equivalent to 10^3 median infectious dose (ID_{50}) units/ml. Under these conditions, we failed to reveal any $UPrP^{Sc}$, even in prionuric mice (Fig. 3 A, C, and D). These negative findings are not unexpected, because urinary infectivity titers were typically ≤1 ID_{50} units per 2 ml of pooled urine (Fig. 1), which is below the detectability of PrP^{Sc} (Fig. 3B).

We then tested whether inflammation of nonexcretory organs leads to prionuria. We administered prions to AlbLTαβ mice, which lack nephritis but develop hepatitis (*6*). Urine from AlbLTαβ and appropriate wild-type control mice (four pools of $n = 4$ mice, 120 dpi) lacked prion infectivity and $UPrP^{Sc}$ (Figs. 1 and 3D; fig. S5, B and C). Thus, extrarenal inflammation, though enabling prion accumulation at the site of inflammation, does not induce prionuria.

Because PrP^{C} is necessary for prion replication (*4*), its expression may be rate-limiting for urinary prion excretion. We assessed prionuria in *tga20* mice, whose renal PrP^{C} content is six to eight times that of wild-type mice (fig. S3F). Pooled urinary proteins (600 μg each) from six terminally scrapie-sick *tga20* mice were inoculated i.c. into *tga20* mice (Fig. 1). None of the recipient *tga20* mice developed scrapie. Upon necropsy (>200 dpi), no scrapie histopathology was detected (fig. S5C). Thus, PrP^{C} overexpression does not induce prionuria. The PrP^{C} content of RIPLTα, NZBW, and MFG-$E8^{-/-}$ kidneys was similar to those of wild-type controls (fig. S3, G and H). RIPLTα and NZBW kidneys contain FDC-$M1^{+}$ cells with high, focal levels of PrP^{C} (*6*), which may facilitate local prion replication (*5*). Inoculation of urinary protein from noninfected mice did not elicit any abnormality in *tga20* mice (fig. S5C).

How do prions enter the urine? Upon extrarenal replication, blood-borne prions may be excreted by a defective filtration apparatus. Alternatively, prions may be produced locally and excreted during leukocyturia. Although prionemia occurs in many paradigms of peripheral prion pathogenesis (*15*, *16*), the latter hypothesis appears more likely, because prionuria was invariably associated with local prion replication within kidneys.

Urine from one CJD patient was reported to elicit prion disease in mice (*17*, *18*), but not in primates (*19*). Perhaps unrecognized nephritic conditions may underlie these discrepant observations. Inflammation-associated prionuria may also contribute to horizontal transmission among sheep, deer, and elk, whose high efficiency of lateral transmission is not understood.

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Supporting Online Material

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Wolbachia Establishment and Invasion in an *Aedes aegypti* Laboratory Population

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A proposed strategy to aid in controlling the growing burden of vector-borne disease is population replacement, in which a natural vector population is replaced by a population with a reduced capacity for disease transmission. An important component of such a strategy is the drive system, which serves to spread a desired genotype into the targeted field population. Endosymbiotic *Wolbachia* bacteria are potential transgene drivers, but infections do not naturally occur in some important mosquito vectors, notably *Aedes aegypti*. In this work, stable infections of *w*AlbB *Wolbachia* were established in *A. aegypti* and caused high rates of cytoplasmic incompatibility (that is, elimination of egg hatch). Laboratory cage tests demonstrated the ability of *w*AlbB to spread into an *A. aegypti* population after seeding of an uninfected population with infected females, reaching infection fixation within seven generations.

Aedes aegypti (yellow fever mosquito) is the principle vector of dengue viruses throughout the tropical world. Without a registered vaccine or other prophylactic measures, efforts to reduce cases of dengue fever and dengue hemorrhagic fever are limited to vector con-

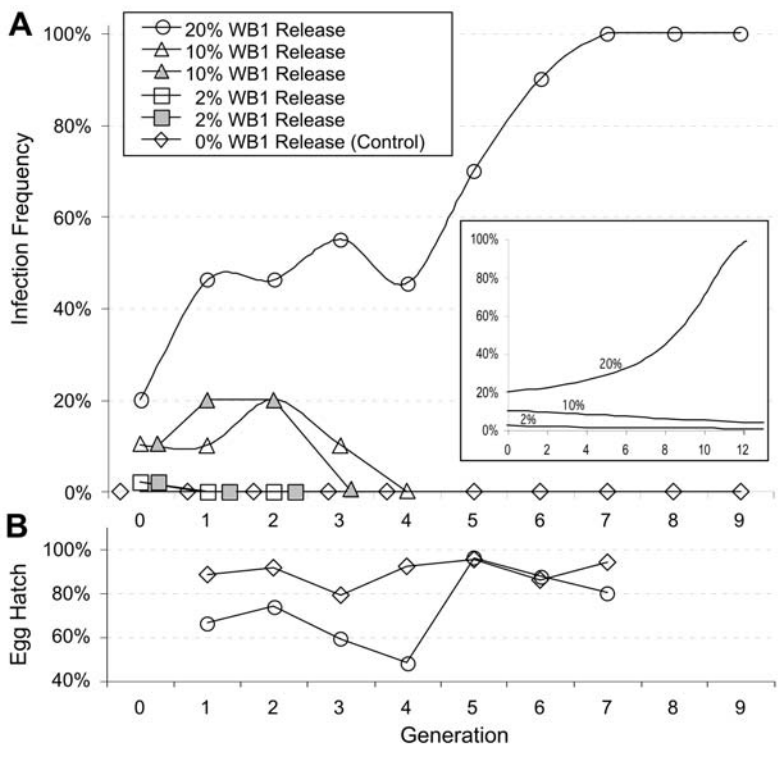

Fig. 1. (**Left**) Oocytes of uninfected Waco and *w*AlbB-infected WB1 stained with a *Wolbachia*-specific FISH probe. FISH staining methods were as previously described (*7*). **Fig. 2.** (**Right**) *Wolbachia* infection frequency (**A**) and egg hatch rates (**B**) after a single release of WB1 females into Waco populations. (Inset) Graph displays model predictions (*8*) of *Wolbachia* infection dynamics assuming complete CI, 100% maternal transmission, and a 15% fecundity cost associated with *Wolbachia* infection.

trol. Unfortunately, traditional mosquito control measures are not succeeding. With an estimated 100 million human cases of dengue fever every year (*1*), substantial effort is being devoted to the development of new strategies to complement existing vector control methods. One such method is population replacement, in which natural *A. aegypti* populations would be replaced with modified populations that are refractory to dengue transmission. Recent advances toward the production of refractory *A. aegypti* strains include the development of methods for stable genetic transformation of *A. aegypti*, RNA interference technology, and genomic sequencing efforts (*2*–*4*). In contrast, there has been relatively little progress toward the development of a vehicle that will serve to drive the refractory genotype into the field population.

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A gene-drive vehicle is an important component of vector population replacement strategies, providing a mechanism for the autonomous spread of desired transgenes into the targeted population. Compared with strategies that rely on inundative releases and Mendelian inheritance, gene-drive strategies would require relatively small "seedings" of transgenic individuals into a field population. Perhaps more important than increased cost efficacy, gene-drive strategies can facilitate population replacement with transgenic individuals that have a lower fitness relative to the natural population.

Cytoplasmic incompatibility (CI), induced by naturally occurring intracellular *Wolbachia* bacteria, has attracted scientific attention as a potential vehicle for gene drive. CI occurs when a *Wolbachia*-infected male mates with an uninfected female, resulting in karyogamy failure and early developmental arrest of the mosquito embryo (*5*). Although CI and other forms of reproductive parasitism have made *Wolbachia* an evolutionary success, with an estimate that infections occur in ~20% of insect species (*6*), *Wolbachia* infections do not naturally occur in *A. aegypti*, raising the question of whether *A. aegypti* can support a *Wolbachia* infection. Although *Wolbachia* infections have been introduced into *Drosophila* and other insects (*7*), *Wolbachia* infection may not cause CI in *A. aegypti* and may not invade an uninfected population. Key parameters in *Wolbachia* infection dynamics include the intensity of CI (number of hatching eggs resulting from an incompatible cross), the maternal inheritance rates (number of uninfected progeny produced by an infected female), and mosquito fitness

Table 1. CI pattern resulting from crosses of the naturally uninfected Waco and the *w*AlbB-transfected WB1 *A. aegypti* strains. Percent egg hatch ± standard deviation and number of cross replicates are shown for each of the four cross types. Crosses were conducted as previously described (*7*).

	Male Waco	Male WB1
Female Waco	92.5 ± 3.7% (n = 9)	0.0 ± 0.0% (n = 15)
Female WB1	69.1 ± 11.7% (n = 9)	50.6 ± 12.9% (n = 15)

costs associated with the infection (*8*). These parameters also determine the infection frequency after a population replacement event, an important consideration because the goal of population replacement is for the entire mosquito population to carry the desired genotype.

A. aegypti were infected by embryonic microinjection with the *w*AlbB *Wolbachia* infection from *A. albopictus* (*7*). In brief, cytoplasm from *A. albopictus* eggs (Hou strain superinfected with *w*AlbA and *w*AlbB) was injected into *A. aegypti* eggs (Waco strain). *Wolbachia* were detected by polymerase chain reaction (PCR) as previously described (*9*) in each of the five females (G_0) that survived from injection to adult. Only three females successfully produced progeny (G_1). PCR tests of G_1 individuals demonstrated the offspring of one female to be uninfected. The lines established from the remaining two females were only infected with the *w*AlbB, and one line (designated WB1) was selected for additional tests. In previous work on *A. albopictus*, *w*AlbB infections were obtained and not *w*AlbA (*7*). This may reflect the lower infection rate of *w*AlbA relative to *w*AlbB (*10*).

PCR assays of WB1 individuals in subsequent generations ($\leq G_{12}$) consistently identified *Wolbachia* infection. As a specific test of the maternal inheritance rate, progeny were collected from isolated WB1 females (G_{12}). After PCR confirmation of *Wolbachia* infection in 10 G_{12} females, the progeny (10 daughters and 10 sons for each G_{12} female) were assayed with PCR. All of the G_{13} progeny ($n = 200$) were infected by *Wolbachia* (95% binomial confidence interval between 0.9851 and 1.0). Fluorescence in situ hybridization (FISH) confirms high *Wolbachia* infection rates in WB1 oocytes (Fig. 1). The infection appears highest in the anterior, posterior, and cortical regions of oocytes, similar to the pattern observed in naturally infected *A. albopictus* (*7*).

Crosses were conducted to determine whether CI occurs as a result of the *Wolbachia* infection in the WB1 strain. The design of the cross experiment was as previously described (*9*). As shown in Table 1, the pattern of egg hatch resulting from crosses is consistent with strong CI, similar to that observed in *A. albopictus*, from which the *w*AlbB infection was derived (*7*). No egg hatch resulted from >3800 eggs examined from crosses of uninfected Waco females and infected WB1 males.

Among the compatible crosses, egg hatches resulting from WB1 crosses [51% and 69% (Table 1)] were significantly lower [Kruskal-Wallis, df (degrees of freedom) = 1, $P < 0.01$] than the egg hatch observed in compatible crosses of Waco individuals (92%). Because the progeny of the WB1 G_0 female were sibling-mated during production of the WB1 isofemale line, the low egg hatch may reflect an inbreeding effect. Therefore, virgin WB1 females (G_3) were mated with uninfected Waco males. After the repeat of this introgression for six generations, the egg hatch increased to an average of 89% (G_9).

Strong CI and high maternal transmission rates suggest that *w*AlbB infection will invade an uninfected population. To test this prediction, we released WB1 females at different ratios into replicate Waco laboratory populations (Fig. 2A). The population cage experimental design was as previously described (*9*). In the 20% initial release cage, the *w*AlbB infection frequency was observed to increase to 100% infection frequency within seven generations. Additional sampling in the eighth and ninth generations demonstrated that the infection frequency remained fixed at 100% (Fig. 2A). Consistent with model predictions (*11*), a transient drop in egg hatch was observed during the cytotype replacement (circa generation four) (Fig. 2B). The latter is expected owing to the frequent occurrence of CI crosses; however, once the infection becomes fixed within the population, CI crosses no longer occur, and the egg hatch rates recover.

In cages established with an initial infection frequency of ≤10%, the infection was detected for up to four generations before its disappearance from the population (Fig. 2A). Infection could not be detected in populations in cages initially infected at a rate of 2% release of WB1 females. Hence, the loss of *Wolbachia* infection from a population is predicted if the initial infection frequency is below a required threshold determined by CI level, fidelity of maternal transmission, and fitness costs associated with *Wolbachia* infection (*8*). Complete CI and no evidence of maternal transmission failure were observed in this study. If no fitness costs were associated with the infection, we would predict *Wolbachia* invasion in all cages in which WB1 females were released. However, we estimated a threshold infection frequency of ~20%, suggesting there is a substantial fitness cost associated with *w*AlbB infection in *A. aegypti*. By using a previously developed model (*8*), we predicted an approximate 15% fecundity cost to be associated with the *w*AlbB infection on the basis of the observed population replacement events (Fig. 2A inset).

An analysis of fecundity costs associated with the *w*AlbB infection neither revealed any differences in egg number ($P > 0.3$, *t* test) in comparisons of WB1 females (57.8 ± 17.6 eggs per female, $n = 12$) with Waco females (52.4 ± 8.5 eggs per female, $n = 14$) nor revealed any differences in egg hatch rate between Waco and WB1 strains (χ^2 test, df = 1, $P > 0.05$). Thus, future experiments need to investigate additional types of fitness costs (e.g., reduced mating competitiveness or immature survivorship). Furthermore, the laboratory population cage tests conducted here represent an initial proof of principle, but future field cage tests will provide a more accurate prediction of infection dynamics in natural populations (*12*).

Nevertheless, important obstacles currently prevent implementation of genetic modification strategies using *Wolbachia* as a vehicle to drive transgenes into *A. aegypti* populations. Notably absent is a method for linking transgenes to *Wolbachia*. However, the ability to artificially infect *A. aegypti* with *w*AlbB into a major disease vector is an important step toward proposed population replacement strategies. The observed high CI rates, high maternal inheritance, and ability of *w*AlbB to invade an uninfected laboratory population to infection fixation represent desired characteristics for population replacement strategies. Additional study is needed to define the fitness of *w*AlbB-infected *A. aegypti* relative to uninfected individuals, and population replacement experiments need to be performed under conditions that resemble the natural environment. Transfection success with *A. aegypti* suggests that the transfection approach may be successful with other medically important disease vectors, including *Anopheles* species.

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