A *Wolbachia* Symbiont in *Aedes aegypti* Disrupts Mosquito Egg Development to a Greater Extent When Mosquitoes Feed on Nonhuman Versus Human Blood

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ABSTRACT A vertebrate bloodmeal is required by female mosquitoes of most species to obtain nutrients for egg maturation. The yellowfever mosquito, *Aedes aegypti* (L.), feeds predominantly on humans, despite having the capacity to use blood from other hosts for this process. Here, we report that female *Ae. aegypti* infected with a virulent strain of the intracellular bacterium *Wolbachia pipientis* (*w*MelPop) from *Drosophila melanogaster* (Meigen) have a reduced ability to use blood for egg development. Blood feeding by *w*MelPop-infected females on mouse, guinea pig, or chicken hosts resulted in a near complete abolishment of reproductive output associated with both a decline in the numbers of eggs oviposited as well as the hatching rate of successfully laid eggs. In contrast, the reproductive output of *w*MelPop-infected females fed human blood was only mildly affected in comparison to individuals fed animal blood sources. Blood-feeding assays over two reproductive cycles definitively illustrated a nutritional interaction between host blood source and egg development in *w*MelPop-infected *Ae. aegypti*. Removal of *Wolbachia* from mosquitoes using antibiotic treatment rescued egg development on all blood sources. Further investigation of this phenotype may provide new insights into the nutritional basis of mosquito anthropophily.

KEY WORDS *Wolbachia*, reproduction, mosquito, anthropophily

Female mosquitoes of most species must ingest a vertebrate bloodmeal to obtain nutrients required for egg development (Attardo et al. 2005). The preference for human blood by a number of mosquito species (anthropophily) aids in the transmission of human pathogens such as dengue and malaria (Besansky et al. 2004). *Aedes aegypti* (L.), the principal vector of dengue, is a highly anthropophilic mosquito that lives in and around human habitation. Previous studies have determined that *Ae. aegypti* feeds predominantly on humans in the field (Scott et al. 1993b, Ponlawat and Harrington 2005), often taking bloodmeals multiple times within a single gonotrophic cycle (Scott et al. 1993a, 2000).

The nutritional components of human blood that differentiate it from other blood sources and subsequently contribute to the preference of *Ae. aegypti* for humans are not fully understood (Harrington et al. 2001). Blood feeding on humans has been shown to increase energy reserves, survival rate, and lifetime fecundity of *Ae. aegypti*, compared with other vertebrate hosts (Scott et al. 1997, Costero et al. 1998, Harrington et al. 2001, Braks et al. 2006). A greater understanding of the nutritional basis of anthropophily may provide insight into important factors associated with host preference for humans, and potentially assist in the rational design of strategies that interfere with this behavior.

A novel method for the control of dengue that has been proposed involves the introduction of strains of the obligate intracellular bacterium *Wolbachia pipientis* into *Ae. aegypti* populations to modify mosquito population age structure (Sinkins and O'Neill 2000, Cook et al. 2008) as well as directly modulate vector competence (Moreira et al. 2009). *Wolbachia* are maternally transmitted endosymbionts that naturally infect a wide diversity of invertebrates (Hilgenboecker et al. 2008), including many medically important mosquito species (Sinkins 2004). Previously, we described the stable introduction of a virulent *Wolbachia* strain (*w*MelPop) from *Drosophila melanogaster* (Meigen) into *Ae. aegypti* (McMeniman et al. 2009), a species not naturally infected with *Wolbachia.*

One major physiological difference between *D. melanogaster* and *Ae. aegypti* is the requirement for the latter species to blood feed to obtain nutrients for oogenesis. In *Drosophila*, *Wolbachia* bacteria are thought to be dependent on their hosts for a range of metabolites including amino acids, iron, and lipids

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(Wu et al. 2004, Brownlie and O'Neill 2006, Brownlie et al. 2009), which are also essential for mosquito egg development (Ziegler and Ibrahim 2001, Attardo et al. 2006, Zhou et al. 2007). Despite this potential conflict, *w*MelPop-infected *Ae. aegypti* are able to use blood from humans, their preferred host, for egg development with minimal costs to reproductive fitness for young females (McMeniman et al. 2009).

In this study, we examined the effect of *w*MelPop infection on the ability of this mosquito to use less preferred nonhuman blood sources for egg development. Here, we report that female *Ae. aegypti* infected with *w*MelPop have a drastically reduced ability to use blood from nonhuman hosts for this process. We used blood from a wide variety of animal hosts, and antibiotic clearance of *Wolbachia* to demonstrate an interaction between blood source and *w*MelPop infection that profoundly influences egg development in *Ae. aegypti*.

Materials and Methods

Mosquito Strains and Rearing. The following *Ae. aegypti* strains were used in this study: 1) the *w*MelPop-infected strains PGYP1 and PGYP2; 2) their uninfected tetracycline-cured counterparts PGYP1.tet and PGYP2.tet; and 3) the uninfected wild-type strain JCU, from which PGYP1 and PGYP2 were originally derived upon transinfection with *w*MelPop (McMeniman et al. 2009). Mosquitoes were maintained at 25°C, 75–85% RH, and a photoperiod of 12:12 (L:D) h. Larvae were reared at set density (150 larvae per 3 liters of distilled water in 30- by 40- by 8-cm plastic trays) and fed 150 mg of fish food (TetraMin Tropical Tablets, Tetra, Melle, Germany) per pan each day until pupation. Adults were kept in screened 30- by 30- by 30-cm cages with ad libitum access to 10% sucrose solution and water. Five day old females were provided with a human bloodmeal (University of Queensland, Human Ethics Approval 2007001379) for egg production. For routine colony maintenance, eggs from the PGYP1 and PGYP2 strains were hatched 5–7 d postoviposition (i.e., without prolonged desiccation) to initiate the next generation.

Tetracycline Treatment. The tetracycline-cured PGYP1.tet and PGYP2.tet strains of *Ae. aegypti* were generated at G_8 and G_{11} posttransinfection by the addition of tetracycline (1 mg/ml) to the adult diet and recolonized with resident gut microflora from wild-type larvae as described previously (McMeniman et al. 2009). Proceeding tetracycline treatment, strains were allowed to recover for at least two generations before being used in experiments.

Blood-Feeding Trials: Laboratory Animals and Membrane Feeding. In blood-feeding assays that used live hosts, premated 5-d-old adult females were bloodfed to repletion using a human forearm (University of Queensland, Human Ethics Approval 2007001379); outbred Quackenbush mice anesthetized with a mixture of ketamine and xylazine (UQ Animal Ethics Approval SIB/549/07/NIH); or guinea pigs anesthetized with Nembutal (Queensland Institute of Medical Research, Animal Ethics Protocol P361). Written consent was obtained from all human subjects used for blood feeding.

To assess the ability of *w*MelPop-infected *Ae. aegypti* to use the blood from a wider range of host species for egg development, we conducted standardized membrane-feeding trials. For membrane feeding, fresh whole mouse, chicken, and sheep blood anticoagulated with trisodium citrate (4 mg/ml), and defibrinated sheep blood, were purchased from a commercial supplier (IMVS Veterinary Services Division, Gilles Plains, SA, Australia). Anticoagulated (4 mg/ml trisodium citrate) and defibrinated pig blood were sourced from a local abattoir (KR Castlemaine, Toowoomba, QLD, Austrailia). Bloodmeals were presented to premated 5-d-old females in glass membrane feeders (Rutledge et al. 1964) maintained at 37°C and fitted with Hemotek collagen membrane (Discovery Workshops, Accrington, Lancashire, United Kingdom). All bloodmeals were supplemented with 1 mM ATP to stimulate engorgement (Kogan 1990). Immediately after a set feeding period of 30 min, unfed females were removed from treatment cages.

In both laboratory animal and membrane feeding trials, sucrose sources were removed from females 5 h before blood feeding. Postfeeding, blood-fed females were provided with constant access to 10% sucrose solution and water until fecundity and fertility assays were done.

Blood-Feeding Trials: Human Volunteers. To examine variation in fecundity associated with blood sources from different individual humans, PGYP1 and PGYP1.tet mosquitoes were fed on eight human volunteers (four male and four female) in four replicate experiments. Sucrose sources were removed 5 h before blood feeding and premated 5-d-old adult females mosquitoes were allowed to feed on exposed forearms for twenty minutes (University of Queensland, Human Ethics Approval 2007001379). In each of the four replicates, the left and right arms were alternated for the *w*MelPop-infected PGYP1 and tetracycline-treated PGYP1.tet mosquitoes. Postblood feeding, the sucrose source was returned.

Fecundity and Egg Viability Assays. Females were isolated individually for egg-laying 96 h postblood meal for a period of 48 h in plastic *Drosophila* vials containing wet filter paper as an oviposition substrate. Eggs were hatched 120 h after oviposition by submersion in nutrient-infused deoxygenated water (75 mg TetraMin/liter) for 48 h. To hatch any remaining eggs, oviposition papers were dried briefly and then resubmersed for a further 5 d before the final numbers of hatched larvae were recorded. Insemination of females was verified by dissection of spermathecae in phosphate buffer saline followed by direct observation of sperm by light microscopy.

Statistics. All statistical comparisons were conducted using Statistica, version 8 (StatSoft, Tulsa, OK). Normality of experimental data was determined using the Kolmogorov–Smirnov test. If data were not normally distributed, nonparametric tests (Mann–Whitney *U* [MWU], one-way Kruksal–Wallis, and Wil-

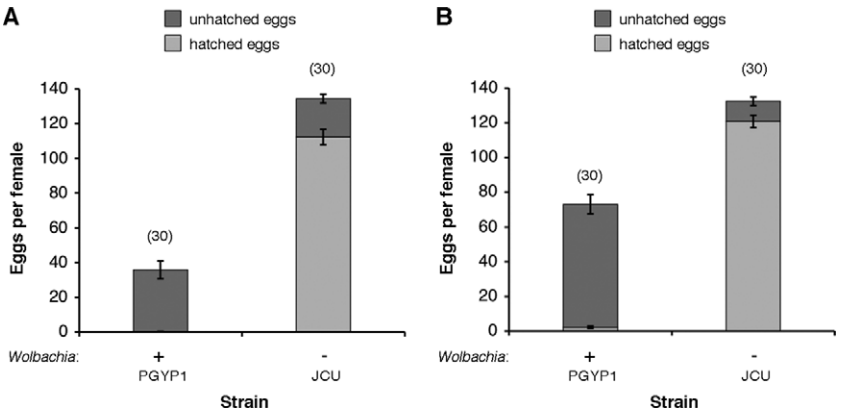

Fig. 1. Effect of rodent blood on egg development in the *w*MelPop-infected PGYP1 and naturally uninfected JCU *Ae. aegypti* strains. Females were blood fed at 5 d posteclosion on mice (A) or guinea pigs (B). The average number of unhatched and hatched eggs ± SE for each treatment is shown, and the numbers of replicates for each strain are denoted in parentheses above error bars. Assays were conducted with G_9 PGYP1 females.

coxon matched pairs tests) were used to analyze data sets. Student's *t*-test, analysis of variance (ANOVA), and post hoc comparisons were used to analyze normally distributed data sets.

Results

Egg Development on Rodent Blood. When females from the *w*MelPop-infected PGYP1 strain (hereafter referred to as PGYP1) were provided with a mouse or guinea pig bloodmeal a dramatic reduction of egg development was observed relative to females from the uninfected wild-type JCU strain (Fig. 1). This was associated with both a decline in the numbers of eggs oviposited by each female and the percentage of hatched larvae. For example, PGYP1 females that were blood fed on mouse blood produced significantly fewer eggs than JCU females (MWU, $U = 3.0$, df = 60, $P < 0.001$; $n = 30$ females per strain). This decline in fecundity was accompanied by an approximate 120-fold decrease in egg viability from 83.7% in the JCU strain to 0.7% in the PGYP1 strain (MWU, $U = 0.0$, df = 60, $P < 0.001$). These trends were similar to those obtained after blood feeding with guinea pigs with both fecundity and egg viability of PGYP1 significantly reduced relative to the JCU strain (MWU, $P < 0.001$ for both comparisons; $n = 30$ females per strain). In general, eggs oviposited by PGYP1 females after rodent bloodmeals were less melanized and smaller than those obtained after a human meal (Fig. 2).

Egg Development in the Absence of *Wolbachia*. To determine whether the reduction in PGYP1 egg development after a rodent bloodmeal was due to *Wolbachia* or differences in host genetic background between the PGYP1 and JCU strains, we cured the *w*MelPop infection from PGYP1 with tetracycline. Af-

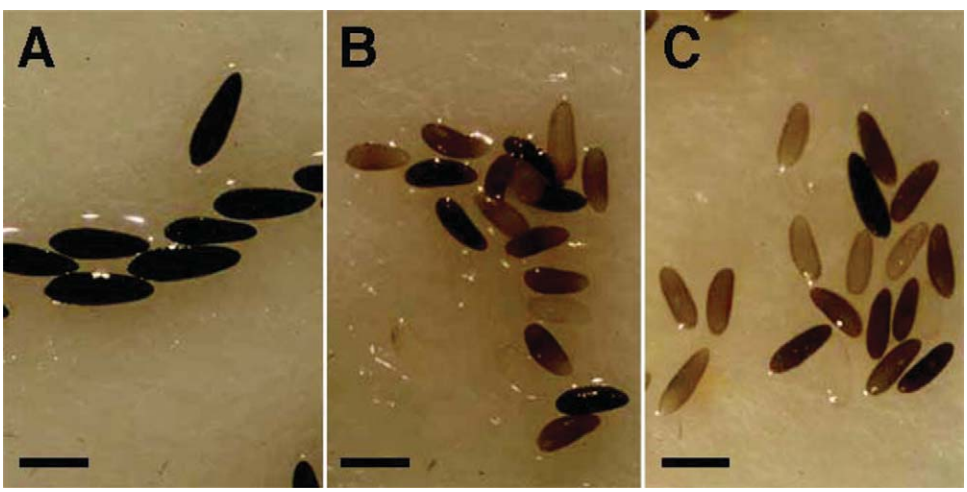

Fig. 2. Phenotypic differences in embryo morphology after blood feeding of *w*MelPop-infected PGYP1 females on different blood sources. PGYP1 females were fed on human (A), mouse (B), or guinea pig (C) blood, and the resulting eggs were photographed 24 h postoviposition. Scale bar = 0.5 mm. (Online figure in color.)

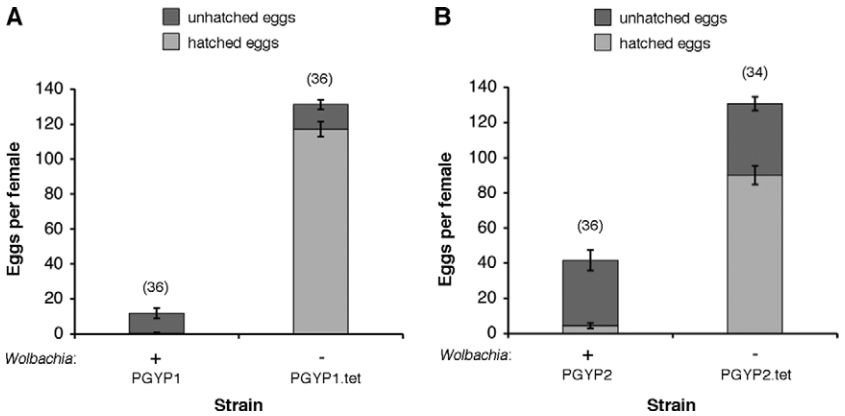

Fig. 3. Tetracycline-induced restoration of egg development. Average number of unhatched and hatched eggs ± SE resulting from *w*MelPop-infected PGYP1 and tetracycline-cured PGYP1.tet females (A); and PGYP2 females and PGYP2.tet females (B), after blood feeding on mice. Numbers of replicates for each strain are shown in parentheses above error bars. Assays were conducted with G_{16} PGYP1 and G_{24} PGYP2, females respectively.

ter antibiotic clearance of *w*MelPop, the ability of females from the resulting uninfected PGYP1.tet strain to use mouse blood for reproduction was fully restored ($n = 36$ females per strain) (Fig. 3A). Moreover, this was also the case after tetracycline-treatment of a second *w*MelPop-infected *Ae. aegypti* strain, PGYP2, which served as an independent biological replicate ($n \geq 34$ females per strain) (Fig. 3B).

Nutritional Interaction Between Blood Source and Egg Development. To better characterize the nutritional interaction between blood source and egg development in the PGYP1 strain, we blood fed cohorts of PGYP1 females on either 1) a human volunteer or laboratory mouse for two successive reproductive cycles (i.e., human–human, mouse–mouse) or 2) alternated the blood source provided for the second cycle (i.e., human–mouse, mouse–human) (Fig. 4). In human–human and mouse–mouse treatments, receiving the same blood source for two successive cycles did not significantly change either the total number of eggs oviposited or egg viability. Human blood resulted in high fecundity and embryo viability for both cycles, whereas mouse blood consistently decreased fecundity and embryo viability. However, in the human-mouse treatment, a mouse bloodmeal on the second cycle resulted in a significant inhibition of both fecundity (Wilcoxon matched pairs test, $T = 5.0$, $Z = 3.95$, $df = 22$, $P < 0.001$) and egg viability ($T = 1.0$, $Z = 4.17$, $df = 23$, $P < 0.001$). The opposite effect was evident in the mouse-human treatment, where a human bloodmeal in the second cycle completely rescued fecundity ($T = 16.0$, $Z = 4.05$, $df = 26$, $P < 0.001$), and significantly restored egg viability ($T = 0.0$, $Z = 4.46$, $df = 26$, $P < 0.001$).

Blood Source Host Range Effect. We investigated the host range of this phenotype using artificial membrane-feeding trials with blood sourced from a variety of common laboratory and agricultural animals. Stan-

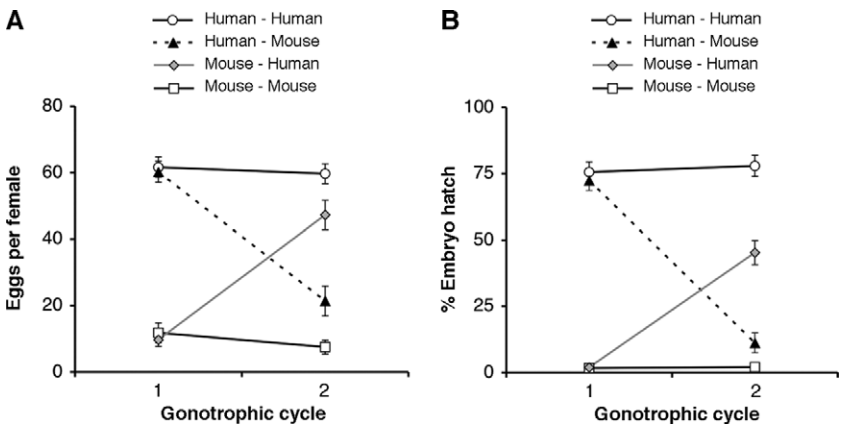

Fig. 4. Nutritional interaction between blood source and egg development in the *w*MelPop-infected PGYP1 strain. (A) Average number of eggs produced per female ± SE. (B) Average percentage of embryo hatch ± SE, after blood feeding PGYP1 females for two successive reproductive cycles on either human or mouse blood, or alternating the blood sources between cycles. Bloodmeals for the first cycle were given at 5 d posteclosion, and the bloodmeals for the second cycle at 11 d posteclosion ($n = 35$ females per treatment). Assays were conducted with G_{24} PGYP1 females.

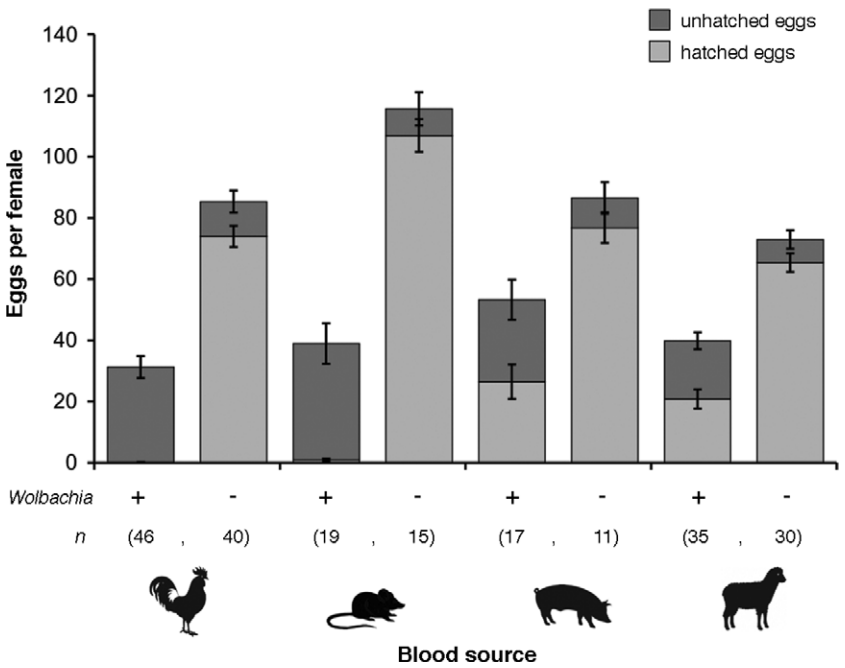

Fig. 5. Effect of different blood sources on PGYP1 egg development. Average number of hatched and unhatched eggs ± SE for *w*MelPop-infected PGYP1 and tetracycline-cured PGYP1.tet strains after blood feeding on anticoagulated chicken, mouse, pig, and sheep blood. Females were blood fed at 5 d posteclosion. The numbers of females (n) per strain for each treatment are shown in parentheses below the bars. Assays were conducted with G_{14} PGYP1 females.

dardized comparisons of fecundity and egg viability were made using the PGYP1 and tetracycline-cured PGYP1.tet strain that share a common genetic background, and the following blood sources: anti-coagulated chicken, mouse, pig, and sheep blood ($n \geq 10$ females per strain per treatment) (Fig. 5). In addition, we also evaluated the effect of defibrination and anticoagulation processes on egg development, by including defibrinated pig and sheep blood in these trials for comparison ($n \geq 25$ females per strain per treatment; data not shown).

Consistent with earlier observations, a significant interaction between blood source and infection status was determined to affect fecundity in membrane feeding trials (ANOVA, $F_{5,312} = 5.99$, $P < 0.001$). On all blood-sources, individual pairwise comparisons revealed the numbers of eggs oviposited by PGYP1 females were significantly reduced relative to PGYP1.tet females (Tukey's honestly significant difference post hoc test, $P < 0.001$ for all comparisons). The same trend also was observed for egg viability, with significant differences in embryo hatch rates detected between PGYP1 and PGYP1.tet strains fed chicken, mouse, pig, and sheep blood (MWU, $P < 0.001$ for all comparisons).

Mean hatch rates of PGYP1.tet eggs were consistently high, exceeding 85% on all blood sources. In contrast, embryo hatch rates varied significantly according to blood source received by PGYP1 females (Kruksal–Wallis, $H_{5,171} = 101.93$, $P < 0.001$). Overall, chicken (0.4 ± 0.2% [SE] hatch) and mouse (4.8 ± 2.7%) blood were the worst blood sources affecting embryo viability, followed by pig (41.3 ± 7.2%) and sheep blood (49.0 ± 5.3%) that produced intermediate levels of hatch.

No significant differences in fecundity or egg viability were found between groups of PGYP1 females fed on either defibrinated or anticoagulated pig and sheep blood (MWU, $P > 0.05$ for all comparisons). The same trend was also evident in comparisons between anticoagulated mouse blood and results using anesthetized mice, where effects on PGYP1 fecundity and egg viability were not statistically different from one another (fecundity: t-test, $t = -0.36$, df = 47, $P = 0.719$; and viability: MWU, $U = 229.0$, df = 49, $P = 0.139$; $n \geq 20$ females per strain).

Influence of Human Host on Fecundity. To examine whether the individual human host used for blood feeding influenced the fecundity of *w*MelPop-infected *Ae. aegypti*, we fed PGYP1 and PGYP1.tet ($n = 35$ females per treatment) on eight different volunteers, both male and female, and examined subsequent egg production. This experiment was replicated four times for each volunteer and mean fecundity is reported in Fig. 6. The presence of *Wolbachia* resulted in a significant reduction in the fecundity of mosquitoes relative to the tetracycline-cured controls (ANOVA, $F_{1,1584} = 74.21$; $P < 0.001$). A significant interaction between human host and infection status was also determined to affect fecundity (ANOVA, $F_{7,1584} = 7.92$; $P < 0.001$). The sex of the human host did not influence fecundity.

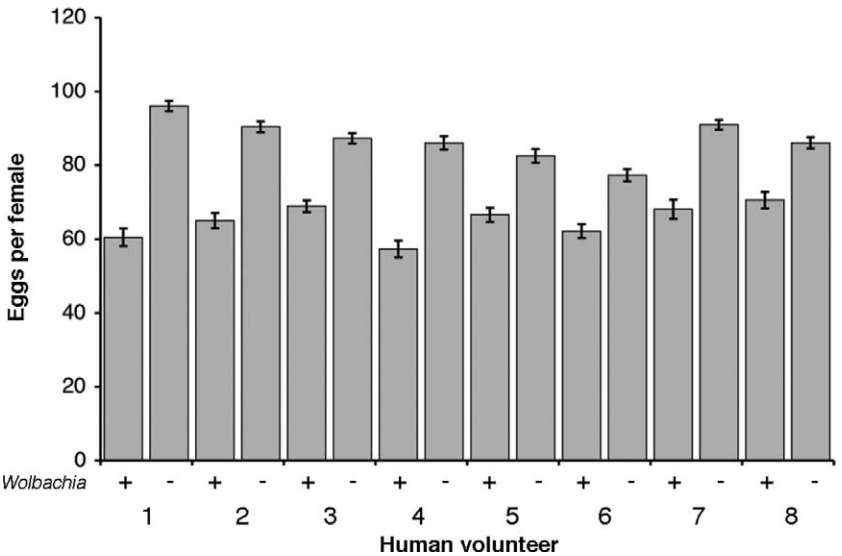

Fig. 6. Effect of human host on fecundity of the *w*MelPop-infected PGYP1 and tetracycline-cleared PGYP1.tet strains. Average number of eggs ± SE for cohorts of female mosquitoes (5 d posteclosion) blood fed on different human volunteers. Volunteers 1–4 and 5–8 were male and female, respectively. Each bar represents the mean of four replicate experiments with $n = 35$ mosquitoes in each trial. Replicate experiments were conducted with PGYP1 females in consecutive generations from G_{29} to G_{33} posttransinfection.

Discussion

This study provides evidence for a nutritional interaction between *w*MelPop infection and host blood that influences *Ae. aegypti* egg development. Both mosquito fecundity and egg viability were severely impacted when *w*MelPop-infected females fed on certain nonhuman blood sources, and, in some cases, this phenotype was associated with gross defects in embryo morphology. These results were unexpected when considered that *Ae. aegypti* is normally able to thrive on a wide variety of nonhuman vertebrates in the lab (Woke 1937, Bennett 1970), including all of the blood sources used in this study. Consistent with the hypothesis that *Wolbachia* was the cause of this phenotype, antibiotic clearance of the *w*MelPop infection restored egg development on all hosts to normal levels. On all blood sources, infected and uninfected mosquitoes fed similarly to repletion, and females as revealed by dissection did not retain embryos in their ovaries (data not shown), suggesting that general defects in feeding or egg laying behavior are unlikely to be contributing factors to this phenomenon.

We also determined that the fecundity of *w*MelPop-infected females was reduced relative to antibiotic treated controls when fed human blood, and varied significantly between human hosts. These results contrast with earlier data obtained in the generations immediately proceeding establishment of *w*MelPop in *Ae. aegypti*, where nonsignificant fecundity costs were observed when PGYP1 females fed on human blood (McMeniman et al. 2009). This discrepancy may possibly reflect 1) an increase in the virulence and associated fitness costs of the *w*MelPop infection over the period following transinfection and/or 2) temporal inbreeding effects in this strain that accentuate the physiological costs of this bacterial infection. Nevertheless, the inclusion of tetracycline-treated mosquito controls in each experiment allowed us to make quantitative comparisons between blood sources within a mosquito generation, and qualitative comparisons between mosquito generations.

In general, the magnitude of fecundity costs when PGYP1 females fed on human blood (18–37% reduction in relative fecundity) was less than that seen when mosquitoes were fed on different animals' blood, and a continuum of host effects was observed. For example, fecundity costs of up to 68% were observed when *w*MelPop-infected females blood fed on either chicken or mouse blood; however, smaller fecundity costs of 38–45% were observed when *w*MelPop-infected mosquitoes were fed on pig and sheep blood. As such the mechanistic basis of this phenotype must rely on factors that vary between individual humans as well as between species.

The enhanced levels of fecundity and egg viability observed when *w*MelPop-infected females are provided with a human bloodmeal in comparison with different animals' blood is intriguing given the natural tendency of this mosquito species to blood feed predominantly on humans in the field (Scott et al. 1993b, Ponlawat and Harrington 2005). The nutritional differences between human and other blood sources that promote anthropophilic behavior are currently unknown (Harrington et al. 2001). Evidence indicates however that human blood may potentially be metabolized with greater energy efficiency than other types of blood in *Ae. aegypti*, leading to an accumulation of energy reserves (Briegel 1990, Harrington et

al. 2001). In this study, variation in the effects of human and other blood sources suggests that potential interactions between these bacteria and biochemical differences in host blood may contribute to disruption of egg development.

A decline in egg development after blood feeding on mice was not observed in a previous study (Xi et al. 2005), where the avirulent *w*AlbB strain of *Wolbachia* from the mosquito *Aedes albopictus* (Skuse) was introduced into *Ae. aegypti.* Likewise, no interactions between any of the blood sources used in this study and egg development have been described for other natural, or artificially-generated *Wolbachia* infections of *Culex* or *Aedes* mosquitoes (Dobson et al. 2002, Rasgon and Scott 2003, Xi et al. 2006). Genotypic differences between *w*MelPop and other *Wolbachia* strains may thus be responsible for this phenotype in *Ae. aegypti.*

From a mechanistic viewpoint the blood source effect may reflect differences between the metabolic response of *w*MelPop relative to other avirulent *Wolbachia* strains to blood feeding. Alternatively, it could relate to the tissue-specific density of *w*MelPop where infection and associated metabolic costs above a phenotypic threshold interferes with the normal process of egg development. The latter of these hypotheses is likely because in *Drosophila* species, *w*MelPop exhibits accelerated growth kinetics and increased densities in certain tissues relative to infection with avirulent *Wolbachia* strains (Min and Benzer 1997, McGraw et al. 2002, McMeniman et al. 2008).

Egg maturation in *Ae. aegypti* is a nutrient limited process that is controlled by a regulated endocrine cascade involving several mosquito tissues, including the midgut, hemolymph, brain, ovaries, and fat body (Attardo et al. 2005), all tissues that *Wolbachia* infect in other insects (Min and Benzer 1997, Dobson et al. 1999). Only after a female mosquito takes a bloodmeal are the major events of egg maturation activated, including the accumulation of egg yolk proteins, lipids, and iron by developing oocytes (Raikhel and Dhadialla 1992, Ziegler and Ibrahim 2001, Zhou et al. 2007). Both sufficient nutritional reserves from larval development, and absorption of nutrients from the bloodmeal are required for egg development to proceed normally (Attardo et al. 2005). Inhibition of egg development may potentially be the result of competition between *Wolbachia* and *Ae. aegypti* for nutrients that are needed for initiation of mosquito oogenesis or the development of oocytes.

In *Ae. aegypti,* a key stage of egg development is vitellogenesis, the tissue-specific expression, synthesis, and secretion of the egg yolk protein precursors, such as vitellogenin (*Vg*), by the mosquito fat body (Attardo et al. 2005). Activation of vitellogenesis is dependent on both the presence of the steroid hormone 20-hydroxyecdysone, and amino acids derived from the digested bloodmeal (Hansen et al. 2004). The presence of several specific amino acids is required for activation of vitellogenesis, with removal of these leading to a decrease in *Vg* gene expression (Attardo et al. 2006).

In *D. melanogaster*, genomic evidence suggests that *Wolbachia* obtains much of its energy through metabolism of amino acids (Wu et al. 2004), including threonine—one amino acid required for activation of *Ae. aegypti Vg* expression (Attardo et al. 2006). Interestingly, threonine occurs at higher levels in human blood relative to guinea pig blood (Chang and Judson 1977)—a suboptimal blood source for egg production in *w*MelPop-infected *Ae. aegypti* in this study. As such, one mechanistic hypothesis may be that competition between *w*MelPop and *Ae. aegypti* for threonine leads to a decrease in *Vg* expression, and a subsequent inhibition of egg development on certain blood sources low in threonine. In line with a potential interaction between *w*MelPop and mosquito vitellogenesis, the avirulent *Wolbachia* strain *w*Mel has recently been shown to induce small decreases in yolk protein gene expression in previtellogenic ovaries of *D. melanogaster* (Sun and Cline 2009).

Another well-documented difference in amino acid levels between the blood sources used in this study is the lower isoleucine content of human blood relative to higher levels in mouse, guinea pig, and chicken blood (Harrington et al. 2001). Previous studies have demonstrated that these higher isoleucine levels contribute to the increased fecundity of *Ae. aegypti* on some hosts relative to human blood (Greenberg 1951, Spielman and Wong 1974, Chang and Judson 1977, Chang and Judson 1979). However, we observed an inhibition of egg development when *w*MelPop-infected females fed on blood sources high in isoleucine (i.e., mouse, guinea pig, and chicken blood), whereas egg development proceeded normally with low isoleucine human blood. The potential for an interaction between isoleucine levels and *w*MelPop is not clear and requires further investigation, because closely related *Wolbachia* strains such as *w*Mel possess biosynthetic pathways for this amino acid (Wu et al. 2004).

Alternatively, inhibition of egg development may be due to reduction of mosquito lipid reserves by *w*MelPop, which are needed for developing oocytes (Ziegler and Ibrahim 2001). It has been hypothesized that *Wolbachia* may acquire cholesterol for synthesis of Lipid A, the major lipid component of the α-proteobacterial cell wall, by catabolizing host lipid molecules (Lin and Rikihisa 2003, Brownlie and O'Neill 2006). If the cholesterol content of blood sources were below a threshold value needed to replenish mosquito lipid stores or supplement egg development fully during *w*MelPop infection, a reduction in fecundity and egg viability could result. A nutritional basis for this hypothesis is possible, as the range in cholesterol content of human blood is much higher than that of other animals (Mitruka and Rawnsley 1977).

Analogous reductions in fecundity and egg viability to the *w*MelPop blood source phenotype have been observed in studies that have used RNA interference to knockdown expression of genes implicated or suspected to function in the nutritional regulation of egg development in *Ae. aegypti.* These include genetic silencing of 1) the target of rapamycin signaling pathway required for the nutrient sensitive-activation of

vitellogenesis in response to amino acids derived from the bloodmeal (Hansen et al. 2004); and 2) the sterol carrier protein AeSCP-2, which is thought to be involved in the uptake of cholesterol and other lipids across the midgut and fat body for mobilization to developing oocytes (Blitzer et al. 2005). A potential interaction between *w*MelPop infection and amino acid or lipid metabolism in *Ae. aegypti* therefore warrants further investigation.

In addition, the recently observed dengue virus resistance phenotype of *w*MelPop-infected mosquitoes (Moreira et al. 2009) as well as antiviral effects observed in *Drosophila* species carrying avirulent *Wolbachia* strains, such as *w*Mel and its close relatives (Hedges et al. 2008, Teixeira et al. 2008, Osborne et al. 2009), also may be potentially mediated by competition for limited nutrient resources. As such, it is tempting to speculate that the fecundity phenotype observed in this study may be mechanistically linked to viral interference effects of these *Wolbachia* strains.

The identification of metabolites that differ between host blood sources, and their interaction with *w*MelPop infection in *Ae. aegypti* is needed to characterize the mechanistic basis of this novel phenotype. We suggest that *w*MelPop infection may have inadvertently unmasked an intrinsic nutritional benefit to *Ae. aegypti* feeding on human blood that may form the basis for its strong anthropophilic behavior. Additional study of this system may provide new insight into the metabolic interplay between *Wolbachia* and insects and the nutritional basis of anthropophily.

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