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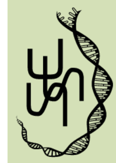

Persistence of a *Wolbachia* infection frequency cline in *Drosophila melanogaster* and the possible role of reproductive dormancy

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Field populations of arthropods are often polymorphic for *Wolbachia* but the factors maintaining intermediate *Wolbachia* frequencies are generally not understood. In *Drosophila melanogaster*, *Wolbachia* frequencies are highly variable across the globe. We document the persistence of a *Wolbachia* infection frequency cline in *D. melanogaster* populations from eastern Australia across at least 20 years, with frequencies generally high in the tropics but lower in cool temperate regions. The results are interpreted using a model of frequency dynamics incorporating cytoplasmic incompatibility (CI), imperfect maternal transmission and *Wolbachia* effects on fitness. Clinal variation is less pronounced in eastern North America which may reflect annual recolonization at higher latitudes. Limited samples from Africa from latitudes matching our tropical and subtropical samples from Australia and North America show comparably high infection frequencies, but some equatorial samples show lower frequencies. Adult dormancy across cold periods may contribute to the Australian *Wolbachia* cline. Infected flies exposed to cold conditions for an extended period had reduced fecundity and viability, an effect not evident in unexposed controls. These fitness costs may contribute to the relatively low *Wolbachia* frequencies in Australian temperate areas; whereas different processes, including CI induced by young males, may contribute to higher frequencies in tropical locations.

KEY WORDS: Cytoplasmic incompatibility, deleterious effects, diapause, mutualism, transmission-selection equilibria.

Wolbachia bacteria are endosymbionts that infect a large fraction of insect species (Jeyaprakash and Hoy 2000; Werren and Windsor 2000; Hilgenboecker et al. 2008; Zug and Hammerstein 2012; Weinert et al. 2015) with the potential to spread rapidly within populations through cytoplasmic incompatibility (CI), male killing and other effects on insect reproduction (O'Neill et al. 1997). Well-documented examples include the very rapid spread of the *w*Ri strain of *Wolbachia* through the *Drosophila simulans* population in California (Turelli and Hoffmann 1991, 1995) and more recently eastern Australia (Kriesner et al. 2013), and the spread of a male-killing strain in the tropical butterfly *Hypolimnas bolina* (Dyson et al. 2002). This invasion ability has become particularly important in mosquito populations where *Wolbachia* suppress arbovirus transmission by mosquitoes (Moreira et al. 2009; Bian et al. 2010; Walker et al. 2011) and where deliberate introductions and invasions are being undertaken (Hoffmann et al. 2011) with the potential to control vector-borne disease agents such as dengue virus.

Some *Wolbachia* infections are known to induce significant CI and reach high frequencies in natural populations associated with a balance between CI and imperfect maternal transmission (Turelli and Hoffmann 1995; Rasgon and Scott 2003; Jaenike 2009; Narita et al. 2009). However, there are also many polymorphic infections that produce little CI or other reproductive manipulation (Hoffmann et al. 1996; Zhang et al. 2013; Hamm et al. 2014). The reasons for these polymorphisms are not entirely

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clear. Although imperfect maternal transmission can account for infection frequencies somewhat below 100% (as in the case of well-studied *Drosophila* infections; Turelli and Hoffmann 1995; Unckless and Jaenike 2012), there has been little progress toward explaining infection frequencies that are low to moderate (e.g., Jiggins et al. 2001; Tagami and Miura 2004) or geographic variation in infection rates within species (Shoemaker et al. 2003; Ahrens and Shoemaker 2005).

Unfortunately, identifying factors that influence *Wolbachia* infection frequency in field populations presents a number of challenges: it requires accurate host species identification, molecular diagnostic tools to distinguish strains and other endosymbionts potentially present in a given population, and capacity to rear hosts in controlled environments to assess effects on host reproduction and maternal transmission fidelity. Consequently, few host-*Wolbachia* systems apart from those in some *Drosophila* are well characterized in nature.

The existence in field populations of weak or non-CI-inducing strains in *Drosophila* such as *w*Au in *D. simulans* (Hoffmann et al. 1996), *w*Mel in *D. melanogaster* (Hoffmann 1988), *w*Suz in *D. suzukii* (Hamm et al. 2014), and other systems including *w*Lug in *Nilaparvata lugens* (Zhang et al. 2010) and *w*Ori in *Tetranychus phaselus* (Zhao et al. 2013) leads to the expectation that some *Wolbachia* variants, at least under some circumstances, provide a net fitness benefit to hosts (Hoffmann and Turelli 1997). Initial attempts to identify positive *Wolbachia* fitness effects either failed (Harcombe and Hoffmann 2004; Montenegro et al. 2006) or produced mixed results (Fry et al. 2004). However, likely candidates for such host fitness benefits have emerged more recently in the form of virus protection (Hedges et al. 2008; Teixeira et al. 2008; Osborne et al. 2009), nutritional supplementation (Brownlie et al. 2009; Hosokawa et al. 2010; Moriyama et al. 2015), and iron metabolism (Gill et al. 2014). In field populations, the magnitude of these effects is not yet characterized and would require multiple samples of individuals across multiple time points. For instance, Kriesner et al. (2013) pointed out that the spread of *Wolbachia* strain *w*Au and subsequently *w*Ri in eastern Australian *D. simulans* detected by repeated population sampling could best be explained by inferring substantial fitness benefits for both strains.

In contrast to the very rapid spread to a high frequency of *Wolbachia w*Ri through *D. simulans* populations in eastern Australia and California, there is variability in *Wolbachia* infection frequencies in *D. melanogaster* populations from the Australian east coast (Hoffmann et al. 1994; Hoffmann et al. 1998), with high infection frequencies in tropical and warmer temperate regions (above ∼26°S latitude) and lower frequencies for mid to cooler temperate regions. For laboratory stocks of *D. melanogaster* that carry *Wolbachia*, infection frequency is generally near 100%. Friberg et al. (2011) found that long-term persistence of *Wolbachia* at high frequency in *D. melanogaster* laboratory stocks could be explained by an absence of fitness costs, near perfect maternal transmission, and significant levels of CI. However, the *Wolbachia* infection in field populations of *D. melanogaster* shows imperfect maternal transmission and induces only weak CI (Hoffmann et al. 1998) unless crosses involve very young males (Reynolds and Hoffmann 2002).

If natural infection frequencies represent a balance between positive and negative fitness effects of *Wolbachia* (potentially context dependent) and imperfect maternal transmission, clinal variation in frequencies might be explained by spatially varying effects with greater net benefits at tropical and warmer temperate latitudes. Alternatively clines may reflect latitudinal variation in maternal transmission rates. *Drosophila melanogaster* is afrotropical in origin and has undergone a relatively recent human-mediated worldwide expansion, having arrived in Europe about 10,000–15,000 years ago after the last glaciation (Lachaise et al. 1988; Stephan and Li 2007) but reaching North America only in the past 150 years (David and Capy 1988; Keller 2007), and invading Australia from the north around 100 years ago (Hoffmann and Weeks 2007). It has adapted relatively recently to temperate climatic conditions with extended cold winter periods.

Although a few species employ polyphenism or undertake long-distance migration, most insects undergo some form of reproductive latency as an adaptation to seasonally adverse conditions encountered during colder months of the year in temperate regions, or at the onset of dry seasons (Tauber et al. 1986). For many species, this entails a distinct physiological condition, usually instigated in embryonic, larval, or pupal stages known as true diapause, with distinct initiation and termination phases. *Drosophila melanogaster* individuals however overwinter at low temperatures as adults (Izquierdo 1991; Mitrovski and Hoffmann 2001). Here, we follow the terminology of Kostal (2006) and refer to this response as dormancy rather than diapause. Reproductive dormancy in females is characterized by a weak developmental arrest of oocyte maturation, generally at previtellogenic stages, induced by ambient temperatures of 12°C or below and a short photoperiod (Saunders et al. 1989).

Schmidt et al. (2005) found polymorphism for incidence of what they termed diapause (defined as oocyte development arrest at or prior to stage 7) in *D. melanogaster*, with frequencies of arrested individuals increasing clinally with latitude along the U.S. east coast under laboratory conditions of 12°C with 10:14 h light:dark photoperiod. However, Lee et al. (2011) did not find a latitudinal cline for this phenotype in flies from eastern Australia, although there was clinal variation in the ability of females to retain eggs (Mitrovski and Hoffmann 2001). Although cold stress does not lead to the removal of *Wolbachia* infections from hosts (Li et al. 2014), it is possible that *Wolbachia* might influence

dormancy responses and thereby the fitness of overwintering flies.

In this article, we revisit clinal patterns in *D. melanogaster* and focus on two main issues. First, we examine the temporal stability of clinal frequency variation in eastern Australia by combining new datasets with published data from the last 20 years when there has been warming in this region (www.bom.gov.au). We also provide information on *Wolbachia* infection frequencies from North America, Africa, and Eurasia for comparison. Second, we investigate fitness effects of natural *Wolbachia* infections during reproductive dormancy using *D. melanogaster* samples from cool temperate locations on three separate continents. We present a theoretical analysis that indicates the magnitude of fitness effects, maternal transmission, and CI needed to explain observed infection frequencies.

Materials and Methods

FIELD COLLECTIONS FOR ESTIMATING *WOLBACHIA* INFECTION FREQUENCY

We assayed *D. melanogaster* field samples collected from various localities along the east coast of mainland Australia and Tasmania during 2004, 2005, 2011, and 2014 for *Wolbachia* infection status. Field flies were collected either from rotting fruit placed out in the field to attract adults, or from piles of rotting fruit located at field sites where thousands of flies were found. Most flies assayed were field males but in some cases isofemale lines were established and field females were assayed by polymerase chain reaction (PCR) after they produced offspring. Species identity was established—directly, or from male F1 offspring in the case of females—by examining male genital arch morphology. Flies were preserved in 100% ethanol and stored at −20°C until use in PCR.

Genomic DNA was extracted from adult flies using a Chelex-based method. We detected *Wolbachia* using either a standard PCR method (for the 2004 and 2005 collections), or a real-time PCR/high-resolution melt (RT/HTM) method. We also screened some samples from North America with the standard method to supplement published data from this continent. The standard screening involved the *wsp* primers wsp81F and wsp691R and a concurrent PCR assay targeting arthropod-specific 28S rDNA (Turelli and Hoffmann 1995). Positive controls using single-copy nuclear genes are essential because failure to detect a *Wolbachia* PCR product could be a failed PCR or failure to extract DNA. PCR products were visualized on 1% agarose gels alongside a standard. We considered an individual infected when both the *Wolbachia*-specific primers and nuclear controls produced fragments of appropriate sizes.

For the RT/HTM method, we amplified a ∼340 bp fragment of the *wsp* gene (Lee et al. 2012). This assay included *D. melanogaster* specific primers for the host gene RpS6 as an internal control to test for the presence of DNA and correct PCR conditions.

BIOINFORMATIC ASSAYS OF *WOLBACHIA* INFECTION FREQUENCIES IN AFRICA

Lack et al. (2015) reported whole genome data from 246 African lines of *D. melanogaster*. We obtained the raw sequencing reads for 122 lines from the National Centre for Biotechnology Information short read archive (http://www.ncbi.nlm.nih.gov/sra). Information on locations and dates of the African samples are provided in Table S2. We determined infection status of these lines as described in Methods S1.

REPRODUCTIVE DORMANCY INDUCTION

To test the hypothesis that *Wolbachia* infection has a fitness effect on flies that undergo reproductive dormancy, we established isofemale laboratory lines from *D. melanogaster* females collected in 2013 from cool temperate locations on three separate continents—Hawthorn (Australia, latitude: 37°49′S), Edinburgh (UK, latitude: 55°56′N), and Portland, ME (US, latitude: 43°40′N). We determined *Wolbachia* infection status for each line using original mothers and F1 offspring with the previously described RT/HTM method. Of these, 12/32 lines from Hawthorn (HAW), 2/13 from Edinburgh (EDIN), and 20/23 from Maine (MNE) were infected. Lines of known status from the respective locations were subsequently used to create infected ($w+$) and uninfected ($w-$) mass-bred lines designated HAW_$w+$, HAW_w-, EDIN_$w+$, EDIN_w-, MNE_$w+$, and MNE_w-.

For each of the HAW_$w+$, HAW_ $w-$, MNE_$w+$, and EDIN_ $w-$ mass-bred lines, we combined F1 offspring of seven mated females from each isofemale line with the appropriate infection status to initiate the mass-bred line. To initiate the MNE_ $w-$ mass-bred line, we collected 30 virgin females from each of the three uninfected Maine isofemale lines and crossed these en masse with five males from each of the remaining 20 infected Maine lines. We then combined these now mated females with a further seven previously mated females from each of the three uninfected Maine lines. To initiate the EDIN_$w+$ mass-bred line, we collected 40 virgin females from each of the two infected Edinburgh isofemale lines, and crossed these en masse with eight males from each of the remaining 11 uninfected Edinburgh lines. We then combined these mated females with a further 12 previously mated females from each of the two infected Edinburgh lines. These steps were taken to ensure that the mass-bred lines being compared had a high level of genetic diversity. All mass-bred lines were maintained in bottles with a census population size >200 at either 25 or 19°C. We then created a further six mass-bred lines by introgressing *Wolbachia* infections from each of the three locations into the genetic background of the other locations by mating 50+ virgin infected females with an equivalent number

Table 1. Mass-bred lines used in dormancy experiments.

Expected nuclear genetic background	Infection status	*Wolbachia* source	Line designation
Hawthorn: 100%	+	Hawthorn	HAW_*w*+
Hawthorn: 100%	–		HAW_ *w*−
Edinburgh: 100%	+	Edinburgh	EDIN_*w*+
Edinburgh: 100%	–		EDIN_ *w*−
Maine: 100%	+	Maine	MNE_*w*+
Maine: 100%	–		MNE_ *w*−
Hawthorn: 96.9%; Maine: 3.1%	+	Maine	HAWw^{MN}
Hawthorn 96.9%; Edinburgh 3.1%	+	Edinburgh	HAWw^{ED}
Edinburgh 96.9%; Hawthorn 3.1%	+	Hawthorn	EDINw^{HW}
Edinburgh 96.9%; Maine 3.1%	+	Maine	EDINw^{MN}
Maine 96.9%; Hawthorn 3.1%	+	Hawthorn	MNEw^{HW}
Maine 96.9%; Edinburgh 3.1%	+	Edinburgh	MNEw^{ED}

of infected males. We then undertook repeated backcrossing for five generations. A summary of mass-bred lines is given in Table 1.

Wolbachia infections of *D. melanogaster* field populations and laboratory stocks are not all identical and can be broadly classified into two monophyletic groups of variants: *w*Mel-like or *w*MelCS-like (Chrostek et al. 2013), with the former being hypothesized to have largely replaced the latter in field populations globally within the past 100 years (Riegler et al. 2005). However, Richardson et al. (2012) in analyzing genomic data from the DGRP and DPGP lines found this replacement commenced several thousand years ago and is not yet complete. There have also been at least some reports of *w*MelCS-like variants found in field populations more recently (Nunes et al. 2008; Richardson et al. 2012; Ilinsky 2013; Versace et al. 2014). As Chrostek et al. (2013) found significant differences in fitness effects between these two categories of variants in a common genetic background, we assayed each infected mass-bred line for *Wolbachia* variant type using primers designed for the WD1310 locus (Riegler et al. 2005). This locus is diagnostic for discriminating *w*Mel and *w*MelCS variants (Riegler et al. 2005; Woolfit et al. 2013) as the latter contain an IS5 element insertion absent in *w*Mel-like variants. Sanger sequence data from each amplicon obtained were invariant from that of the published *w*Mel genome (Wu et al. 2004).

OVARIOLE DEVELOPMENTAL ARREST

Virgin females collected within 4 h of eclosion were placed in glass vials (7–13 ♀ per vial) together with males in a controlled temperature (CT) cabinet under two separate treatments, consisting of 10:14 h light:dark with temperature range of 10.0–10.6°C, or 9½:14½ h light:dark with temperature range of 9.0–9.6°C. Both treatments were maintained for 28–31 days. Temperature inside the CT cabinet was monitored with data loggers (Thermocron DS1922L, iButtonLink Technology, WI). Immediately after treatments were terminated, females were removed and individually placed in chilled phosphate buffered saline Tween 20 (PBST) buffer. Ovaries were dissected and examined under 40–100× magnification. The most advanced oocyte from each set of ovaries was determined following King (1970). Treatment differences in arrest stages were assessed using logistic regression models (glm) in R version 3.2 (2015).

FECUNDITY AND VIABILITY

Batches of one-day-old mated females from each experimental line were subjected to 9½:14½ h light:dark with temperature range of 9.0–9.6°C. For each line, 15–17 females were transferred individually to glass vials with plastic spoons holding ∼2 ml of treacle medium, brushed with 5% w/v live baker's yeast solution and allowed to air dry for 1–2 h. These vials were held at 25°C under 12:12 h light:dark. Females were transferred to new vials with fresh spoons daily for seven days. Eggs were counted, and then left to develop in vials with culture medium at 25°C for a further 12 days to assess egg to adult viability. Most emerging progeny were preserved in 100% ethanol at −20°C.

Fecundity and viability data were not normally distributed. Wilcoxon rank sum tests (wilcox.test in R) were therefore used to test for differences between infected and uninfected lines, whereas Kruskal–Wallis rank sum tests (kruskal.test in R) were used to detect differences in lines grouped by genetic background or *Wolbachia* origin. Pairwise comparisons between all lines were performed with Wilcoxon rank sum tests following Holm correction (pairwise.wilcox.test in R).

WING SIZE

Wings from F1 progeny of females subjected to dormancy-inducing conditions and control flies (one wing per fly) were mounted on microscope slides and examined under 100× magnification. Two distances between landmarks representing wing length and wing width (see Fig. S1) were scored using Nikon NIS

Elements v3.2 imaging software with distance standardized using a 200 × 0.01 mm microscope graticule.

To test for differences between infected and uninfected lines, we performed *t*-tests (t.test in R) and one-way ANOVA (oneway.test in R) with Tukey's tests (TukeyHSD in R) for pairwise comparisons.

WOLBACHIA DENSITY

To obtain ratios of relative *Wolbachia* density compared to a single-copy host nuclear gene, we used a quantitative PCR (qPCR) method with the Roche LightCycler 480 system in a 384-well format and primers designed to amplify a 139 bp fragment of the *wsp* gene region conserved across a variety of Supergroup A strains (wspFQALL 5′-GCATTTGGTTAYAAAATGGACGA-3′ and wspRQALL 5′-GGAGTGATAGGCATATCTTCAAT-3′) for *Wolbachia* (Osborne et al. 2009) and a second set of primers to amplify a 135 bp region of the rpII215 gene region which encodes the large RNA polymerase II subunit (qrpII215F 5′-AGGCGTTTGAGTGGTTGG-3′ and qrpII215R 5′-TGGAAGGTGTTCAGTGTCATC-3′) for the host (Correa and Ballard 2012). Amplification efficiency of each gene was determined by constructing a standard curve using serial dilutions of gDNA from infected flies. Reactions were performed in triplicate and relative copy number of *Wolbachia* was calculated by the Pfaffl Method (Pfaffl 2001) using Drosophila rpII215 as a reference gene for *wsp*. Genomic DNA was extracted from adult whole bodies using a Chelex-based method. The qPCR protocol consisted of an initial denaturation step of 10 min at 95°C, with 42 cycles of 10 sec at 95°C, 15 sec at 58°C, and 30 sec at 72°C. Melting curves were analyzed after each run to confirm specificity of amplified products.

Differences in relative *Wolbachia* density between experimental lines were performed with a linear model fit (lm in R), and the different lines were compared pairwise with a Tukey's test.

Results

WOLBACHIA INFECTION FREQUENCY ESTIMATES

Our data for east Australian *D. melanogaster* populations (Fig. 1B–E) exhibit a pattern: *Wolbachia* frequency is generally high in the tropics, but declines with more southerly latitude in temperate regions. This is broadly consistent with earlier results (Hoffmann et al. 1994; Hoffmann et al. 1998; Fig. 1A). Detailed results are shown in Table S1.

To assess the consistency of the relationship between *w*Mel infection frequency and latitude for the eastern Australian data, we performed logistic regression. There was a highly significant negative association between *w*Mel infection frequency and southerly latitude overall ($b = -0.173 \pm 0.006$, $P < 2 \times 10^{-16}$), and for each of the six sampling periods considered separately ($P < 2.47 \times 10^{-8}$; see Fig. 2A). There was no significant association when data only for tropical locations (i.e., north of latitude 23.5°S) were considered ($P = 0.657$). For the data from subtropical and temperate locations only (i.e., below latitude 23.5°S), the negative association between infection frequency and latitude was greater ($b = -0.198 \pm 0.008$, $P < 2 \times 10^{-16}$). When "year" was included as a factor, the logistic regression model for the 1994–1996 sampling period differed significantly from 1993 ($P = 0.019$), 2004 ($P = 7.26 \times 10^{-7}$), 2011 ($P = 0.00025$), and 2014 ($P = 3.83 \times 10^{-6}$). The average gradient (latitude: year interaction) for the 1994–1996 model also differed significantly from that of models for each of the other sampling periods. However, when the 1994–1996 data are excluded, differences between the remaining models are not significant (see Fig. 2B). In a number of cases, the 1994–1996 data (Hoffmann et al. 1998) comprised samples obtained from the same location at several different time points. Infection frequencies at two subtropical locations (Gold Coast and Coffs Harbour) varied over time (19.6–95% and 11.1–80% infected, respectively) during this period. Reasons for this temporal variation are unclear, but there is no obvious seasonal effect.

To investigate whether a clinal pattern might be found elsewhere, we assayed field *D. melanogaster* adults from various localities along the east coast of North America during 2011 and 2013. These results, combined with previously published data (Verspoor and Haddrill 2011; Early and Clark 2013; Huang et al. 2014; Webster et al. 2015), are summarized by latitude of collection locality in Figure 1F, with a complete list in Table S1. We did not find a significant linear association between northerly latitude and *w*Mel frequency for the North American data as a whole ($P = 0.148$). However, when data from the seven locations above 38°N were excluded, there was a significant negative association ($b = -0.\ 266 \pm 0.051$, $P = 1.52 \times 10^{-7}$) which, along with high infection frequencies observed in Panama City (latitude ~9°N; 28/30 = 93.3% infected) and at three separate tropical locations in Brazil (latitude ~8-23°S; 284/300 = 94.7% infected; Ventura et al. 2012) is consistent with the Australian pattern. For locations above 38°N, average daily minimum temperatures (e.g., worldweatheronline.com 2015) are expected to consistently fall well below freezing during winter months, whereas locations further south (down to ~33°N) behave less consistently with rare freezing conditions.

We also examined *Wolbachia* infection frequencies from Africa and Eurasia, combining estimates from our bioinformatics analyses with previously published data (Ilinsky and Zakharov 2007; Verspoor and Haddrill 2011; Richardson et al. 2012; Early and Clark 2013; Webster et al. 2015). These data are summarized in Figure 3A and B and a full list provided in Table S2.

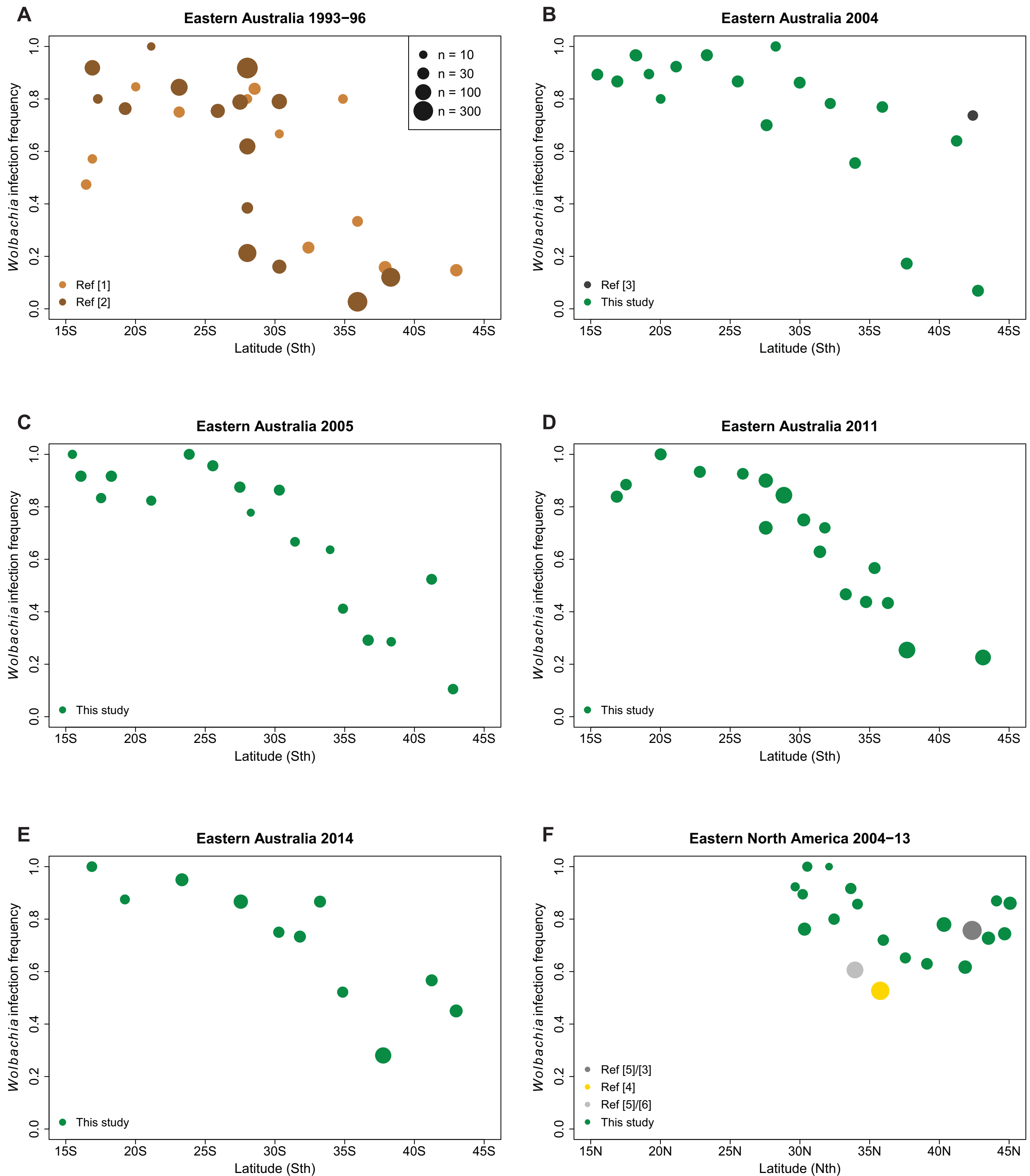

Figure 1. Infection frequencies of *D. melanogaster* individuals sampled from Eastern Australia in: (A) 1993–1996, (B) 2004, (C) 2005, (D) 2011, (E) 2014, and from North America in (F) 2004–2013. Size of circles is proportional to log(N), where N = sample size. Data sourced from [1] Hoffmann et al. (1994), [2] Hoffmann et al. (1998), [3] Early and Clark (2013), [4] Huang et al. (2014), [5] Webster et al. (2015), and [6] Verspoor and Haddrill (2011). Data were combined for samples obtained from the same or proximate localities (≤40 km apart), and where infection frequencies are statistically homogeneous.

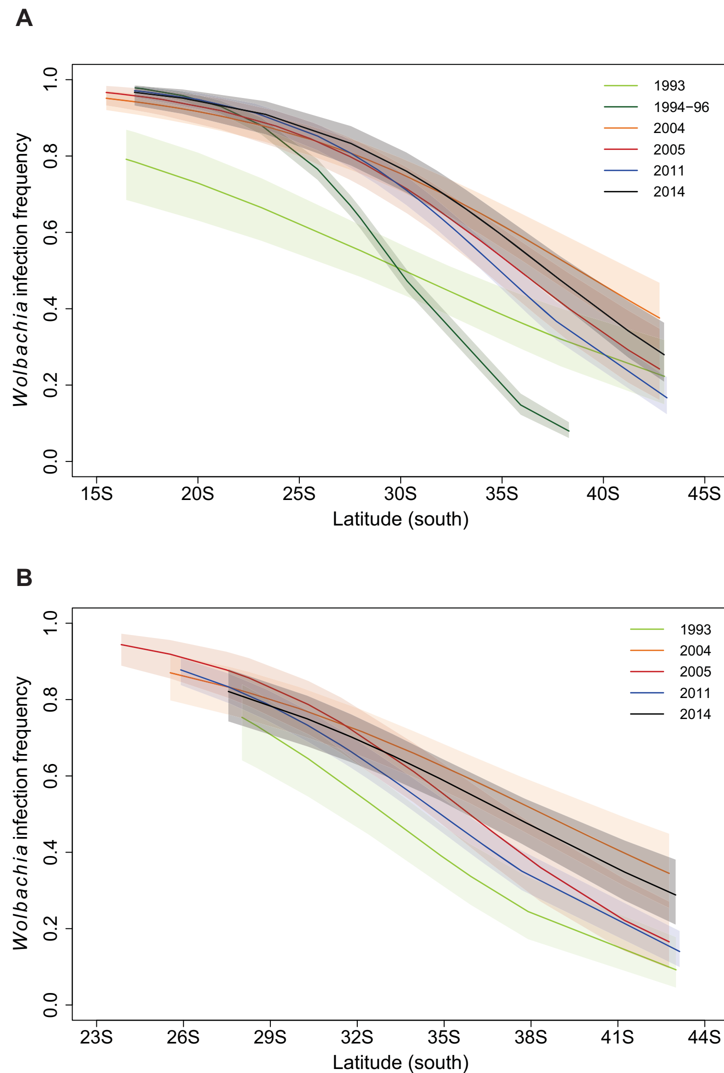

Figure 2. Logistic regression models with 95% confidence intervals for each of the five sampling periods from eastern Australia for locations below (south of) latitude 24°S.

The latitudes sampled in southern Africa below about 16.5°S correspond to the tropical and subtropical regions sampled in eastern Australia and North America and show similarly high infection frequencies. There are likely to be few if any locations in southern Africa where conditions are sufficiently cold to induce extended periods of reproductive dormancy among fly populations. African equatorial samples show highly variable infection levels, for reasons we do not understand—from over 96% in samples from Rwanda to less than 10% (and statistically heterogeneous) in Accra, Ghana, where large samples were available. Two separate samples obtained from one North African location (Marrakesh) also had variable infection frequencies (83.3 and 34.1%).

Ilinsky and Zakharov (2007) analyzed *D. melanogaster* isofemale lines from almost 100 locations throughout Eastern Europe and Central Asia over a considerable timespan. There was no latitudinal or other geographic pattern apparent in their data. However, in nearly all cases, the original populations sampled were from locations that experience prolonged periods below freezing during winter. Western Europe generally experiences very different climatic conditions to those in central Asia at equivalent latitudes. Limited sampling from Western Europe suggests infection frequencies that roughly correspond to those found in temperate regions in eastern Australia, but again there were variable infection frequencies for separate samples from each of the two most northern locations from Western Europe (Table S2: Sussex and Edinburgh, UK; 10.8–68.4%, and 24.2–98% infected, respectively). Taken together, our data suggest that *Wolbachia* infection frequencies are mostly high to very high for *D. melanogaster* populations from tropical and subtropical climates, but with some notable exceptions in Africa. For populations in cool temperate climates, there is evidence for lower infection frequencies

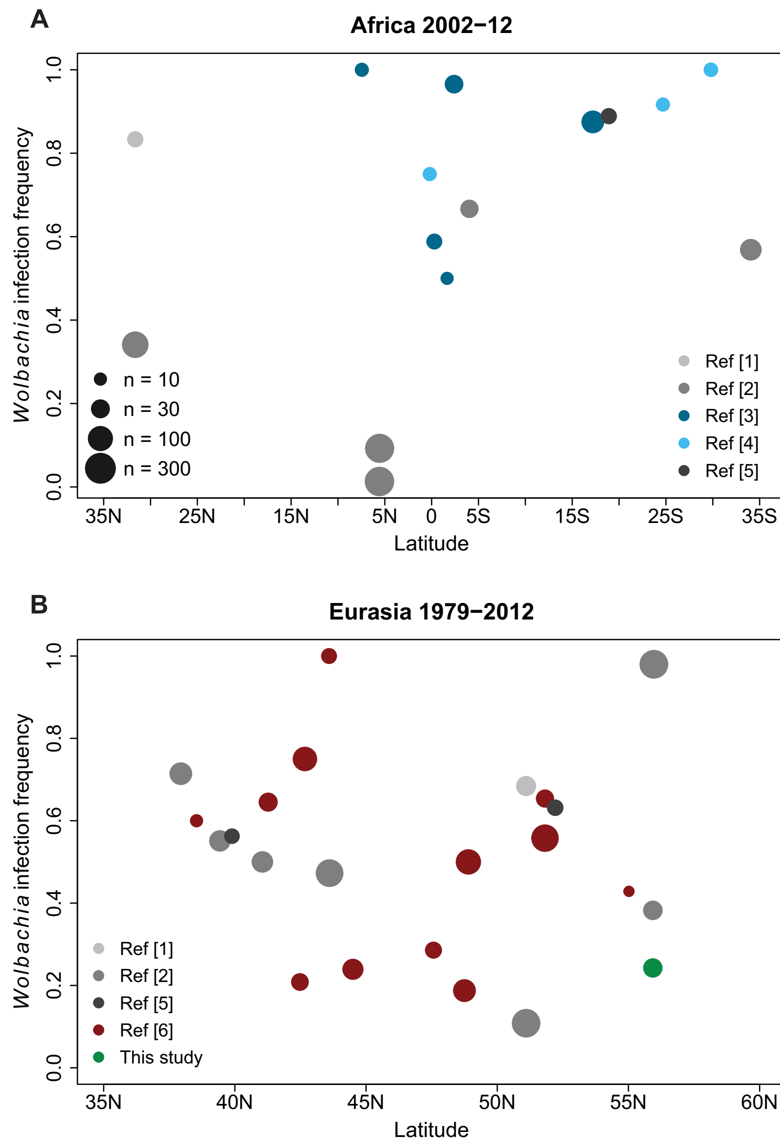

Figure 3. Infection frequencies of *D. melanogaster* individuals sampled from (A) Africa and (B) Eurasia. Size of circles is proportional to log(*N*), where *N* = sample size. Data sourced from [1] Verspoor and Haddrill (2011), [2] Webster et al. (2015), [3] Richardson et al. (2012) and our extraction of *Wolbachia* data from the sequences reported therein, [4] our extraction of *Wolbachia* data from the sequences reported by Lack et al. (2015), [5] Early and Clark (2013), and [6] Ilinsky and Zakharov (2007). Data were combined for samples obtained from the same or reasonably proximate localities (<400 km apart), and where infection frequencies are statistically homogeneous.

possibly related to conditions experienced during winter months, but higher frequencies at very high latitudes where permanent populations are unlikely outdoors.

REPRODUCTIVE DORMANCY INDUCTION

We investigated dormancy responses of infected and uninfected flies from each of our mass-bred lines (see Methods) by subjecting newly eclosed females to 10:14 h light:dark at 10.0–10.6°C; or 9½:14½ h light:dark at 9.0–9.6°C. In many cases, the most advanced oocyte dissected was either previtellogenic or on the cusp of vitelline formation and between stages 7 and 8 in size.

Significantly, fewer females had ovaries containing at least one oocyte advanced beyond stage 7/8 under the second dormancy condition (Fisher's exact test [fisher.test in R], $P = 0.0007$; Fig. 4). Under the first condition, a few more infected females produced oocytes that had developed past stage 7/8 than uninfected females did, but this was not significant (Fisher's exact test, $P = 0.118$). In this case, the smaller sample size ($N = 180$) provides reduced power (pwr.chisq.test from pwr package in R [Champely 2015], power $= 0.7446$ at the $\alpha = 0.05$ level) to detect an effect size (0.195) at least as large as that observed between the two dormancy conditions.

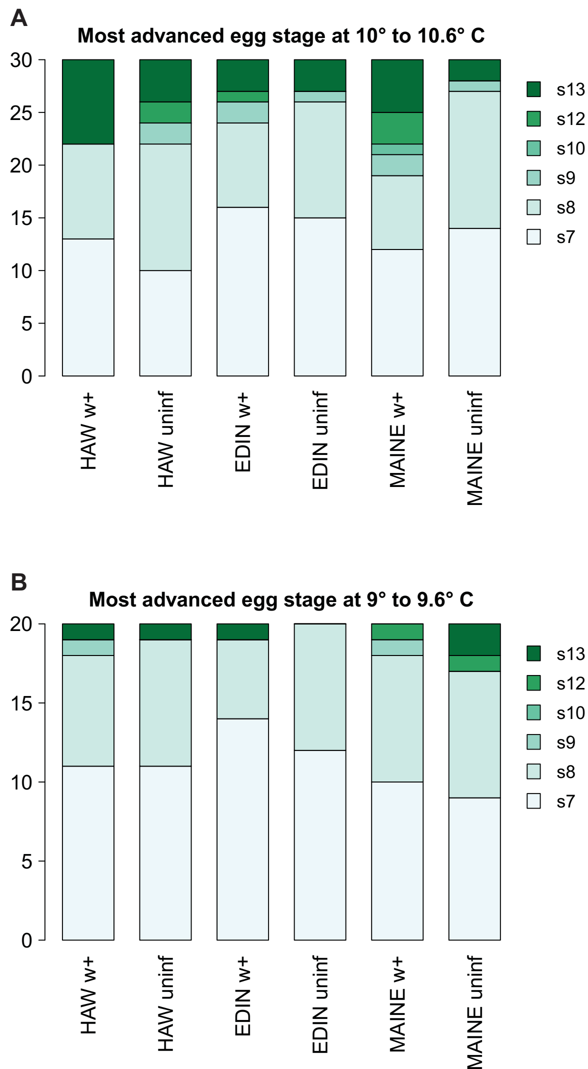

Figure 4. Frequency distribution of the most advanced egg development stage among females sampled from *D. melanogaster* infected and uninfected mass-bred lines. The flies underwent one of two reproductive dormancy-inducing conditions: (1) temperature range 10.0–10.6°C with 10:14 h light:dark (left) and (2) 9.0–9.6°C with 9½:14½ h light:dark (right).

FECUNDITY AND VIABILITY ASSAYS

We introgressed the *Wolbachia* infection from each mass-bred line into each of the other two genetic backgrounds to create a further six infected mass-bred lines denoted HAWw^{ED}, HAWw^{MN}, EDINw^{HW}, EDINw^{MN}, MNEw^{HW}, and MNEw^{ED} (see Methods). We then subjected females from these lines to conditions inducing dormancy and scored for daily fecundity over seven days and viability of F1 offspring. Overall, very few eggs were produced within the first 24 h for any of the lines (n_{max} = 14, mean = 0.357).

As expected for this type of assay (e.g., Weeks et al. 2007), overall fecundity was variable, but increased significantly (P = 0.020, adjusted r^2 = 0.631) over the postdormancy period, peaking around day 5. There was little change in the number of viable offspring per female until after day 5 when it tended to decline (Fig. S2A and B).

Uninfected females from the original mass-bred lines produced significantly more eggs (on average 1.365× more) than infected females from these lines (Wilcoxon rank sum test, P = 0.017; Fig. 5A) and this pattern is consistent across the postdormancy period (Fig. S2A). In contrast, the difference between uninfected and infected females from these same lines without the induction period was small (0.996×) and not significant (Wilcoxon rank sum test, P = 0.814; Fig. 5C).

When all infected females (from the three original and six introgressed lines) that underwent dormancy induction are considered together, uninfected females produced 1.48× the number of eggs compared to infected females (Wilcoxon rank sum test, P = 0.0005). The difference in average fecundity between infected females from the original and introgressed lines was not significant (Wilcoxon rank sum test, P = 0.265), and there was also no significant effect among exposed females for either genetic background or *Wolbachia* origin—overall, or when original or introgressed lines were considered separately (Kruskal–Wallis rank sum tests, $P > 0.05$).

The number of viable offspring produced by uninfected females was higher (on average 1.58× more) than the number produced by infected females from the original lines when exposed to dormancy induction (Wilcoxon rank sum test, P = 0.046; Fig. 5B), whereas the difference between uninfected and infected unexposed control females from these same lines was small (1.013×) and not significant (Wilcoxon rank sum test, P = 0.882; Fig. 5D). When the infected females from all lines (original and introgressed) were considered, the uninfected females produced 2.17× the number of viable F1 offspring compared to infected females following dormancy induction (Wilcoxon rank sum test, P = 0.0002; Fig. 5B) and there was a difference between the original and introgressed infected females (Wilcoxon rank sum test, P = 0.029). There was no significant effect for either genetic background or *Wolbachia* origin on this trait (data not shown). The difference between uninfected and infected females from these same lines without induction was small (0.996×) and not significant (Wilcoxon rank sum test, P = 0.814).

EFFECT OF *WOLBACHIA* ON BODY (WING) SIZE OF F1 OFFSPRING

We used wing size (length and width) as a proxy for overall body size (Karan et al. 1998) from 84 individuals for each of the lines (N = 504 in total) using wing landmarks. These measures were strongly correlated (r^2 = 0.89). Each batch of 84 comprised F1

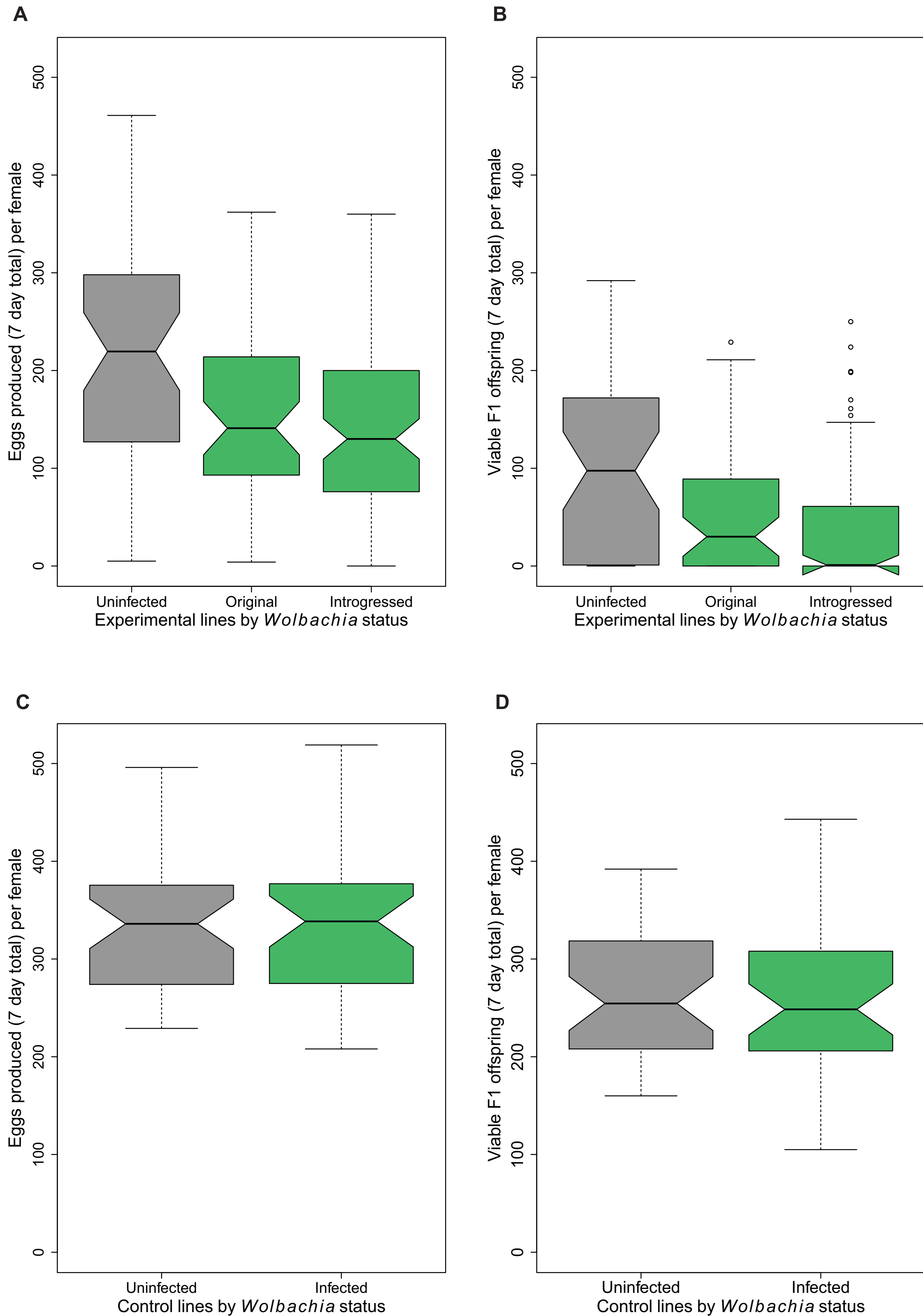

Figure 5. (A) Total fecundity of females over seven days following release of dormancy induction, and (B) number of viable F1 offspring that resulted from the eggs produced, contrasted with (C) fecundity and (D) viability for unexposed control lines over the same period.

offspring arising from eggs produced on day 2 ($N = 36$) or day 6 ($N = 32$) postdormancy and unexposed controls ($N = 16$).

Females subjected to dormancy produced significantly smaller ♀ offspring than controls did for both wing length and width ($P < 0.0001$) but ♀ F1s arising from day 2 eggs were similar in size to those arising from day 6 eggs (Tukey's test of pairwise comparisons; Fig. 6A). Similar results were obtained for ♂ F1s (Fig. S3A). Infected females were significantly larger than uninfected females for wing length ($P = 0.001$). This difference was evident both in unexposed controls (Fig. 6B) and in ♀ F1s produced by dormancy-exposed mothers (Fig. 6C). Size differences between infected and uninfected males were not significant (Fig. S3B and C).

EFFECT OF *WOLBACHIA* DENSITY ON FECUNDITY AND EGG VIABILITY

For the infected mothers from the original ($N = 42$) and introgressed ($N = 72$) lines that underwent induced dormancy, along with some female F1 offspring ($N = 14$) and other females from the original mass-bred lines ($N = 18$), we assayed *Wolbachia* density using qPCR. For the mothers that experienced the dormancy-induction period, there was a difference in average *Wolbachia* density between the original and introgressed lines (Wilcoxon rank sum test, $P = 4.1 \times 10^{-9}$), and these were therefore treated separately.

All females from the original lines produced at least some eggs; and there was a significant negative correlation (adjusted $r^2 = 0.1073$; Spearman's rank correlation, $P = 0.027$; Fig. 7A) between number of eggs produced over seven days and *Wolbachia* density. Of the 42 females, 29 produced viable offspring, and the relative *Wolbachia* density of females that did not produce offspring was on average 1.40× that of females which did (marginally nonsignificant, Wilcoxon rank sum test, $P = 0.060$). However, there was no correlation between the number of viable F1 offspring with *Wolbachia* density (Spearman's rank correlation, $P = 0.3$) and no effect of genetic background (Kruskal–Wallis rank sum test, $P = 0.169$).

For females from the introgressed lines, 68 of 72 individuals produced at least some eggs. For females producing eggs, there was a significant negative correlation (adjusted $r^2 = 0.067$; Spearman's rank correlation, $P = 0.009$) between number of eggs over six days and *Wolbachia* density (Fig. 7B). For the introgressed-line females overall, 40 of 72 produced some viable offspring. The mean relative *Wolbachia* density of introgressed-line females that did not produce viable offspring was significantly higher (1.72×) than that of females from the same lines which did (Wilcoxon rank sum test, $P = 0.002$). There was no significant effect of either genetic background (Kruskal–Wallis rank sum test, $P = 0.142$) or *Wolbachia* origin (Kruskal–Wallis rank sum test, $P = 0.358$). For females from the introgressed lines which produced at least some viable offspring, those with higher relative *Wolbachia* density tended to produce fewer viable offspring (adjusted $r^2 = 0.065$) but this was not significant (Spearman's rank correlation, $P = 0.093$).

MATHEMATICAL ANALYSES

To explore the forces that may be responsible for the ubiquitous *w*Mel polymorphisms and the persistent cline in eastern Australia, we consider an idealized discrete-generation model for *Wolbachia* infection-frequency dynamics introduced in Hoffmann et al. (1990). The model incorporates imperfect maternal transmission, CI, and effects of *Wolbachia* infection on host fitness, modeled as differences in fecundity (Hoffmann and Turelli 1997). We assume that on average a fraction μ of the ova produced by an infected female are uninfected, and that uninfected ova from infected females are as susceptible to CI as are ova from uninfected females (see Carrington et al. 2011 for empirical support in *D. simulans*). Embryos produced from fertilizations of uninfected ova by sperm from infected males hatch with frequency $H = 1 - s_h$ relative to the fraction of embryos that hatch from the three compatible fertilizations, all of which are assumed to produce equal hatch frequencies. Finally, we assume that the relative fecundity of infected females is F.

From Hoffmann et al. (1998) and Reynolds and Hoffmann (2002), we know that *w*Mel is imperfectly maternally transmitted in natural Australian populations and that it causes little CI unless males are extremely young. Hence, it may seem plausible that the frequency of *w*Mel can be understood by ignoring the low level of CI and considering an equilibrium between imperfect transmission and positive fitness effects, $F > 1$ (Hoffmann and Turelli 1997). If $F(1 - \mu) > 1$, the stable transmission-selection equilibrium frequency is

$$\hat{p} = 1 - \frac{\mu F}{F - 1}, \tag{1}$$

which increases from 0 toward $1 - \mu$ as F increases from $1/(1 - \mu)$. However, as shown below, even very weak CI can appreciably increase equilibria predicted from equation (1) with $s_h = 0$.

Turelli and Hoffmann (1995) focused on the properties of equilibria produced by imperfect transmission and CI when infected females suffer reduced fecundity, that is, ($F < 1$). Here, we revisit the equilibria assuming that *Wolbachia* enhances fecundity. Hoffmann and Turelli (1997) conjectured that advantageous *Wolbachia*-induced fitness effects must be common for infections that do not induce CI. Now, based on the observed spatial and temporal dynamics of *w*Au and *w*Ri in Australian *D. simulans* (Kriesner et al. 2013), it seems plausible that even CI-causing natural *Wolbachia* infections routinely enhance host fitness. According to the Hoffmann et al. (1990) model, adult infection

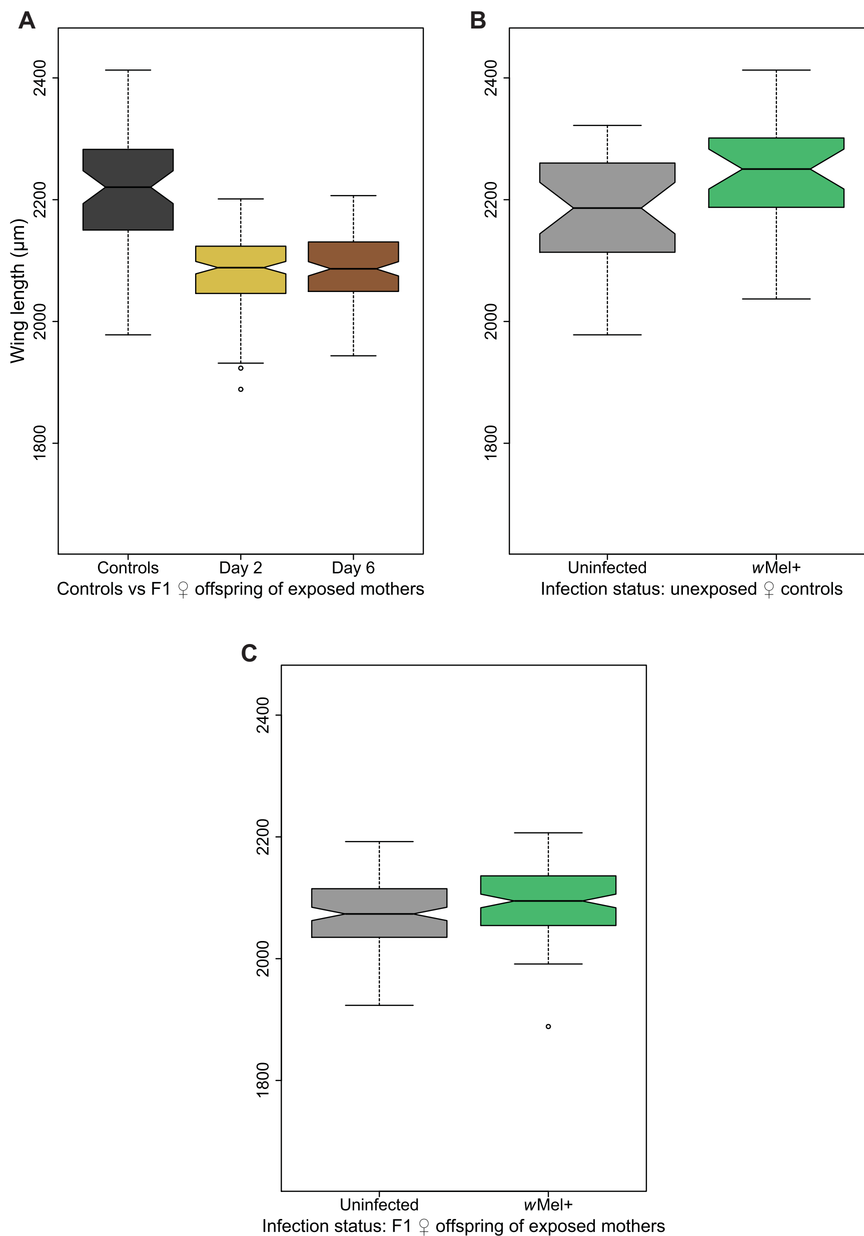

Figure 6. **Wing length for unexposed control females and F1 females arising from eggs produced by dormancy-exposed mothers. (A) Controls compared to F1s arising from eggs produced on day 2 or day 6 following dormancy release, (B) uninfected compared to infected controls, and (C) uninfected compared to infected F1s.**

frequencies, denoted p_t, change between generations as

$$p_{t+1} = \frac{p_t F(1-\mu)}{1 + p_t(F - 1 - s_h) + p_t^2 s_h (1 - \mu F)} \approx p_t F(1-\mu) \quad \text{for } p_t \approx 0. \tag{2}$$

Whether it causes CI, a *Wolbachia* infection will tend to increase when rare only if

$F(1-\mu) > 1$. The fecundity parameter F approximates more general fitness effects. Given that our interest is in understanding polymorphic variation in *Wolbachia* infections, we need to understand the stable (and unstable) polymorphic equilibria (i.e., $0 < \hat{p} < 1$) produced by (2). Nonzero equilibria of (2) satisfy

$$p^2 s_h (1 - F\mu) + p(F - 1 - s_h) + 1 - F(1-\mu) = 0. \tag{3}$$

First note that if $F < 1$, the coefficients of the powers of p in equation (3) are positive, negative, and positive, respectively. Thus, by Descartes' rule of signs, equation (3) has either two positive roots or none. With small μ and moderate CI (see conditions (4) in Turelli and Hoffmann 1995), there are two positive

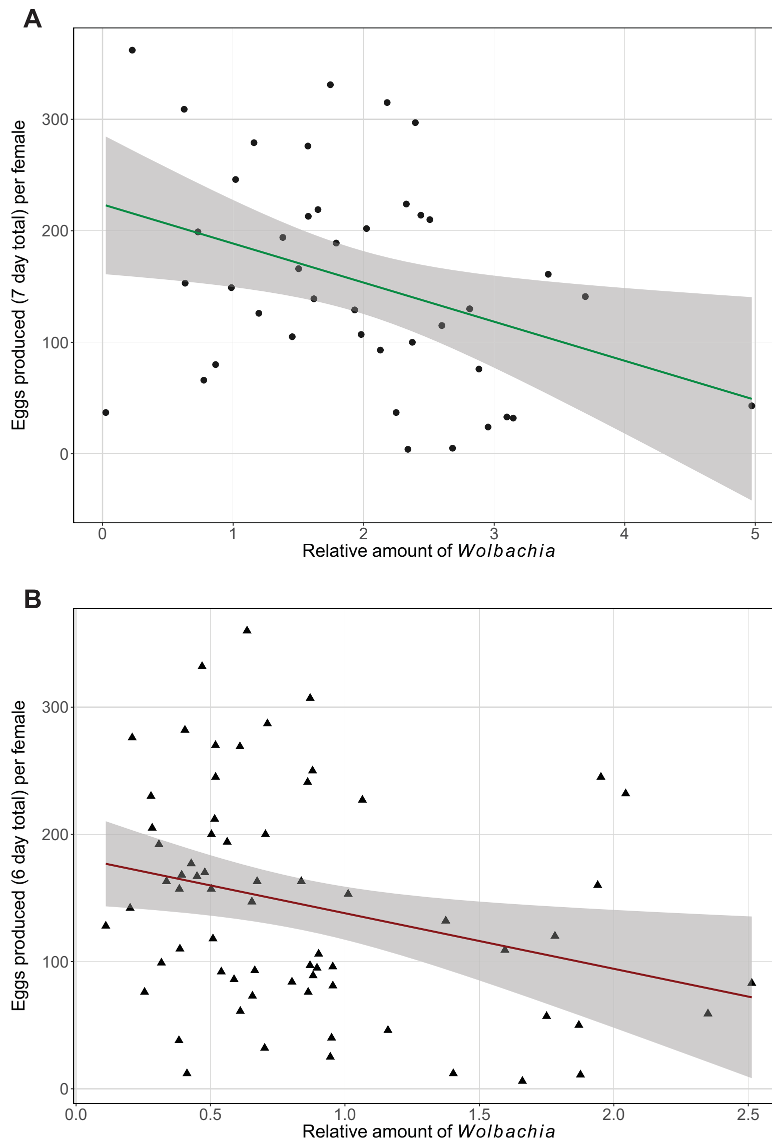

Figure 7. **Relative amount of *Wolbachia* genomic DNA (calculated using host rpII215 as a reference gene) for mothers that underwent induced dormancy compared to number of eggs produced for: (A) original mass-bred lines and (B) introgressed mass-bred lines. Each point represents an average of three PCR replicates. Shaded areas indicate 95% confidence intervals for the gradient of fitted linear models.**

solutions between 0 and 1, corresponding to unstable and stable polymorphic equilibria satisfying $0 < \hat{p}_\mu < \hat{p}_s < 1$. In contrast, with positive fitness effects such that $F(1 - \mu) > 1$, CI (i.e., $H < 1$), and $F\mu < 1$, the coefficients of equation (3) change sign only once. This produces only one equilibrium between 0 and 1, it is stable and given by

$$\hat{p} = \frac{s_h + 1 - F + \sqrt{(s_h + 1 - F)^2 + 4s_h[F(1-\mu) - 1](1 - F\mu)}}{2s_h(1 - F\mu)}. \quad (4)$$

As expected, $\hat{p} = 1$ if $\mu = 0$. With $F(1 - \mu) > 1$ and $\mu > 0$, the stable equilibrium can be anywhere in (0, 1); whereas for $F < 1$, the stable equilibrium generally exceeds ½. To understand the implications of (4), we need plausible parameter values for *w*Mel.

The intensity of CI (s_h) and maternal loss (μ) have been estimated in natural Australian populations. Hoffmann et al. (1998) used field-collected males and females from four different locations ranging from near the middle of the cline (Coffs Harbour, latitude 30°19′S) to the tropical end in far north Queensland (Cairns, latitude 16°54′S). None of the assays found statistically significant evidence for CI, but the resulting confidence intervals are generally broad, with four of six estimates being consistent with low levels of CI, on the order of $s_h = 0.05$. In contrast, Reynolds

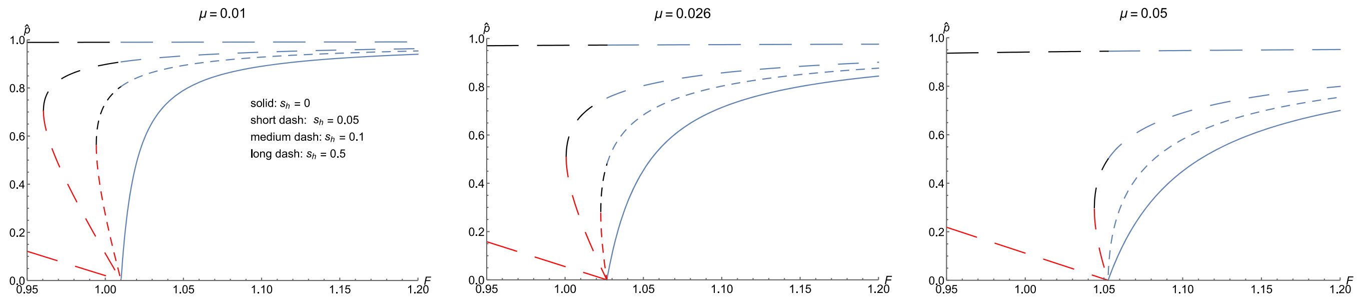

Figure 8. **Equilibrium frequencies of *Wolbachia* infections among adults assuming imperfect maternal transmission (quantified by μ the loss parameter), fitness effects (quantified by *F*, the relative fecundity of infected females), and cytoplasmic incompatibility (quantified by $s_h = 1 - H$, where *H* is the relative hatch rate of embryos produced by incompatible fertilizations). The polymorphic equilibria are solutions of equation (4). For $F(1 - \mu) > 1$, blue lines show the unique stable equilibrium. For $F(1 - \mu) < 1$, black and red lines show the stable and unstable polymorphic equilibria, respectively, and zero is the alternative stable equilibrium.**

and Hoffmann (2002) used one-day-old infected males derived from wild-collected larvae and pupae (from Coffs Harbour) and found average egg hatch frequencies of only 0.39, with 95% confidence interval (0.15, 0.64). Given that CI declines rapidly with male age in *D. melanogaster*, these data collectively suggest that for tropical and subtropical populations, s_h values from 0 to 0.05 are plausible, but $s_h > 0.1$ is unlikely. Hoffmann et al. (1998) used wild-collected females from a mid-cline population (Gold Coast, latitude 30°19′S) to estimate the fidelity of maternal transmission. Their point estimate was $\mu = 0.026$, with 95% confidence interval (0.008, 0.059).

Figure 8 illustrates the polymorphic equilibria produced by equation (3), focusing on values of μ (i.e., 0.01–0.05) and s_h (i.e., 0–0.1) consistent with these data. For comparison, we present equilibria produced with $s_h = 0.5$, typical for *Wolbachia w*Ri in field populations of *D. simulans* (Turelli and Hoffmann 1995; Carrington et al. 2011). A range of *F* values is considered to illustrate combinations of parameters that might produce observed frequencies as stable polymorphisms. As noted above, without CI ($s_h = 0$), $\hat{p}$ increases from 0 toward 1 as *F* increases. The rise is very steep if μ is very small, for example, $\mu = 0.01$, but becomes increasingly gradual for larger μ, for example, $\mu = 0.05$. When $F(1 - \mu) > 1$, 0 is no longer a stable equilibrium (cf. eq. (2)). As *F* increases, the new stable equilibrium can rise continuously from 0, as illustrated with $\mu = 0.05$ and $s_h = 0.05$; or increase discontinuously, as shown with $\mu = 0.05$ and $s_h = 0.1$. To understand this discontinuity, note that when $F(1 - \mu) = 1$, equation (3) produces a linear equation for the equilibrium whose positive solution is

$$\hat{p} = \frac{s_h - \dfrac{m}{1-\mu}}{s_h\left(1 - \dfrac{\mu}{1-\mu}\right)} \tag{5}$$

whenever $s_h > \mu/(1 - \mu)$. Note that equation (5) provides the minimum stable polymorphic equilibrium for a given s_h and μ,

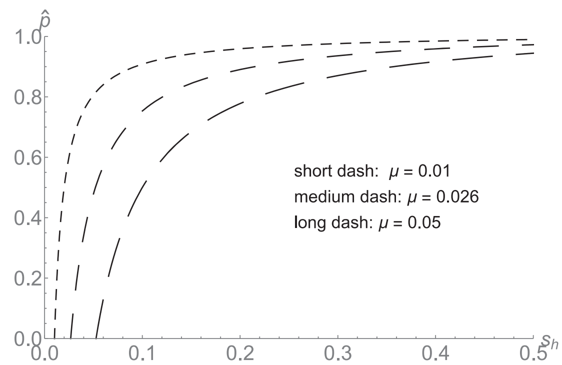

Figure 9. **Minimum stable polymorphic equilibrium frequencies, $\hat{p}$, of *Wolbachia* infections among adults (from eq. 5), assuming $F(1 - \mu) > 1$. The minima are produced by assuming that $F = 1/(1 - \mu)$. The plots show $\hat{p}$ as a function of the magnitude of CI (i.e., values of s_h) for three values of μ.**

because $\hat{p}$ increases with *F* beyond the threshold $F = 1/(1 - \mu)$. Using values of s_h and μ that seem plausible for *D. melanogaster*, Figure 9 shows that the minimal stable $\hat{p}$ rises sharply with small increases in s_h.

In the Australian cline, observed frequencies range from about 0.3 up to about 0.9, but both lower and higher frequencies occur in equatorial Africa (see Fig. 3A). Several qualitative features of the predicted equilibria are notable. If maternal loss is uncommon, for example, $\mu = 0.01$, a stable equilibrium near 0.3 can be produced only if CI is essentially nonexistent, that is, $s_h << 0.05$. Indeed, even with $\mu = 0.026$, a stable $\hat{p} = 0.3$ cannot be produced if $s_h \geq 0.05$. Stable equilibria near 0.3 are much easier to explain with higher rates of maternal loss, for example, $\mu = 0.05$. Thus, if rates of maternal loss are low (e.g., $\mu \leq 0.026$), we expect that field populations which show consistently low *Wolbachia* frequencies are unlikely to exhibit detectable CI—unless *Wolbachia* effects other than viability, fecundity and imperfect transmission are significant (e.g., *Wolbachia*-mediated sperm displacement, cf.

Wade and Chang 1995). The equilibrium predictions (see Fig. 8) suggest that $\hat{p} = 0.3$ is easier to produce with maternal loss closer to $\mu = 0.05$, as in California *D. simulans*. With no CI, a relatively small fitness advantage, on the order of $F = 1.03$, suffices to produce $\hat{p} = 0.3$ with $\mu = 0.026$. In contrast, to produce equilibria near 0.9 with imperfect transmission near $\mu = 0.026$ and little or no CI ($s_h \leq 0.055$), very strong selection, comparable to $F = 1.3$, favoring *Wolbachia* infection must be occurring. Equilibria as large as $\hat{p} = 0.9$ are more easily explained with significant CI ($s_h > 0.05$) or little maternal loss ($\mu < 0.026$).

Discussion

The widespread variation in *Wolbachia* infection frequency previously observed among many *D. melanogaster* populations worldwide represents a conundrum. Simple models including CI predict invasion or loss depending on initial frequencies (Caspari and Watson 1959) or a balance between maternal leakage and CI leading to persistent uninfected individuals at a low frequency (Turelli and Hoffmann 1995). Yet intermediate frequencies appear typical of many *Wolbachia* infections (Ahmed et al. 2015; Weinert et al. 2015) and suggest that factors other than reproductive manipulation often dominate *Wolbachia* dynamics. Here we demonstrate that the *Wolbachia* infection first detected in *D. melanogaster* in eastern Australia (Hoffmann 1988; Hoffmann et al. 1994) shows a stable geographic pattern across 20 years. The stable cline is in sharp contrast to the rapid sweep in both California and eastern Australia of *w*Ri in *D. simulans*. Those sweeps were driven by strong CI (Turelli and Hoffmann 1991; Kriesner et al. 2013). Unlike the relative stability of the *Wolbachia* infection frequencies in *D. melanogaster*, the *w*Au infection in *D. simulans* increased in eastern Australia prior to being eliminated by the *w*Ri invasion (Kriesner et al. 2013).

The clinal pattern in eastern Australia contrasts with data from North America. Where outdoor temperatures are likely to fall significantly below freezing for extended periods, fly populations may be re-established in the spring / summer from individuals that persist in artificially heated, human-constructed environments where a dormancy response is unlikely to be required. Alternatively where few or no flies survive winter outdoors, populations may be significantly supplemented via human-mediated fruit transport. Patterns of genetic variation in sigma viruses suggest persistent overwintering populations in cold locations (Wilfert and Jiggins 2014). This supports the hypothesis that populations in colder temperate regions are repopulated from local refugia rather than being replenished with summer migrants from warmer regions. In contrast, in south-eastern Australia, although eggs did not survive when placed outside over winter, adults persist and overwinter successfully to produce eggs (Mitrovski and Hoffmann 2001; Hoffmann et al. 2003). Therefore selection pressures at particularly high latitudes where no overwintering is possible may be different to those in areas where adults overwinter outside.

Our results indicate that *Wolbachia*-infected adult *D. melanogaster* females which survived an induced period of reproductive dormancy produced fewer eggs overall, fewer viable offspring and generally had a lower proportion of viable ova than uninfected females after the dormancy-inducing condition was released. This provides a clear advantage to uninfected individuals, particularly if there are abundant resources available directly after winter. Furthermore this cost was not simply related to the presence/absence of the infection but reflected a quantitative effect: among infected females there was a negative correlation between *Wolbachia* density and both fecundity and viability of offspring. Thus, our results indicate a fitness deficit for *Wolbachia*-infected mothers over-wintering in climates with temperatures of 9–10°C or below for extended periods. If this time-limited cost is important in south-eastern Australian populations, it may drive annual cycles of *Wolbachia* infection frequency, with frequencies falling between late autumn and early spring. Our capacity to assess this prediction is limited. Sampling from the Melbourne region in early summer and again in late autumn did not indicate an infection frequency increase (actually a decrease, but not significantly so).

Previous research has shown that fecundity can be decreased by the *w*Mel infection when flies are held in field cages in the tropics and temperate areas, although interactions between the infection and temperate/tropical genetic backgrounds may also occur (Olsen et al. 2001). Favorable fecundity effects of *w*Mel have also been detected, although again with genetic background effects (Fry et al. 2004). Our dormancy results point to a *w*Mel fitness cost, in specific environmental conditions, independent of genetic background. The quantitative relationships between *Wolbachia* and fecundity suggest that infection titer affects this cost, rather than just infection presence. The wide tissue distribution and high density of the *w*MelPop infection in *D. melanogaster* is thought to be associated with substantial fitness costs (Min and Benzer 1997) although a relationship between *Wolbachia* and fitness costs is not always clear cut (Hoffmann et al. 2015).

The influence of thermal dormancy on *Wolbachia* fitness effects has not been previously investigated. However in *Aedes*, *Wolbachia* influences fitness particularly during egg diapause, at least for the high-density *w*MelPop strain (McMeniman and O'Neill 2010; Yeap et al. 2011). Keller et al. (2004) found a stable clinal pattern of infection for one of two *Wolbachia* strains (*w*Calt2) infecting Neotropical *Chelymorpha alternans* beetles based on sampling over four years in Panama and mtDNA haplotype analysis. They hypothesized that this pattern was associated with the incidence of dry season diapause in the Pacific side of the beetles' range. In a meta-analysis of *Wolbachia* occurrence in moths and butterflies, Ahmed et al. (2015) found that intraspecific infection frequencies were systematically lower in colder

climates (and the fraction of species infected was also lower). A high proportion of species studied were found to harbor some level of *Wolbachia* infection suggesting these endosymbionts may be particularly influential for lepidopteran ecology. This raises the general issue of whether *Wolbachia* costs are more likely in areas where seasonal dormancy is protracted. Also, Morrow et al. (2015) detected *Wolbachia* infection at low to very low frequencies among Australian tephritid fruit fly populations in tropical locations, whereas the infection was absent in temperate populations. These authors hypothesized a relatively higher incidence of horizontal transfer in tropical regions as a possible explanation of this pattern, associated with higher biodiversity and transience of resulting infections. However, based on the current results, other factors such as overwintering dormancy could also be involved. The challenge is to collect phenotypic data from other insect groups on the impact of *Wolbachia* on hosts and particularly on their host's dormancy. If species cannot be easily cultured or cured of *Wolbachia*, it might still be possible to test for changes in *Wolbachia* frequency before and after a dormant phase.

Although the effect of *Wolbachia* on viability appears to be related to density, the mechanism involved remains unclear. Total egg development time for *Drosophila* is expected to be 24 h or less at 25°C. Thus, flies released from dormancy-inducing conditions might be expected to rapidly purge any partly developed eggs that have accrued a high *Wolbachia* load or suffered deleterious effects, and replace these with fresh oocytes that did not experience any developmental delay. However, longer term effects might be expected if high *Wolbachia* densities influence the provisioning of eggs.

Although our data may help to explain the relatively lower frequency of the infection in southern areas, there remains the issue of accounting for the persistence of *Wolbachia* at low latitudes and the variability in *Wolbachia* frequencies in some equatorial locations. Field collections of *Drosophila* can utilize a variety of methods but these are not always specified. Some sampling methods may not provide a good representation of *Wolbachia* frequencies in a natural population, such as sampling multiple larvae from one breeding site which will often represent the offspring of one or a few females (Hoffmann and Nielsen 1985). It would be interesting to re-sample from equatorial locations with apparently low frequencies across time. On the other hand, some *D. melanogaster* populations may experience relatively higher viral prevalence so that having virus protection as a consequence of *Wolbachia* may be an advantage. The *w*Mel strain provides virus protection in laboratory settings (Hedges et al. 2008; Teixeira et al. 2008), but there are no field data demonstrating protection (Webster et al. 2015). If the age distributions of mating males tend to be younger in tropical locations, our theoretical analysis indicates that even weak average CI produced by young *w*Mel-infected males (Reynolds and Hoffmann 2002; Yamada et al. 2007) may appreciably raise *w*Mel infection frequencies. As noted previously, if selection-transmission (eq. 1) produces a low equilibrium frequency, for example, $\hat{p} = 0.32$ with $\mu = 0.026$ and $F = 1.04$, adding even weak CI will appreciably raise the equilibrium infection frequency, for example, with $s_h = 0.05$, $\mu = 0.026$ and $F = 1.04$, $\hat{p} = 0.63$ (eq. 3). However, maintaining a high infection frequency, on the order of 90%, with even a moderate level of imperfect transmission (e.g., $\mu = 0.015$) and weak CI (less than 10% increase in embryo death from incompatible fertilizations) requires significant net fitness advantage from *Wolbachia* infections. Such effects remain to be identified in nature.

In conclusion, we have demonstrated a stable cline of *Wolbachia* in *D. melanogaster* in eastern Australia, despite high rates of gene flow along this climatic gradient. We find a shallower cline in the eastern United States and no cline in Africa or Eurasia, but in these cases sampling has been more limited and other continents may not provide conditions that produce reproductive dormancy induced selection against *w*Mel we expect in Australia. It will be interesting to track changes in the Australian cline across the next few years, particularly as there is evidence of genetic changes occurring in nuclear markers (Umina et al. 2005). As winter conditions become milder, an increase in *Wolbachia* frequency might be expected in southern parts of the gradient, unless other selective factors such as life-history changes act against them.

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DATA ARCHIVING

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.7t5r1.

LITERATURE CITED

Ahmed, M. Z., E. V. Araujo-Jnr, J. J. Welch, and A. Y. Kawahara. 2015. *Wolbachia* in butterflies and moths: geographic structure in infection frequency. Front. Zool. 12:1–9.

Ahrens, M. E., and D. Shoemaker. 2005. Evolutionary history of *Wolbachia* infections in the fire ant *Solenopsis invicta*. BMC Evol. Biol. 5:1–11.

Bian, G. W., Y. Xu, P. Lu, Y. Xie, and Z. Y. Xi. 2010. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. PLoS Pathog. 6:e1000833.

Brownlie, J. C., B. N. Cass, M. Riegler, J. J. Witsenburg, I. Iturbe-Ormaetxe, E. A. McGraw, and S. L. O'Neill. 2009. Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis*, during periods of nutritional stress. PLoS Pathog. 5:e1000368.

Carrington, L. B., J. R. Lipkowitz, A. A. Hoffmann, and M. Turelli. 2011. A re-examination of *Wolbachia*-induced cytoplasmic incompatibility in California *Drosophila simulans*. PLoS One 6:e22565.

Caspari, E., and G. S. Watson. 1959. On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution 13:568–570.

Champely, S. 2015. pwr: basic functions for power analysis. Available from https://CRAN.R-project.org/package=pwr.

Chrostek, E., M. S. P. Marialva, S. S. Esteves, L. A. Weinert, J. Martinez, F. M. Jiggins, and L. Teixeira. 2013. *Wolbachia* variants induce differential protection to viruses in *Drosophila melanogaster*: a phenotypic and phylogenomic analysis. PLoS Genet. 9:e1003896.

Correa, C. C., and J. W. O. Ballard. 2012. *Wolbachia* gonadal density in female and male *Drosophila* vary with laboratory adaptation and respond differently to physiological and environmental challenges. J. Invertebr. Pathol. 111:197–204.

David, J. R., and P. Capy. 1988. Genetic variation of *Drosophila melanogaster* natural populations. Trends Genet. 4:106–111.

Dyson, E. A., M. K. Kamath, and G. D. D. Hurst. 2002. *Wolbachia* infection associated with all-female broods in *Hypolimnas bolina* (Lepidoptera : Nymphalidae): evidence for horizontal transmission of a butterfly male killer. Heredity 88:166–171.

Early, A. M., and A. G. Clark. 2013. Monophyly of *Wolbachia pipientis* genomes within *Drosophila melanogaster*: geographic structuring, titre variation and host effects across five populations. Mol. Ecol. 22:5765–5778.

Friberg, U., P. M. Miller, A. D. Stewart, and W. R. Rice. 2011. Mechanisms promoting the long-term persistence of a *Wolbachia* infection in a laboratory-adapted population of *Drosophila melanogaster*. PLoS One 6:e16448.

Fry, A. J., M. R. Palmer, and D. M. Rand. 2004. Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster*. Heredity 93:379–389.

Gill, A. C., A. C. Darby, and B. L. Makepeace. 2014. Iron necessity: the secret of *Wolbachia*'s success? PLoS Negl. Trop. Dis. 8:e3224.

Hamm, C. A., D. J. Begun, A. Vo, C. C. R. Smith, P. Saelao, A. O. Shaver, J. Jaenike, and M. Turelli. 2014. *Wolbachia* do not live by reproductive manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. Mol. Ecol. 23:4871–4885.

Harcombe, W., and A. A. Hoffmann. 2004. *Wolbachia* effects in *Drosophila melanogaster*: in search of fitness benefits. J. Invertebr. Pathol. 87:45–50.

Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson. 2008. *Wolbachia* and virus protection in insects. Science 322:702.

Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren. 2008. How many species are infected with *Wolbachia*?—a statistical analysis of current data. FEMS Microbiol. Lett. 281:215–220.

Hoffmann, A. A. 1988. Partial cytoplasmic incompatibility between 2 Australian populations of *Drosophila melanogaster*. Entomol. Exp. Appl. 48:61–67.

Hoffmann, A. A., and K. M. Nielsen. 1985. The effect of resource subdivision on genetic variation in *Drosophila*. Am. Nat. 125:421–430.

Hoffmann, A. A., and M. Turelli. 1997. Cytoplasmic incompatibility in insects. Pp. 42–80 *in* S. L. O'Neill, A. A. Hoffmann, and J. H. Werren, eds. Influential passengers: inherited microorganisms and arthropod reproduction. Oxford Univ. Press, New York.

Hoffmann, A. A., and A. R. Weeks. 2007. Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. Genetica 129:133–147.

Hoffmann, A. A., M. Turelli, and L. G. Harshman. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. Genetics 126:933–948.

Hoffmann, A. A., D. J. Clancy, and E. Merton. 1994. Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. Genetics 136:993–999.

Hoffmann, A. A., D. Clancy, and J. Duncan. 1996. Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility. Heredity 76:1–8.

Hoffmann, A. A., M. Hercus, and H. Dagher. 1998. Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. Genetics 148:221–231.

Hoffmann, A. A., M. Scott, L. Partridge, and R. Hallas. 2003. Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. J. Evol. Biol. 16:614–623.

Hoffmann, A. A., B. L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P. H. Johnson, F. Muzzi, M. Greenfield, M. Durkan, Y. S. Leong, Y. Dong, et al. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476:454–457.

Hoffmann, A. A., P. A. Ross, and G. Rašić. 2015. *Wolbachia* strains for disease control: ecological and evolutionary considerations. Evol. Appl. 8:751–768.

Hosokawa, T., R. Koga, Y. Kikuchi, X. Y. Meng, and T. Fukatsu. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proc. Natl. Acad. Sci. USA 107:769–774.

Huang, W., A. Massouras, Y. Inoue, J. Peiffer, M. Ramia, A. M. Tarone, L. Turlapati, T. Zichner, D. H. Zhu, R. F. Lyman, et al. 2014. Natural variation in genome architecture among 205 *Drosophila melanogaster* genetic reference panel lines. Genome Res. 24:1193–1208.

Ilinsky, Y. 2013. Coevolution of *Drosophila melanogaster* mtDNA and *Wolbachia* genotypes. PLoS One 8:e54373.

Ilinsky, Y. Y., and I. K. Zakharov. 2007. The endosymbiont *Wolbachia* in Eurasian populations of *Drosophila melanogaster*. Russ. J. Genet. 43:748–756.

Izquierdo, J. I. 1991. How does *Drosophila melanogaster* overwinter. Entomol. Exp. Appl. 59:51–58.

Jaenike, J. 2009. Coupled population dynamics of endosymbionts within and between hosts. Oikos 118:353–362.

Jeyaprakash, A., and M. A. Hoy. 2000. Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. Insect Mol. Biol. 9:393–405.

Jiggins, F. M., J. K. Bentley, M. E. N. Majerus, and G. D. D. Hurst. 2001. How many species are infected with *Wolbachia*? Cryptic sex ratio distorters revealed to be common by intensive sampling. Proc. R. Soc. B Biol. Sci. 268:1123–1126.

Karan, D., J. P. Morin, B. Moreteau, and J. R. David. 1998. Body size and developmental temperature in *Drosophila melanogaster*: analysis of body weight reaction norm. J. Therm. Biol. 23:301–309.

Keller, A. 2007. *Drosophila melanogaster's* history as a human commensal. Curr. Biol. 17:R77–R81.

Keller, G. P., D. M. Windsor, J. M. Saucedo, and J. H. Werren. 2004. Reproductive effects and geographical distributions of two *Wolbachia* strains infecting the Neotropical beetle, *Chelymorpha alternans* Boh. (Chrysomelidae, Cassidinae). Mol. Ecol. 13:2405–2420.

King, R. C. 1970. Ovarian development in Drosophila melanogaster. Academic Press, New York.

Kostal, V. 2006. Eco-physiological phases of insect diapause. J. Insect Physiol. 52:113–127.

Kriesner, P., A. A. Hoffmann, S. F. Lee, M. Turelli, and A. R. Weeks. 2013. Rapid sequential spread of two *Wolbachia* variants in *Drosophila simulans*. PLoS Pathog. 9:e1003607.

Lachaise, D., M. L. Cariou, J. R. David, F. Lemeunier, L. Tsacas, and M. Ashburner. 1988. Historical biogeography of the *Drosophila melanogaster* species subgroup. Evol. Biol. 22:159–225.

Lack, J. B., C. M. Cardeno, M. W. Crepeau, W. Taylor, R. B. Corbett-Detig, K. A. Stevens, C. H. Langley, and J. E. Pool. 2015. The *Drosophila* genome nexus: a population genomic resource of 623 *Drosophila melanogaster* genomes, including 197 from a single ancestral range population. Genetics 199:1229–1241.

Lee, S. F., C. M. Sgro, J. Shirriffs, C. W. Wee, L. Rako, B. van Heerwaarden, and A. A. Hoffmann. 2011. Polymorphism in the couch potato gene clines in eastern Australia but is not associated with ovarian dormancy in *Drosophila melanogaster*. Mol. Ecol. 20:2973-2984.

Lee, S. F., V. L. White, A. R. Weeks, A. A. Hoffmann, and N. M. Endersby. 2012. High-throughput PCR assays to monitor *Wolbachia* infection in the dengue mosquito (*Aedes aegypti*) and *Drosophila simulans*. Appl. Environ. Microbiol. 78:4740–4743.

Li, Y. Y., K. D. Floate, P. G. Fields, and B. P. Pang. 2014. Review of treatment methods to remove *Wolbachia* bacteria from arthropods. Symbiosis 62:1–15.

McMeniman, C. J., and S. L. O'Neill. 2010. A virulent *Wolbachia* infection decreases the viability of the dengue vector *Aedes aegypti* during periods of embryonic quiescence. PLoS Negl. Trop. Dis. 4:e748:1–6.

Min, K. T., and S. Benzer. 1997. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. Proc. Natl. Acad. Sci. USA 94:10792–10796.

Mitrovski, P., and A. A. Hoffmann. 2001. Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions. Proc. R. Soc. Lond. B Biol. Sci. 268:2163–2168.

Montenegro, H., A. S. Petherwick, G. D. D. Hurst, and L. B. Klaczko. 2006. Fitness effects of *Wolbachia* and *Spiroplasma* in *Drosophila melanogaster*. Genetica 127:207–215.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, et al. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium. Cell 139:1268–1278.

Moriyama, M., N. Nikoh, T. Hosokawa, and T. Fukatsu. 2015. Riboflavin provisioning underlies *Wolbachia's* fitness contribution to its insect host. MBio 6:1–8.

Morrow, J. L., M. Frommer, J. E. Royer, D. C. A. Shearman, and M. Riegler. 2015. *Wolbachia* pseudogenes and low prevalence infections in tropical but not temperate Australian tephritid fruit flies: manifestations of lateral gene transfer and endosymbiont spillover? BMC Evol. Biol. 15:1–16.

Narita, S., Y. Shimajiri, and M. Nomura. 2009. Strong cytoplasmic incompatibility and high vertical transmission rate can explain the high frequencies of *Wolbachia* infection in Japanese populations of *Colias erate poliographus* (Lepidoptera: Pieridae). Bull. Entomol. Res. 99:385–391.

Nunes, M. D. S., V. Nolte, and C. Schlötterer. 2008. Nonrandom *Wolbachia* infection status of *Drosophila melanogaster* strains with different mtDNA haplotypes. Mol. Biol. Evol. 25:2493–2498.

Olsen, K., K. T. Reynolds, and A. A. Hoffmann. 2001. A field cage test of the effects of the endosymbiont *Wolbachia* on *Drosophila melanogaster*. Heredity 86:731–737.

O'Neill, S. L., A. A. Hoffmann, and J. H. Werren. 1997. Influential passengers: inherited microorganisms and arthropod reproduction. Oxford Univ. Press, New York.

Osborne, S. E., Y. S. Leong, S. L. O'Neill, and K. N. Johnson. 2009. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. PLoS Pathog. 5:e1000656.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:e45:2002–2007.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.R-project.org/.

Rasgon, J. L., and T. W. Scott. 2003. *Wolbachia* and cytoplasmic incompatibility in the California *Culex pipiens* mosquito species complex: parameter estimates and infection dynamics in natural populations. Genetics 165:2029–2038.

Reynolds, K. T., and A. A. Hoffmann. 2002. Male age, host effects and the weak expression or nonexpression of cytoplasmic incompatibility in *Drosophila* strains infected by maternally transmitted *Wolbachia*. Genet. Res. 80:79–87.

Richardson, M. F., L. A. Weinert, J. J. Welch, R. S. Linheiro, M. M. Magwire, F. M. Jiggins, and C. M. Bergman. 2012. Population genomics of the *Wolbachia* endosymbiont in *Drosophila melanogaster*. PLoS Genet. 8:e1003129.

Riegler, M., M. Sidhu, W. J. Miller, and S. L. O'Neill. 2005. Evidence for a global *Wolbachia* replacement in *Drosophila melanogaster*. Curr. Biol. 15:1428–1433.

Saunders, D. S., V. C. Henrich, and L. I. Gilbert. 1989. Induction of diapause in *Drosophila melanogaster*—Photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. Proc. Natl. Acad. Sci. USA 86:3748–3752.

Schmidt, P. S., L. Matzkin, M. Ippolito, and W. F. Eanes. 2005. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution 59:1721–1732.

Shoemaker, D. D., M. Ahrens, L. Sheill, M. Mescher, L. Keller, and K. G. Ross. 2003. Distribution and prevalence of *Wolbachia* infections in native populations of the fire ant *Solenopsis invicta* (Hymenoptera : Formicidae). Environ. Entomol. 32:1329–1336.

Stephan, W., and H. Li. 2007. The recent demographic and adaptive history of *Drosophila melanogaster*. Heredity 98:65–68.

Tagami, Y., and K. Miura. 2004. Distribution and prevalence of *Wolbachia* in Japanese populations of Lepidoptera. Insect Mol. Biol. 13:359–364.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal adaptations of insects. Oxford Univ. Press, New York.

Teixeira, L., A. Ferreira, and M. Ashburner. 2008. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. PLoS Biol. 6:e1000002.

Turelli, M., and A. A. Hoffmann. 1991. Rapid spread of an inherited incompatibility factor in California *Drosophila*. Nature 353:440–442.

———. and ———. 1995. Cytoplasmic incompatibility in *Drosophila simulans*—dynamics and parameter estimates from natural populations. Genetics 140:1319–1338.

Umina, P. A., A. R. Weeks, M. R. Kearney, S. W. McKechnie, and A. A. Hoffmann. 2005. A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. Science 308:691–693.

Unckless, R. L., and J. Jaenike. 2012. Maintenance of a male-killing *Wolbachia* in *Drosophila innubila* by male-killing dependent and male-killing independent mechanisms. Evolution 66:678–689.

Ventura, I. M., A. B. Martins, M. L. Lyra, C. A. C. Andrade, K. A. Carvalho, and L. B. Klaczko. 2012. *Spiroplasma* in *Drosophila melanogaster* populations: prevalence, male-killing, molecular identification, and no association with *Wolbachia*. Microb. Ecol. 64:794–801.

Versace, E., V. Nolte, R. V. Pandey, R. Tobler, and C. Schlotterer. 2014. Experimental evolution reveals habitat-specific fitness dynamics among *Wolbachia* clades in *Drosophila melanogaster*. Mol. Ecol. 23:802–814.

Verspoor, R. L., and P. R. Haddrill. 2011. Genetic diversity, population structure and *Wolbachia* infection status in a worldwide sample of *Drosophila melanogaster* and *D. simulans* populations. PLoS One 6:e26318.

Wade, M. J., and N. W. Chang. 1995. Increased male-fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. Nature 373:72–74.

Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. S. Leong, Y. Dong, J. Axford, P. Kriesner, et al. 2011. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature 476:450–453.

Webster, C. L., F. M. Waldron, S. Robertson, D. Crowson, G. Ferrari, J. F. Quintana, J.-M. Brouqui, E. H. Bayne, B. Longdon, A. H. Buck, et al. 2015. The discovery, distribution, and evolution of viruses associated with *Drosophila melanogaster*. PLoS Biol. 13:e1002210.

Weeks, A. R., M. Turelli, W. R. Harcombe, K. T. Reynolds, and A. A. Hoffmann. 2007. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biol. 5:e114.

Weinert, L. A., E. V. Araujo-Jnr, M. Z. Ahmed, and J. J. Welch. 2015. The incidence of bacterial endosymbionts in terrestrial arthropods. Proc. R. Soc. B Biol. Sci. 282:1–6.

Werren, J. H., and D. M. Windsor. 2000. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? Proc. R. Soc. Lond. B Biol. Sci. 267:1277–1285.

Wilfert, L., and F. M. Jiggins. 2014. Flies on the move: an inherited virus mirrors *Drosophila melanogaster's* elusive ecology and demography. Mol. Ecol. 23:2093–2104.

Woolfit, M., I. Iturbe-Ormaetxe, J. C. Brownlie, T. Walker, M. Riegler, A. Seleznev, J. Popovici, E. Rances, B. A. Wee, J. Pavlides, et al. 2013. Genomic evolution of the pathogenic *Wolbachia* strain, *w*MelPop. Genome Biol. Evol. 5:2189–2204.

worldweatheronline.com. 2015. Poolesville weather, United States of America.

Wu, M., L. V. Sun, J. Vamathevan, M. Riegler, R. Deboy, J. C. Brownlie, E. A. McGraw, W. Martin, C. Esser, N. Ahmadinejad, et al. 2004. Phylogenomics of the reproductive parasite *Wolbachia pipientis w*Mel: a streamlined genome overrun by mobile genetic elements. PLoS Biol. 2:327–341.

Yamada, R., K. D. Floate, M. Riegler, and S. L. O'Nein. 2007. Male development time influences the strength of *Wolbachia*-induced cytoplasmic incompatibility expression in *Drosophila melanogaster*. Genetics 177:801–808.

Yeap, H. L., P. Mee, T. Walker, A. R. Weeks, S. L. O'Neill, P. Johnson, S. A. Ritchie, K. M. Richardson, C. Doig, N. M. Endersby, et al. 2011. Dynamics of the "Popcorn" *Wolbachia* infection in outbred *Aedes aegypti* informs prospects for mosquito vector control. Genetics 187:583–U346.

Zhang, H., K. J. Zhang, and X. Y. Hong. 2010. Population dynamics of noncytoplasmic incompatibility-inducing *Wolbachia* in *Nilaparvata lugens* and its effects on host adult life span and female fitness. Environ. Entomol. 39:1801–1809.

Zhang, Y. K., K. J. Zhang, J. T. Sun, X. M. Yang, C. Ge, and X. Y. Hong. 2013. Diversity of *Wolbachia* in natural populations of spider mites (genus Tetranychus): evidence for complex infection history and disequilibrium distribution. Microb. Ecol. 65:731–739.

Zhao, D. X., D. S. Chen, C. Ge, T. Gotoh, and X. Y. Hong. 2013. Multiple infections with *Cardinium* and two strains of *Wolbachia* in the spider mite *Tetranychus phaselus* Ehara: Revealing new forces driving the spread of *Wolbachia*. Plos One 8:e54964:1–9.

Zug, R., and P. Hammerstein. 2012. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS One 7:e38544.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. *Wolbachia* infection frequencies for *D. melanogaster* populations from eastern Australia and North America by location.
Table S2. *Wolbachia* infection frequencies for *D. melanogaster* populations from Africa and Eurasia by location.
Methods S1. Bioinformatic analysis.
Figure S1. Wing size measurements taken based on landmarks.
Figure S2. Trend in (A) average egg production per female per day after transfer from the dormancy inducing conditions to 25°C and 12:12 h light:dark, and (B) average number of viable F1 offspring per female that resulted from those eggs.
Figure S3. Wing length for unexposed control males and F1 males arising from eggs produced by dormancy-exposed mothers.