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ORIGINAL ARTICLE

Population dynamics and rapid spread of *Cardinium*, a bacterial endosymbiont causing cytoplasmic incompatibility in *Encarsia pergandiella* (Hymenoptera: Aphelinidae)

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Cytoplasmic incompatibility (CI) is a common phenotype of maternally inherited bacterial symbionts of arthropods; in its simplest expression, uninfected females produce few or no viable progeny when mated to infected males. Infected females thus experience a reproductive advantage relative to that of uninfected females, with the potential for the symbiont to spread rapidly. CI population dynamics are predicted to depend primarily on the strength of incompatibility, the fitness cost of the infection and how faithfully symbionts are inherited. Although the bacterial symbiont lineage *Wolbachia* has been most identified with the CI phenotype, an unrelated bacterium, *Cardinium* may also cause CI. In the first examination of population dynamics of CI-inducing *Cardinium*, we used population cages of the parasitic wasp *Encarsia pergandiella* (Hymenoptera: Aphelinidae) with varying initial infection frequencies to test a model of invasion. *Cardinium* was found to spread rapidly in all populations, even in cases where the initial infection frequency was well below the predicted invasion threshold frequency. The discrepancy between the modeled and actual results is best explained by weaker CI than measured in the lab and a cryptic fitness benefit to the infection.
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Introduction

A large number of insect species harbor maternally inherited bacterial symbionts that have diverse effects on their hosts (Moran *et al*., 2008). Many symbionts are beneficial to their hosts, providing them with essential nutrients (Buchner, 1965; Baumann, 2005) or protection from parasitoids or pathogens (Oliver *et al*., 2003; Scarborough *et al*., 2005). On the other hand, some symbionts are known as reproductive manipulators, and do not confer direct benefits to their hosts, but instead manipulate their host's reproduction in ways that increase their transmission.

The most common reproductive manipulation strategy is cytoplasmic incompatibility (CI), whereby uninfected females produce few or no viable progeny when mated with infected males. Infected females, in contrast, can mate with either uninfected or infected males without negative effects. In this way, infected females experience a reproductive advantage relative to that of uninfected females, and rapid spread of the CI symbiont can result (Caspari and Watson, 1959; Turelli and Hoffmann, 1991; Engelstadter and Telschow, 2009). The ability of CI symbionts to spread has many potential applications, including population suppression or the introduction of beneficial genes into an insect population (Sinkins *et al*., 1997; Zabalou *et al*., 2004; Xi *et al*., 2005; Brelsfoard *et al*., 2008; McMeniman *et al*., 2009).

Two bacterial endosymbionts have been found to be able to induce CI in arthropods. *Wolbachia*, in the α-proteobacteria, is the best known reproductive manipulator, infecting at least 16–24% of all arthropod species (Werren *et al*., 1995; Weeks *et al*., 2003; Zchori-Fein and Perlman, 2004) and potentially as many as 66%, when low prevalence infections are considered (Hilgenboecker *et al*., 2008). *Wolbachia* has been shown to cause CI in all of the major insect orders, as well as in mites and isopods (Stouthamer *et al*., 1999). More recently, *Cardinium*, a member of the phylum Bacteroidetes, has been found to cause CI in three hosts: in the parasitoid wasp *Encarsia pergandiella* (Hunter *et al*., 2003), and in the spider mites *Eotetranychus suginamensis* and *Bryobia sarothamni* (Gotoh *et al*., 2007; Ros and Breeuwer, 2009). Since its discovery, *Cardinium* has been found in four orders and approximately 6–7% of arthropods (Weeks *et al*., 2003; Zchori-Fein and Perlman, 2004; Duron *et al*., 2008). *Wolbachia* and *Cardinium* are primarily vertically transmitted (Hoffmann *et al*., 1990; Perlman *et al*., 2008), although rare horizontal transmission events are thought to occur on an evolutionary timescale (Werren, 1997; Zchori-Fein and Perlman, 2004).

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The spread and equilibrium frequency of a CI-inducing symbiont within a population will depend on its relative costs and benefits to the host (Caspari and Watson, 1959; Turelli, 1994; Turelli and Hoffmann, 1995; Vavre *et al*., 2000). The symbiont's ability to induce incompatible matings will facilitate its spread within a population, because infected females experience a reproductive advantage relative to uninfected females. However, if there is a cost associated with the infection, for example, decreased fecundity, this will reduce the symbiont's ability to spread. Consequently, the infection is expected to spread when the benefits to hosts of being infected (that is, higher reproductive success) outweigh the fitness costs. Although fitness costs, such as reduced fecundity, are not expected to vary with infection frequency, the reproductive benefits associated with the infection are frequency dependent. In a population with a high infection frequency, a female is very likely to mate with an infected male, and thus benefits greatly from being infected. However, in a population where the infection is rare, a female is not very likely to mate with an infected male, and therefore receives little benefit in harboring the infection. At low frequency, therefore, fecundity costs might be expected to outweigh potential reproductive benefits.

CI invasion and spread are thought to depend primarily on three key parameters: any losses in maternal transmission (μ), the relative fitness of infected females compared to uninfected females (F) and the offspring production of incompatible crosses relative to compatible crosses (H) (Caspari and Watson, 1959; Turelli, 1994; Turelli and Hoffmann, 1995; Vavre *et al*., 2000). Models of CI dynamics make two key predictions. First, the equilibrium frequency of infection is predicted to be stable in a population at two points: when the infection is at or near fixation and when it is completely lost from the population (Turelli, 1994). Second, an unstable equilibrium, also termed the invasion threshold, is found between these two stable infection frequencies. When the frequency of infection is higher than the invasion threshold, the infection is expected to spread until it is at or near fixation. Alternatively, if the infection frequency is lower than the invasion threshold, the symbiont will be lost from the population (Turelli, 1994).

Understanding the invasion and spread of CI symbionts is critical for the successful application of CI for disease and pest control (Rasgon, 2008). Moreover, virtually no studies to date have tested theoretical models of CI spread, and such studies are essential to link our theoretical knowledge of CI with empirical data (Engelstadter and Telschow, 2009). In addition, cytoplasmic incompatibility models may be inaccurate if other factors are influencing CI spread, such as population subdivision or cryptic fitness costs or benefits (Egas *et al*., 2002). For instance, Xi *et al*. (2005) used population cages to monitor the spread of CI *Wolbachia* within a mosquito population. They found that *Wolbachia* did not spread until the initial infection frequency was well above the predicted invasion threshold, suggesting that there was a cryptic fitness cost associated with the infection that was not revealed in laboratory assays of individuals (Xi *et al*., 2005).

In the parasitoid wasp *E. pergandiella*, the CI symbiont *Cardinium* was found to induce a high fitness cost, resulting in a relatively high predicted invasion threshold of 20–24% (Perlman *et al*., 2008). The prediction of a high invasion threshold is surprising, because field populations are found at or near fixation (Perlman *et al*., 2008). To examine this prediction, we established *E. pergandiella* populations with varying initial *Cardinium* infection frequencies and monitored the dynamics of the infection for nine generations. In addition to testing a model of CI invasion using laboratory estimates of parameter values, this study allowed us to more accurately estimate the threshold frequency and host fitness effects of *Cardinium* under more realistic conditions.

Methods

Cultures

E. pergandiella is a solitary hymenopteran parasitoid of whiteflies with an unusual 'autoparasitic' biology (Hunter and Woolley, 2001); females lay single female eggs in whitefly nymphs, whereas male eggs are laid in developing wasp larvae or pupae, either their own species, or other primary parasitoids, enclosed within the whitefly cuticle. The infected line was collected from *Bemisia tabaci* hosts in the Rio Grande Valley in Texas in 2003, and was maintained in the laboratory on *B. tabaci* reared on cowpea plants (*Vigna unguiculata*). This line is fixed for *Cardinium* infection and is not infected with *Wolbachia* (Hunter *et al*., 2003). The infection is primarily maternally transmitted, and a previous study by Perlman *et al*. (2008) suggests that paternal and/or horizontal transmission is absent or rare. An uninfected line was obtained by curing a subpopulation of the infected line by treating adult wasps with 50 mg ml^{-1} rifampicin in honey for three generations.

Strength of cytoplasmic incompatibility

The number of offspring produced by the incompatible cross relative to the compatible cross will strongly influence the invasion and spread of a CI symbiont (Turelli, 1994). To determine the strength of CI in *Cardinium*-infected *E. pergandiella*, we mated freshly emerged uninfected virgin females individually to 1- to 2-day-old virgin males of known infection status. Each female was then transferred to a 35 mm Petri dish containing 1% agar, upon which a cowpea leaf disk was placed. Females were allowed to oviposit on leaf disks infested with 50–100 whiteflies in the third to early fourth nymphal stage for 24 h.

Following the oviposition period, infested leaves were incubated at 27 °C until *E. pergandiella* pupae could be counted and removed (8–10 days). When an *E. pergandiella* egg fails to develop due to CI, the parasitized whitefly is developmentally arrested (Hunter *et al*., 2003). Therefore, the number of arrested whiteflies served as an indicator of CI. Whiteflies were considered developmentally arrested if, by the time of scoring, they had not developed eyespots or wing buds. The majority of whiteflies in the control treatment had already emerged at the time of scoring. Females that did not produce any pupae and produced <2 whiteflies scored as developmentally arrested were presumed to be unmated and were removed from the analysis.

The average reduction in the number of pupae produced by the incompatible cross (infected

male × cured female) relative to the average number of pupae produced by the compatible cross (cured male × cured female) was measured as H.

Invasion models

The spread of *Cardinium* was modeled using the Hoffmann–Turelli model (Turelli, 1994; Turelli and Hoffmann, 1995), modified by Vavre *et al*. (2000) for haplodiploid hosts with the female mortality type of CI:

$$f_{t+1} = \frac{Ff_t(1-\mu)}{Ff_t[1-\mu(1-H)m_t] + (1-f_t)[1-(1-H)m_t]}$$

$$m_{t+1} = \frac{Ff_t(1-\mu)}{Ff_t + (1-f_t)}$$

where f_t is the female infection frequency at time t, f_{t+1} the female infection frequency one generation after time t, F the number of offspring produced by infected females relative to uninfected females and can thus describe a fitness cost ($F<1$) or a fitness benefit ($F>1$) of the infection, μ the loss in maternal transmission efficiency, H the proportion of offspring produced by the incompatible cross relative to the compatible cross, m_t the male infection frequency at time t and m_{t+1} the male infection frequency one generation after time t. The invasion threshold frequency was calculated using equation A5 in Vavre *et al*. (2000).

Population cages

E. pergandiella wasps of known infection status were introduced to 50 cm^3 cages (four cages per treatment) to establish populations with low (15%), medium (36% for females and 31% for males) and high (55%) initial infection frequencies. The infection frequencies used to establish the medium treatment were at the predicted invasion threshold, when using the following laboratory estimates of infection parameter values. In a previous study, *Cardinium*-infected female *E. pergandiella* produced 18% fewer offspring than uninfected females when supplied with unlimited whitefly hosts over a period of 4 days (Perlman *et al*., 2008). Therefore, as an upper limit to F, we estimated the fitness cost of the infection to be 18% ($F=0.82$). Maternal transmission efficiency was nearly perfect among field-caught females (Perlman *et al*., 2008), so we used the conservative estimate of 99% ($\mu=0.01$). In this study we found that, on average, the incompatible cross produced 38% of the offspring of the control cross ($H=0.38$). Given these parameter estimates, we predicted that the invasion frequency required for *Cardinium* to spread was 36% for females and 31% for males.

The initial infection frequencies were established using three successive weekly introductions of 50 female and 24–33 male adult wasps. All wasps were 3–5 days old at the time of introduction. Because *E. pergandiella* is autoparasitic, plants bearing whitefly hosts for female eggs and heterospecific parasitoid pupae for male eggs were both provided in the cages. The majority of whiteflies were third instar nymphs at the time that plants were introduced to the cages, whereas the majority of the parasitoids, *Eretmocerus eremicus*, were early pupae. Each week, wasps were provided with two cowpea plants infested with *B. tabaci* and an additional plant infested with *E. eremicus* developing on *B. tabaci*. Plants were removed from the cages before adult wasps of either *E. pergandiella* or *E. eremicus* emerged, the leaves placed in an emergence jar and a subset of 50 female and 30 adult males from the new generation were re-introduced to the cage. In generation three, there was a shortage of males in some cages due to the poor health of plants infested with *E. eremicus*. Therefore, male introductions in two cages of the 'low treatment', two cages of the 'medium treatment' and one cage of the 'high treatment' were supplemented with 30, 30, 25, 13 and 8 males from the previous week of the same cage, respectively. We expect the effect of using males of the previous week to be minimal and, if anything, to slow down any increases in infection. Infection frequencies in each cage were estimated using PCR at generations 2, 4, 6, 8 and 9 (see below). Wasps were frozen or stored in 95% ethanol until DNA extraction.

DNA extractions

To assess *Cardinium* infection, we performed single wasp DNA extractions on at least 50 female *E. pergandiella* wasps from each cage for each generation tested. DNA was extracted by grinding individual wasps in 3 µl of 20 mg ml^{-1} proteinase k, and adding the homogenate to 50 µl of 5–10% w/v Chelex (White *et al*., 2009). Samples were incubated at 37 °C for 1 h and then at 96 °C for 8 min, with periodic vortexing. Samples were stored at −20 °C for a maximum of 3 months.

Diagnostic PCR

Wasps were screened for the presence of *Cardinium* using diagnostic PCR. Two sets of *Cardinium*-specific primers were used, Ch441F (GTACAGGAGCAAAACAATCCC) and either Ch665R (TATTCTTAACTCAAGCCTAAT) or Ch1017R (ATTTTTCAAAGTAGCAAAATA), which amplified an ~200- and 600-bp region of 16S rDNA, respectively (developed by Stephan Schmitz-Esser, University of Vienna). PCR conditions for Ch441F and Ch665R were a 3 min initial denaturation at 94 °C, followed by 40 cycles of 94 °C for 30 s, 53 °C for 45 s and 72 °C for 45 s, and a final extension of 72 °C for 5 min. PCR conditions for Ch441F and Ch1017R were a 3 min initial denaturation at 94 °C, followed by 40 cycles of 94 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 6 min. Samples that appeared to be negative for *Cardinium* were screened twice more. A positive control of an infected *E. pergandiella* and a negative control of a cured *E. pergandiella* were included in each run.

Samples determined to be negative for *Cardinium* were screened for single- or double-copy *E. pergandiella* genes (EF1α, opsin or histone) as a positive control for the DNA extraction. An ~380-bp segment of EF1α was amplified using EpergEF-F and EpergEF-R (Perlman *et al*., 2008) with PCR conditions of 95 °C for 3 min, followed by 30 cycles of 95 °C for 60 s, 50 °C for 60 s and 72 °C for 90 s, with a final extension of 72 °C for 10 min. An ~1.5-kb fragment of the opsin gene was amplified using LWRh-F and LWRh-R according to Mardulyn and Cameron (1999), and a faint band of ~700 bp was often also observed, likely because this gene is duplicated in Hymenoptera. An ~400-bp region of histone was amplified using H3Af and H3Ar (Colgan *et al*., 1998)

with PCR conditions of 94 °C for 3 min, followed by 40 cycles of 94 °C for 45 s, 65 °C for 45 s and 72 °C for 60 s, with a final extension of 72 °C for 6 min. A positive control of *E. pergandiella* and a negative no-DNA control were included in each EF1α, opsin and histone PCR. Samples that appeared negative for *E. pergandiella* DNA were excluded from further analysis. At least one PCR product of each gene was sequenced for confirmation (Macrogen Inc., Seoul, South Korea). For sequencing, all PCR products were purified using a QIAquick PCR purification kit (Qiagen), except for opsin, which was first cloned into *Escherichia coli* using a StrataClone PCR cloning kit (Stratagene, La Jolla, CA, USA). Novel opsin and histone sequences were deposited in GenBank under the accession numbers FJ483848 and FJ842099, respectively.

Statistical analysis

A Mann–Whitney test was used to determine if wasp offspring number was significantly different between the predicted incompatible and compatible crosses. To determine if there were significant changes in infection frequency over the nine-generation period, we calculated a replicated goodness-of-fit test. Logistic regression analysis was performed using the binomial response variable of the presence or absence of infection within individual wasps, where each population cage was nested within treatment (SAS Institute, Cary, NC, USA). This analysis allowed for the identification of variables that were significant predictors of the probability of infection with *Cardinium*. The Hosmer and Lemeshow goodness-of-fit test was used to examine fit between the logistic regression model and the data.

To determine the infection parameter values of the model that best fit our observed data, we simultaneously varied all three parameters ($0 \leqslant F \leqslant 2$; $0 \leqslant H \leqslant 1$; $0 \leqslant \mu \leqslant 1$) using increments of 0.01, and calculated the global sum of squares between the modeled predictions and the observed infection frequencies for all treatments. We selected the parameter values of best fit as the combination of values that resulted in the lowest sum of squares (MATLAB 7.4; The MathWorks, MA, USA). We also determined the parameter values that best fit the observed data when H was fixed at the experimentally determined value of 0.38 in a similar fashion.

Results

Wasp offspring number was significantly lower when uninfected females were mated with infected males (that is, the incompatible cross) compared with when they were mated with uninfected males (that is, the compatible cross; Mann–Whitney test; $U = 55.5$; $n = 47$; $P < 0.0001$). On average, incompatible crosses produced 38% of the offspring produced by the compatible crosses ($H = 0.38$).

Cardinium was found to spread rapidly in *E. pergandiella* for all three initial infection frequencies (Figure 1). Initial infection frequencies were significantly different from the infection frequencies at generation nine for the treatment below ($G_P = 111.37$; df = 1; $P < 0.001$), at ($G_P = 257.45$; df = 1; $P < 0.001$) and above the predicted invasion threshold ($G_P = 176.28$; df = 1; $P < 0.001$). There was considerable variation in infection frequencies between cages within treatments (Figure 1), and this

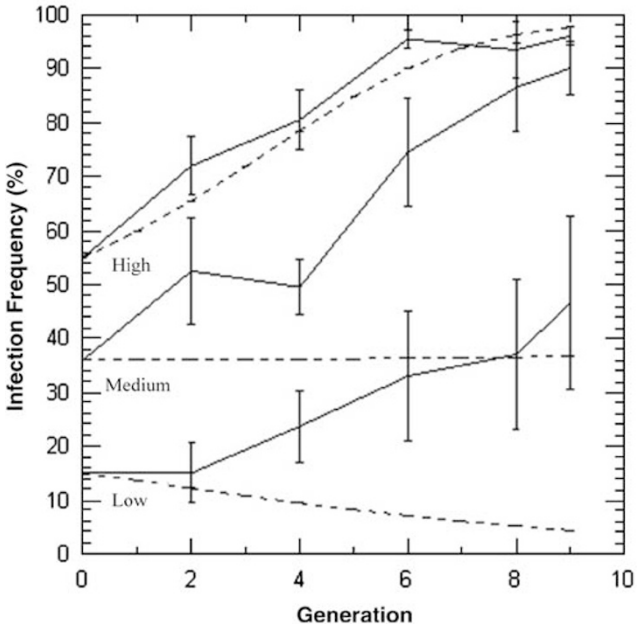

Figure 1 Spread of the bacterial symbiont *Cardinium* in *Encarsia pergandiella* population cages with initial infection frequencies of 15, 36 and 55% (solid lines) compared with predicted spread based on infection parameter estimates of fitness cost $F = 0.82$, maternal transmission efficiency $\mu = 0.01$ and cytoplasmic incompatibility (CI) strength $H = 0.38$ (dotted lines). Data represent the mean ± standard error, $n = 4$.

was particularly true in generation 2, where the infection frequency of one cage in the medium treatment was 29% higher than the average for the other cages in this treatment. Generation ($P < 0.001$), treatment ($P < 0.001$), cage ($P < 0.002$) and the interaction between generation and cage ($P < 0.0001$) were significant predictors of the probability of *Cardinium* infection. The logistic regression model generated using these parameters showed a good fit to the data (Hosmer and Lemeshow goodness–of-fit test $\chi^2 = 9.01$; df = 8; $P > 0.34$). The interaction between treatment and generation was not associated with the probability of infection (logistic regression; $P > 0.05$).

The observed data best fit a model with a fitness benefit of $F = 1.09$, a loss in maternal transmission of $\mu = 0.01$ and a reduction in incompatible offspring of $H = 0.61$ (Figures 2a and 3a). When H was fixed to the experimentally determined value of 0.38, an optimal fit to the observed data was found using a fitness benefit ($F = 1.12$) and a loss in maternal transmission of $\mu = 0.07$ (Figures 2b and 3b). Although these parameter value combinations fit optimally, a range of parameter values provided good fit to the observed data (that is, sum of squares <1), as seen in Figure 3.

Discussion

Cardinium was found to spread rapidly in *E. pergandiella* for all initial infection frequencies (Figure 1), suggesting that the invasion threshold is much lower than that of our modeled predictions. Although this is the first study on population dynamics of *Cardinium*-induced CI, a few studies have documented the invasion and spread of CI *Wolbachia*. The rapid increase in infection prevalence observed for *Cardinium* in *E. pergandiella* is similar to that

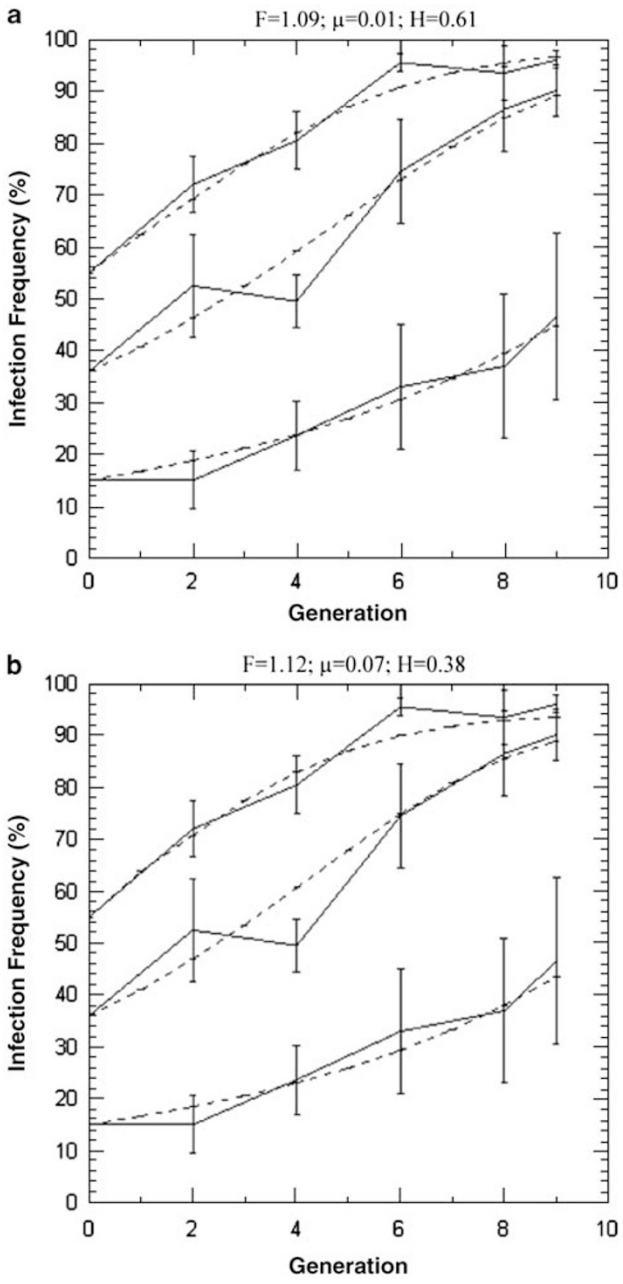

Figure 2 Spread of *Cardinium* using model parameter values (dotted lines) compared with observed infection frequencies in population cages (solid lines). Data represent the mean ± standard error, $n = 4$. (**a**) Model of best fit where the fitness benefit $F = 1.09$; the proportion of offspring produced by the incompatible cross relative to the compatible cross $H = 0.61$ and infidelity of maternal transmission $\mu = 0.01$. (**b**) Model of best fit when H is fixed at 0.38 (determined experimentally); fitness benefit $F = 1.12$ and infidelity of maternal transmission $\mu = 0.07$.

found in populations infected with a CI-inducing *Wolbachia*. For example, in their population cage study, Nigro and Prout (1990) showed that a *Wolbachia* causing CI in *Drosophila simulans* increased in prevalence from an initial infection frequency of 20% within a few generations. Similarly, a *Wolbachia* causing CI in *Drosophila melanogaster* increased in prevalence from 50% to near fixation within five generations (Reynolds and Hoffmann, 2002). Turelli and Hoffmann (1991) also reported

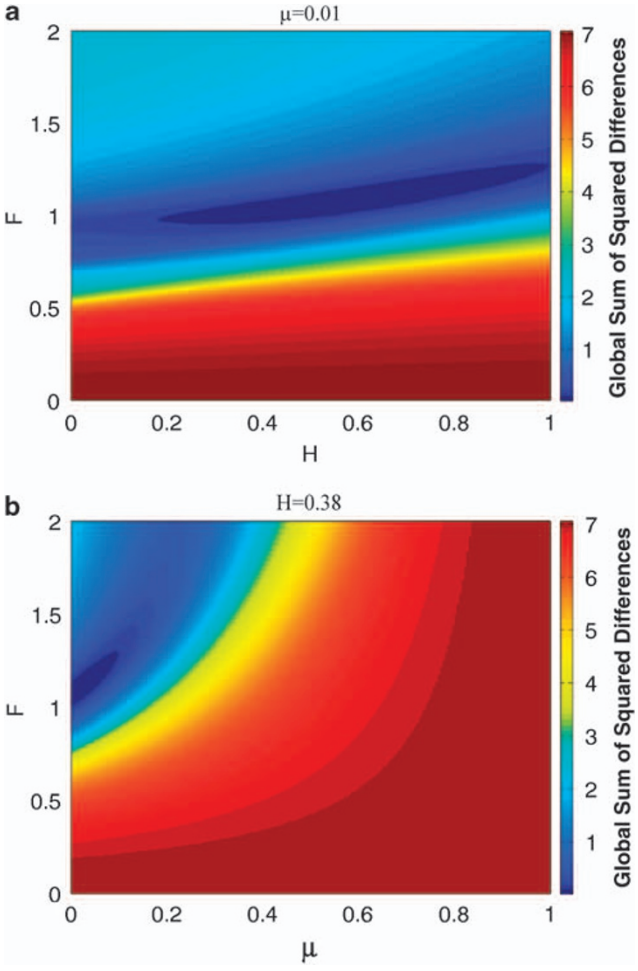

Figure 3 Sum of squared differences between observed infection frequencies and those predicted by a range of parameter values. (**a**) μ is fixed at 0.01 and (**b**) H is fixed at 0.38 (as determined experimentally). Best fits (i.e., lowest sums of squares) are in blue.

the rapid geographical spread of *Wolbachia* in wild populations of *D. simulans* across California, where the infection was found to spread upward of 100 km per year. In addition, infection parameters estimated from field-collected *Culex pipiens* predicted the rapid spread of a CI *Wolbachia* to near fixation (Rasgon and Scott, 2004). Invasion thresholds were clearly evident in other studies. For example, a *Wolbachia* strain inducing CI in *Aedes aegypti* did not spread when initial infection frequencies were below 20% (Xi *et al*., 2005). In addition, Johanowicz and Hoy (1999) found that a *Wolbachia* associated with CI in the mite *Metaseiulus occidentalis* was not able to spread within 12 generations when started at an introductory frequency of 10%, likely because of high fitness costs of the infection in this host. Interestingly, *M. occidentalis* has since been found to be infected with *Cardinium* as well, and it is not clear which symbiont (or both) causes CI (Weeks *et al*., 2003).

Stochastic factors are likely responsible for some of the variation in infection cage frequencies in our study (Figure 1). For example, random sampling effects could have caused the upsurge in infection frequency in one of the cages in the medium treatment in generation 2, as it could explain jumps or dips in infection frequencies in nature. Stochastic events may allow infections to spread

even when the initial frequency is lower than the invasion threshold, particularly in the case of a small or subdivided population (Jansen *et al*., 2008). However, it is unlikely that random genetic drift was responsible for the spread of the infection in all replicate cages that were initiated below the predicted threshold.

Interestingly, the strength of CI in *Cardinium*-infected *E. pergandiella* was found to be much weaker with $H = 0.38$ in this study compared with previous studies, where H ranged from 0.07 to 0.13 (Hunter *et al*., 2003; Perlman *et al*., 2008). The best model fit was found to have an even weaker CI strength, at $H = 0.61$ (Figure 1). Several factors have been found to affect the expression of CI, including bacterial density (Noda *et al*., 2001; Clark *et al*., 2003), temperature (Clancy and Hoffmann, 1998), male age (Turelli and Hoffmann, 1995) and host density (Sinkins *et al*., 1995). Although temperature and male age were similar between experiments, differences in infection density, host density or plant quality could have been important factors affecting the change in CI strength.

It is also possible that the host or symbiont has evolved toward weaker CI. Although a decrease in CI strength over time has not, to our knowledge, been reported before, theory suggests that host selection will favor variants that induce weaker CI if they also exhibit reduced fitness costs in infected females (Turelli, 1994; Vavre *et al*., 2003). A recent study showed rapid evolution in *D. simulans* infected with *Wolbachia*, with a shift from a 15–20% fecundity cost to a 10% fecundity benefit within 20 years (Weeks *et al*., 2007). Backcrossing experiments indicate that this change in fitness effects was not due to changes in host genotype, which therefore suggests that the *Wolbachia* strain had evolved (Weeks *et al*., 2007). Although it is possible that host–parasite evolution may also have resulted in a change in CI strength or host fitness in *Cardinium*-infected *E. pergandiella*, it seems unlikely that these differences would have evolved within a period of only 5 years.

Changes in the host fitness effects of *Cardinium* had a large impact on fitting the model to the observed data. The best overall model fit to the observed data used a slight fitness benefit of $F = 1.09$, and a fitness benefit of $F = 1.12$ was found to fit optimally when H was fixed at 0.38 (Figure 2). A good fit to the observed data (that is, sum of squares <1) was found using values of F ranging from a slight fecundity cost ($F \sim 0.95$) to a fecundity benefit ($F \sim 1.30$; Figure 3). In contrast, Perlman *et al*. (2008) reported that *Cardinium* infection reduced *E. pergandiella* host fecundity by approximately 18% within the first 4 days of reproduction (F=0.82). In that study, female *E. pergandiella* were provided with high-density arenas of unlimited whitefly hosts. It is likely that this observed fitness cost is not realized in circumstances where hosts are scarce. In addition, cryptic fitness benefits of the infection, such as an increased ability to find hosts or an increased fertilization rate or competitive ability, may not have been detected in the confined arenas of the laboratory study, whereas these effects may be detected in the more natural environment of the population cages. It is not uncommon for fitness effects to vary between laboratory and more natural populations. For example, a higher fitness cost of CI symbiont infection was noted in the laboratory compared with the field for *Wolbachia*-infected *D. simulans* populations (Hoffmann *et al*., 1990). Recently it has been found that a CI-inducing *Wolbachia* in *D. melanogaster* protects its host from viruses (Hedges *et al*., 2008; Teixeira *et al*., 2008), and it is therefore possible that the apparent fitness benefit of *Cardinium* is also related to host defense.

It is interesting to note that mutually compatible CI symbionts are expected to evolve to reduced fitness costs (or increased fitness benefits), even if this also results in a decrease in CI strength (Turelli, 1994). This is because increased host fitness will result in a greater number of infected offspring (Turelli, 1994). Thus, symbiont selection does not act directly on the strength of CI, and the symbiont is expected to ultimately evolve to be benign and beneficial (Hoffmann and Turelli, 1997).

The fitness effects of a CI infection remain notoriously difficult to estimate, often due to large discrepancies between the laboratory and the field (Hoffmann and Turelli, 1988; Hoffmann *et al*., 1990; Turelli and Hoffmann, 1995). Monitoring infection frequencies in population cages allows for a more realistic measure of this infection parameter, relative to single individual assays (Hoffmann and Turelli, 1988; Xi *et al*., 2005). Our study further shows the utility that invasion threshold experiments, coupled with current invasion models, have in estimating the fitness effects of a CI infection.

The observed data best fit a model with a slight infection benefit ($F = 1.09$), near-perfect maternal transmission ($\mu = 0.01$) and a reduction in offspring of the incompatible cross of $H = 0.61$ (Figure 2). On the basis of these parameter values, there is no invasion threshold, meaning the infection is able to spread at any initial infection frequency. It would be useful to test this prediction by studying *E. pergandiella* populations with very low ($\sim$1–2%) initial infection frequencies to determine if *Cardinium* is able to spread. The stable equilibrium for these infection parameter values is 98.6%. Because the *Cardinium* infection frequency was able to increase from 55% to an average of 96% within nine generations, a stable equilibrium frequency of $\sim$99% seems plausible. In the laboratory, *Cardinium* appears to be fixed in *E. pergandiella* (Hunter *et al*., 2003), lending further support to a stable equilibrium near fixation. In addition, the predicted stable equilibrium infection frequency of $\sim$99% is only slightly higher than the estimate of 92% (95% confidence interval of 81.4–97.9%) infection frequency for *E. pergandiella* in the field (Perlman *et al*., 2008).

Overall, the revised model ($F = 1.09$, $\mu = 0.01$ and $H = 0.61$) describes the observed invasion and spread of *Cardinium* very well (Figure 2). This is the first study examining the invasion and spread of *Cardinium*, and our results indicate that its infection dynamics appear similar to those of *Wolbachia*. The ability of *Cardinium* to rapidly spread within a host population shows its potential for future CI applications. These applications include releasing mass numbers of incompatible males into a pest population, thereby reducing offspring production (Laven, 1967). In addition, virulent strains of CI that induce severe fitness costs could be used to reduce the survival of disease vectors (Rasgon *et al*., 2003). For example, the *popcorn* strain of *Wolbachia* has been found to reduce the life span of *D. melanogaster* by 50% (Min and Benzer, 1997), and was recently successfully introduced into the mosquito *A. aegypti* (McMeniman *et al*., 2009). Furthermore, it has been suggested that CI

symbionts could potentially be used to drive genes of interest, for example, a gene that decreases the longevity of a vector, into a population (Beard *et al*., 1993). Our study suggests that, in addition to *Wolbachia*, the symbiont *Cardinium* may be a good candidate for use in these potential applications.

Conflict of interest

The authors declare no conflict of interest.

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