Evolutionary Ecology Research, 2011, **13**: 323–345

The prevalence and persistence of sigma virus, a biparentally transmitted parasite of *Drosophila melanogaster*

Marta L. Wayne[1,2,3], Gabriela M. Blohm[1], Mollie E. Brooks[1], Kerry L. Regan[1,4], Brennin Y. Brown[1], Michael Barfield[1], Robert D. Holt[1,2] and Benjamin M. Bolker[1,5]

[1]*Department of Biology, University of Florida, Gainesville, Florida, USA,*
[2]*Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA,*
[3]*UF Genetics Institute, University of Florida, Gainesville, Florida, USA,*
[4]*Department of Biological Sciences, University of Notre Dame, South Bend, Indiana, USA and* [5]*Department of Mathematics, McMaster University, Hamilton, Ontario, Canada*

ABSTRACT

Question: How do vertically transmitted parasites persist?

Organisms: *Drosophila melanogaster* (host) and sigma virus (parasite).

Field site: Peach stands in northern Georgia, USA, on a transect between Macon and Athens.

Empirical methods: We estimated prevalence in the field. We also estimated male and female transmission in the laboratory, using field-collected animals as parents. We further quantified patrilineal (father to son) transmission in the laboratory, and estimated cost of infection (virulence) by quantifying decreased egg production of infected flies.

Mathematical methods: Discrete-time, deterministic models for prevalence; analysis of stability of disease-free and endemic equilibria; numerical computation of equilibria based on empirical estimates.

Key assumptions: Random mating, discrete generations, cost of infection to females only.

Predictions and conclusions: The model allows persistence under parameter estimates obtained for this population. Uncertainty in parameters leads to wide confidence intervals on the predicted prevalence, which may be systematically underestimated due to Jensen's inequality. Male transmission is required for persistence, and multiple generations of strictly patrilineal transmission are possible in the laboratory, albeit with decreasing transmission efficiency.

Keywords: *Drosophila melanogaster*, evolution of virulence, host–pathogen co-evolution, persistence, prevalence, vertical transmission.

Correspondence: M.L. Wayne, Department of Biology, University of Florida, Gainsville, FL 32611, USA. e-mail: mlwayne@ufl.edu

INTRODUCTION

The prevalence and persistence of a parasite are constrained by the host's population density (Bjornstad *et al.*, 2002), life history and genotype, by the transmission mechanism of the parasite, and by the impact of the parasite upon its host. Virulence, or the reduction of host fitness by a parasite, may impose a cost on the parasite as well as the host, if decreased host lifespan or fecundity results in decreased rates of parasite transmission. However, increased virulence may also benefit the parasite, as virulence is often closely linked to the diversion of host resources for parasite reproduction. The costs and benefits of virulence to the parasite are thus expected to balance each other to some extent, giving rise to an optimal (for the parasite) intermediate level of virulence [i.e. the trade-off theory for the evolution of virulence (Ebert, 1999; Combes, 2001; Alizon and van Baalen, 2005)]. Other factors, of course, can influence virulence (Ebert and Bull, 2003), but in some systems the nature of transmission makes costs for the host likely to be costs for the parasite as well.

For strictly vertically transmitted parasites, virulence affecting either pre-reproductive viability or fecundity is particularly costly, because parasite fitness is so closely tied to host fitness. Hosts that die before reproduction doom their parasites as well. And, where parasite reproduction involves passage through host gametes, reduced host fecundity also reduces parasite fitness unless a complementary drive mechanism is present, such as is observed in *Wolbachia* (reviewed in Serbus *et al.*, 2008). The persistence of vertically transmitted parasites that remain virulent despite their mode of transmission thus presents both an ecological puzzle (how does the parasite persist?) and an evolutionary puzzle (why doesn't selection act to reduce virulence, if it is costly to both host and parasite?). Observing such a system often leads to a working hypothesis of the existence of some form of cryptic horizontal transmission [i.e. transmission within generations rather than from parent to offspring (Mangin *et al.*, 1995)]. Parasites that combine vertical transmission with horizontal transmission, involving both within- and among-species transmission, are not uncommon. Examples of viruses that include both vertical transmission within a dipteran population and horizontal transmission between dipteran vectors and human hosts include West Nile and yellow fever viruses (Mishra and Mourya, 2001; Goddard *et al.*, 2003; Murray *et al.*, 2010; Sall *et al.*, 2010).

Another factor that can affect persistence is diversity in the modes of vertical transmission. In sexual host species, vertical transmission can be uniparental (typically maternal) or biparental (either parent can infect the offspring). Biparental inheritance permits persistence of virulent parasites with no horizontal transmission, at least if transmission efficiency is high relative to the cost of infection (Fine, 1975), and moreover changes the selective pressures on both the host and the parasite relative to uniparental inheritance. Biparentally transmitted parasites have epidemiological and evolutionary similarities to horizontally transmitted parasites (Fine, 1975). Systems with biparental transmission may provide an unusual opportunity to understand the ecology and evolution of virulence, because transmission to new host lineages occurs *via* sexual reproduction, which can be easier to document and to experimentally manipulate in these systems (which necessarily are structured by mating pairs) than in many modes of horizontal transmission.

The sigma virus–*Drosophila melanogaster* model system provides an ideal opportunity to investigate the mechanisms permitting parasite persistence without horizontal transmission. Sigma virus (Rhabdoviridae) is a vertically transmitted, virulent parasite that infects natural populations of *D. melanogaster* worldwide (reviewed in Fleuriet, 1996). It has long been known to be transmitted biparentally (L'Héritier, 1958, 1970). Sigma is virulent:

infected hosts express a variety of symptoms consistent with lowered fitness, including a decrease in fecundity (Fleuriet, 1981). This is the kind of fitness cost in the host that also imposes a potential fitness cost on the parasite. Interestingly, sigma virus infections have been observed in several species of *Drosophila* in addition to *D. melanogaster*, including *D. affinis* and *D. athabasca* (Williamson, 1961; Félix *et al.*, 1971b; Longdon *et al.*, 2010), but has not been found outside the genus.

The specific goal of this study is to ascertain if transmission efficiency and virulence as estimated from field and laboratory studies are consistent, even broadly, with levels of prevalence observed in nature. To achieve this goal, we develop dynamic, deterministic, discrete-generation models that incorporate sex-specific transmission efficiency and cost of infection with respect to fecundity for projecting disease prevalence across time. [Despite the potential for complex patterns of interference and conflict among host reproductive biology and vertically transmitted symbionts (Engelstädter and Hurst, 2009; Yamauchi *et al.*, 2010), there is little current evidence for such conflicts in the *D. melanogaster*–sigma system at present, and hence we treat the system in a largely epidemiological rather than evolutionary framework.] Using data from a natural population of flies, we estimate prevalence, track transmission efficiency across generations and lineages, and also quantify the effect of sigma infection on female fecundity (a key aspect of virulence). We use the results of these laboratory studies to parameterize the models, and then compare the equilibrium prevalence predicted by the models to the prevalence measured in our field samples. In the Discussion, we sketch future extensions of the modelling framework that may be needed to account for some of the empirical patterns we observe in the laboratory studies.

Model of the dynamics of sigma virus prevalence

Our first model is broadly based on models presented by L'Heritier (1970) and Yampolsky *et al.* (1999), but differs from the former in that we do not distinguish among hosts differing in level of infection (what L'Heritier refers to as the stability of the infection), and from the latter in that we incorporate costs to female flies of being infected, and also permit a broader range of transmission efficiency when both members of a mating pair are infected. Our second model (see Appendix and Results) allows the transmission efficiencies to depend not only on the sex of the fly transmitting the infection, but also on the sex of the parent from which this fly received the infection.

The first model requires that we track only the prevalence. Given the current prevalence (p_t, the fraction of flies that are infected, which is assumed to be independent of sex), the prevalence in the next generation (p_{t+1}) is the ratio of the total number of infected offspring produced to total number of all offspring. Infected offspring are produced in three ways: (1) an infected female with fecundity n_i and transmission efficiency e_F mates with an uninfected male; (2) an uninfected female with fecundity n_u mates with an infected male with transmission efficiency e_M; or (3) two infected flies mate with fecundity n_i and transmission efficiency e_B (the probability that an offspring of two infected parents is infected). We assume that infection affects only female fecundity, resulting in an asymmetry in the equations below with respect to male and female transmission parameters. We further assume that there is no sex-specific effect of infection on survivorship, thus infected, fertilized eggs have an equal chance of entering the mating pool, regardless of their sex.

We also assume that mating is random with respect to infection status; therefore, the probability that a randomly chosen mating pair consists of an infected female and an

uninfected male (or vice versa) is $p_t(1-p_t)$, and the probability of both parents being infected is p_t^2. The number of infected eggs (per adult female) is the sum of products of mating probabilities, fecundities, and transmission efficiencies for events producing infected offspring, which is $[p_t(1-p_t)(n_i e_F + n_u e_M) + p_t^2 n_i e_B]$. The number of eggs produced (per adult female) is the weighted average of the uninfected and infected fecundities $[(1-p_t)n_u + p_t n_i]$. Taking the ratio of these quantities gives the following recursion:

$$p_{t+1} = \frac{p_t(1-p_t)(n_i e_F + n_u e_M) + p_t^2 n_i e_B}{(1-p_t)n_u + p_t n_i} = \frac{p_t(1-p_t)(q e_F + e_M) + p_t^2 q e_B}{1 - p_t + p_t q} \quad (1)$$

where $q = n_i/n_u$ is the fecundity of infected relative to uninfected females, which could include differential oviposition rate and even egg viability (as long as egg viability depends on infection of the female, not the eggs). As q decreases, the cost of the virus to the host (virulence) increases.

We analyse the properties of this model in the Results section below. We then relate this model to data, including estimates of prevalence, sex-specific transmission efficiency, and the impact of infection on female fecundity.

METHODS

Our focal population for the study consisted of a population of *D. melanogaster* in north-central Georgia, USA. To measure the prevalence of sigma virus, we sampled natural populations of *D. melanogaster* from six sites along US Highway 129/441 (see Table 1 for exact locations). We collected five times during the summer of 2009: 12 June, 24 June, 10 July, 24 July, and 18 September. Twenty-four hours before sampling, we placed three baits containing fruit and yeast at each site to attract *D. melanogaster*. We then swept for all visible flies using *Drosophila* fly nets (Bioquip®). We transferred the animals to plastic shell vials containing standard molasses-cornmeal medium, and brought them to the University of Florida in Gainesville.

All animals were assigned to individual vials in the laboratory within 72 h of collection. Because exposure to CO_2 is lethal to flies that are infected with sigma virus (L'Héritier and Teissier, 1945), animals were anaesthetized using cold treatment, a standard alternative to the more common procedure of CO_2 anaesthesia. We first placed the insects in empty

Table 1. Location of collection sites

Site ID	Distance (miles)	Latitude
6	50	33°94.671′ N
5	35	33°49.463′ N
4	23	33°46.528′ N
3	22	33°46.264′ N
2	20	33°44.447′ N
1	0	33°24.578′ N

Note: All sites are along US Highway 441/129 between Eatonton and Athens, Georgia. Distance is indicated in miles along the highway from the southernmost site (site 1).

vials in ice for up to 5 min. Once the insects stopped moving, we transferred them to custom-made metal blocks that had been chilled for >1 h at 0°C and covered with moist KimWipes. We discarded all species except *D. melanogaster*, which we placed individually in *Drosophila* plastic vials with standard molasses-cornmeal medium.

Carbon dioxide assay for sigma virus infection

Infection status was determined by CO_2 assay, as exposure to CO_2 causes paralysis and/or death in infected flies. Because of the negligible lethality from CO_2 treatment of uninfected flies, CO_2 is routinely used for anaesthesia in *Drosophila* laboratories. In addition, there are no other known pathogens in the *Drosophila* group that cause CO_2 sensitivity. Thus, we expect the rate of false positives to be extremely low. A precise estimate of the rate of false negatives in the field is not known; however, 'defective' mutant virus that does not confer sensitivity has been created in the laboratory (Brun and Plus, 1980). Accordingly, while we do not know the false negative rate for this assay, it is likely that it is non-zero.

Each fly to be assayed was placed in an empty vial, which was flooded with CO_2 for 5 min, and then returned to ambient oxygen and CO_2 levels for 15 min. Flies that returned to normal activity (walking and flying) were scored as uninfected; flies that were either dead or paralysed (unable to walk or fly but still moving) after CO_2 exposure were scored as infected. Because the assay kills or paralyses infected flies, flies were allowed to reproduce (see below) before they were assayed for infection.

Transmission assays

Field-collected adults will hereafter be referred to as the parental (*P*) generation. These flies were held in individual vials and allowed to produce offspring (the F_1 generation) before CO_2 assay (which is lethal to infected animals). Females captured from the field are usually inseminated, and thus can produce viable offspring without mating in the laboratory. We therefore placed the field-collected females individually in vials and allowed them to oviposit for 5 days. The infection status of the sires of these F_1 was thus unknown. To determine rates of transmission by field-collected males, we crossed each male with two uninfected virgin females from the effectively isogenic *58* stock (Wayne *et al.*, 2007), and left them in the vial together for 5 days. F_1 progeny with an infected parent were reared, and on day 14 after the vials were established they were collected and assayed for sigma virus infection. The transmission efficiencies were estimated as the fraction of F_1 offspring produced by an infected parent that were infected (calculated separately for male and female parents; see Fig. 1). We determined the single-generation transmission efficiency of males and of females obtained from three collection trips: 24 June (trip 2), 10 July (trip 3), and 24 July (trip 4), 2009.

We checked our data and the residuals graphically for deviations from the assumptions of linear models [normality, homogeneity of variance: plot.lm function in R (R Development Core Team, 2008)], and observed no large deviations. We analysed the fraction of infected offspring according to a fixed effect of sex and a random effect of trip, using a mixed model approach [R lme4 package (Bates *et al.*, 2011). lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-39. http://CRAN.R-project.org/package = lme4], because the data were strongly unbalanced (10, 12, and 19 individuals from each trip). A preliminary attempt to assess the interactive effect of sex × trip showed that this model was overfitted,

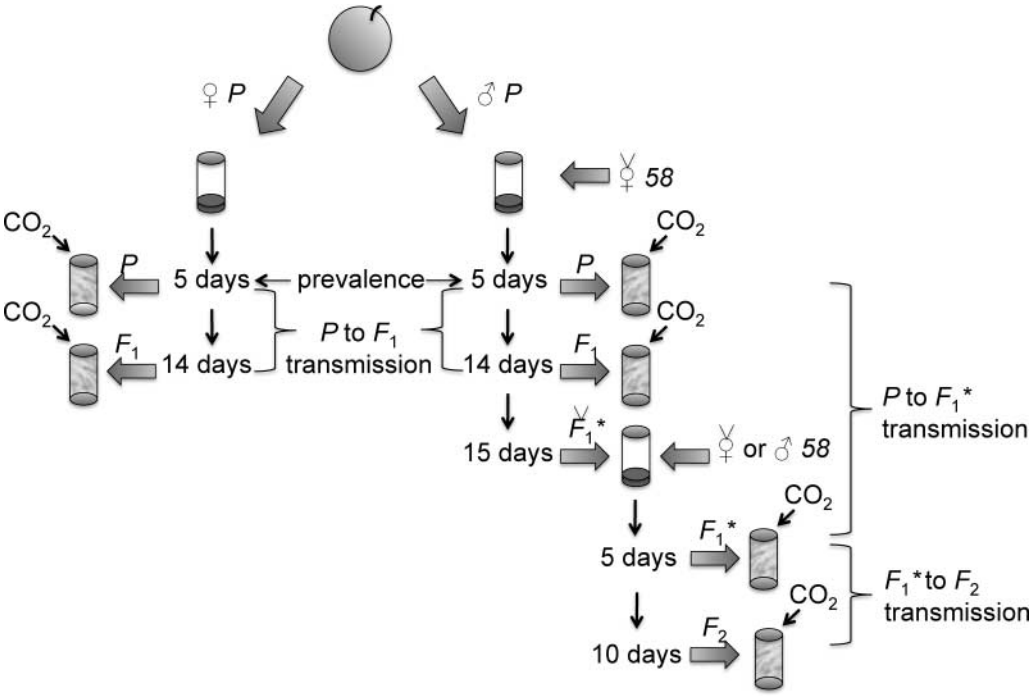

Fig. 1. How prevalence and transmission were measured, in females (left) and males (right). Heavy arrows denote movement of animals; thin arrows denote passage of time. *P* denotes the generation captured from peach stands (i.e. from the field). The F_1 are the progeny of the field-caught animals and the F_2 are their grand-progeny. Prevalence and *P* to F_1 transmission were measured for both female and male *P* animals (see text in middle of figure). The multi-generational patrilineal transmission efficiency experiment procedure (see text on right of figure) differs from the *P* to F_1 transmission assay described above in a few important ways. First, multi-generational patrilineal transmission efficiency was only calculated for 11 independent lineages derived from males (i.e. isomale lines), as the dynamics of female multi-generational transmission are better understood. The progeny of these 11 isomale lines (all of which were from trip 4) are denoted F_1*. Second, *P* to F_1* includes only virgin F_1* animals (as indicated by the V) from day 15 (in contrast to *P* to F_1 transmission efficiency, which included non-virgin progeny from days 10 to 14). Individual virgin F_1* animals were paired with a virgin of the opposite sex from line *58* to create the F_2. Only vials from infected F_1* were used to estimate F_1* to F_2 transmission efficiency.

so this term was discarded. The analysis was weighted by the total number of offspring collected per trip. We used the Wald *t*-statistic to test the effect of sex (see Results for details) and a simulation-based test (Scheipl, 2011) to test the random effect of trip.

Multi-generational patrilineal transmission

As noted in the Introduction, given the complete lack of evidence for any horizontal transmission, biparental transmission is required for persistence of the sigma virus in natural populations. To further characterize biparental transmission, we monitored patrilineal transmission efficiency across three generations originally sired by infected male

P flies from the 24 July trip (trip 4; see Fig. 1). A subset of 11 infected males assayed for transmission as described above was used to create 11 independent 'isomale' lines. After the original transmission assay using F_1 progeny collected on day 14, additional unmated progeny (denoted as F_1* virgins) were collected 12 h later (10–13 F_1* virgins per vial). We next set up single pair matings by pairing each F_1* fly with a single uninfected virgin of the opposite sex from the effectively isogenic *58* stock. Five days after establishment of the F_1* vials, the parents were removed and assayed for infection using the CO_2 assay. Only F_1* vials of infected parents were retained. The percentage of F_1* flies infected is referred to as the *P* to F_1* transmission efficiency. When the offspring (the F_2) flies eclosed 12–14 days after the vials were established, we determined the infection status of 10 haphazardly chosen F_2 flies from each vial using CO_2 assay. The sexes of the F_2 progeny were not recorded. The percent infected F_2 offspring is referred to as the transmission efficiency from the F_1* to the F_2 generation.

We analysed the data from these experiments using three separate paired *t*-tests; all comparisons were made within each of the 11 lineages. We first determined whether the probability of acquiring sigma virus infection from the *P* males was equal for both male and female F_1 flies. We then compared the transmission efficiency (F_1* to F_2) from infected F_1* female flies to that of infected F_1* male flies, to test for sex-specific differences in transmission efficiency in animals that acquired infection from the sire (male parent). Finally, we compared the transmission efficiency of *P* males to that of their male F_1* progeny, to estimate changes in patrilineal efficiency from one generation to the next.

Prevalence analysis

All flies that were collected were assayed for sigma infection using a CO_2 assay (described earlier). Prevalence was calculated as the fraction of flies infected, for each sex separately. Because the data are strongly unbalanced [flies were caught at only 23 (77%) of a total of 30 trip/site combinations], a classical analysis of variance (ANOVA) table is difficult to construct. Instead, we fitted a mixed-model ANOVA, weighted by the numbers of individuals in each sex/trip/site category, to estimate the differences in prevalence between sexes (a fixed effect) and the variance among sites, trips, and site × trip combinations (crossed random effects). In this case, the test of the fixed effect of sex is nearly equivalent to a classical paired *t*-test of the difference between male and female prevalences within trip/site combinations, although the mixed model approach allows us to include trip/site combinations for which only one sex or the other was caught.

Effect of sigma virus on female fecundity

With vertical transmission, impairment of female fecundity hampers the persistence of the parasite. Accordingly, we measured the effect of sigma virus on female fecundity (number of eggs per female). We compared four infected isofemale lines to four uninfected isofemale lines of *D. melanogaster* collected near Athens, Georgia in the summer of 2007. Isofemale lines are created by placing a single, inseminated wild-caught female in a vial and propagating her offspring *en masse*. Thus, these lines represent a small random sample from the population, and are expected to be genetically distinct from one another. Lines were monitored by CO_2 assay every generation to ensure that high prevalence (95% or greater) was maintained. We conducted four replicate blocks of measurements, conducted at

different times, each with four replicate bottles (total: 4 lines × 2 levels of infection status × 4 blocks × 4 replicate vials/block = 128 vials). For two generations prior to assay, flies were propagated in vials set up at constant density (5 females + 5 uninfected males per vial), ovipositing for 5 days on standard cornmeal-molasses medium. For the assay, individual female flies, 4 days post-eclosion, were placed in inverted milk bottles over small Petri dishes containing cornmeal-molasses food. After 6 h, we counted the number of eggs laid by each female.

We fitted a mixed model for the mean number of eggs for each line × block × infection status combination (i.e. aggregating over vials within block), with a fixed effect of infection and crossed random effects of line and block; we also considered the possibility of an interaction between infection status and block. Because the classical denominator degrees of freedom for an *F*-test are not precisely defined in the case of crossed random effects, we used a parametric bootstrap to test significance of the infection effect. We simulated repeatedly (1000 times) from the null model without an effect of infection (but with the observed among-line and among-block variances), fitting a model with a fixed effect of infection, and recording the *t*-statistic for the effect of infection. We then used this simulated distribution of *t*-statistics as the null distribution for a two-tailed test on the observed *t*-statistic from the full model fitted to the data.

Relating model to experiments

We parameterized the prevalence model (equation 1) with the fecundities and transmission efficiency measured in the laboratory to predict sigma prevalence at the non-zero equilibrium (see Results). To obtain point estimates and confidence intervals for transmission efficiencies, we used maximum likelihood estimation [bbmle: Tools for general maximum likelihood estimation. R package version 0.9.5.1; http://CRAN.R-project.org/package=bbmle (Bolker, 2008)] to fit beta distributions [parameterized in terms of mean and variance parameters (Morris, 1997)] to the proportions of offspring infected from either an infected female (for e_F) or an infected male (for e_M). We based our model parameters on the estimates and confidence intervals (CIs) for the means of these distributions. We used the same method to derive a point estimate and confidence intervals for the observed prevalence. We similarly used maximum likelihood, but based on a negative binomial distribution, to derive point estimates and confidence intervals for the fecundity of infected and uninfected flies.

We calculated the expected prevalence and rate of increase at low prevalence by substituting the mean observed parameter values in the expression for the equilibrium prevalence (see Results). We used non-parametric bootstrapping ($n = 10^7$) using all observed values of fecundity and transmission efficiency to find confidence intervals on these quantities.

RESULTS

Transmission efficiency

We measured transmission efficiency (proportion of offspring that were infected) for male and female parents. Both sexes transmitted the virus, but with different efficiency. Transmission efficiency was higher for females than males (0.915 vs. 0.497; Fig. 2); the

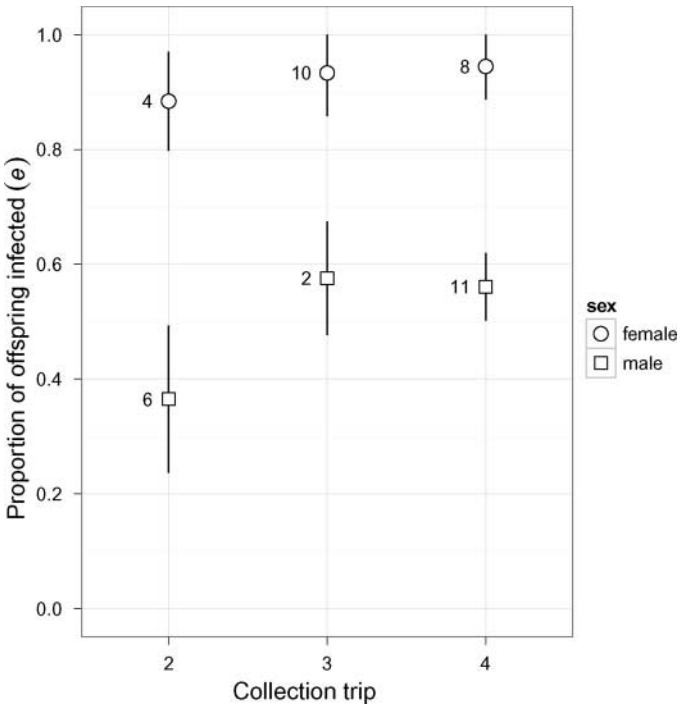

Fig. 2. Transmission efficiency of sigma virus (single generation). Mean transmission efficiency by females (circles) and males (squares), calculated from the first ten F_1 offspring from each *P* fly to eclose (±2 s.e.). Sample sizes (number of flies) are indicated next to each point. Flies from collection trip 1 were not assayed for transmission efficiency; while transmission efficiency was assayed from collection trip 5, a different protocol was used such that the data were not comparable.

difference was highly significant (0.42, s.e. = 0.011, $t_{37} = 37.5$, $P < 0.0001$). The estimated among-trip variance was quite small (variance = 5.01×10^{-4}, s.d. = 0.0224, or 4% of among-trip + residual variance), and statistically insignificant ($P = 0.069$, restricted likelihood ratio simulation test). Transmission efficiency for both males and females was lowest on 24 June, and approximately the same on 10 July as on 24 July (Fig. 2).

Multi-generational, patrilineal propagation of sigma virus was possible for three generations in the laboratory, using 'isomale' lines [each of which was propagated from a unique infected sire obtained in the field (*P* generation), mated with a single virgin female from the effectively isogenic laboratory stock *58*]. All 11 *P* generation males transmitted sigma virus to some offspring (Fig. 3, left panel). Sons and daughters (F_1* flies) were equally likely to become infected with sigma virus when the sire was infected (paired *t*-test; $P = 0.79$). Intriguingly, daughters of infected males had higher transmission efficiency than did sons (F_1* to F_2 transmission efficiency; paired *t*-test, $P = 0.032$) (Fig. 3, middle panel). Moreover, transmission efficiency from sire to offspring (ignoring the sex of the offspring) was significantly lower in the F_1* to F_2 generation than in the *P* to F_1* generation (paired *t*-test, $P = 1.149 \times 10^{-6}$) (Fig. 3, right panel).

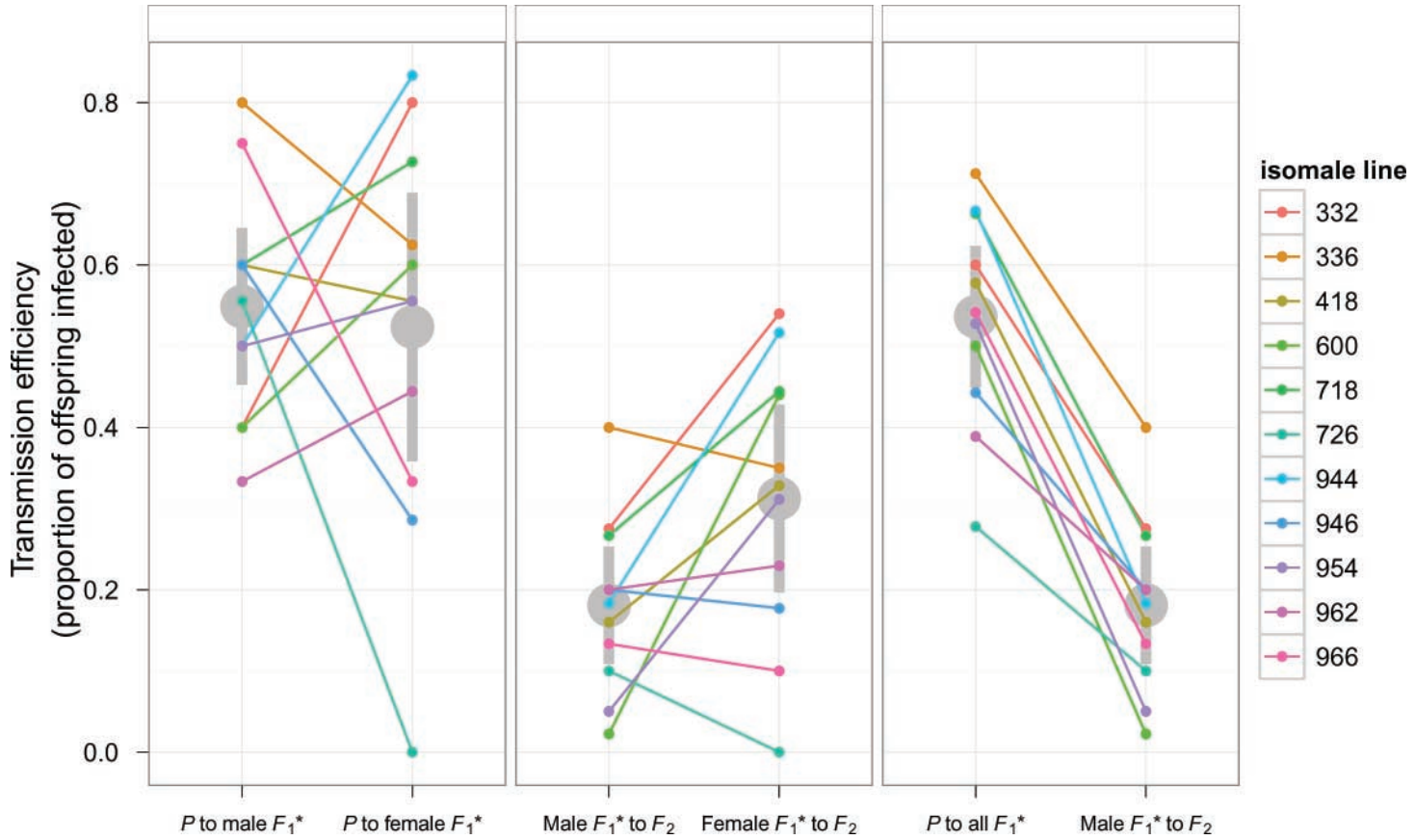

Fig. 3. Patrilineal transmission of sigma virus. Transmission efficiency (the proportion of offspring infected) is illustrated on the ordinate. Coloured lines correspond to each of the 11 isomale lineages. The grand means (±2 S.E.) of the lines are shown as heavy grey lines. The left-hand panel represents transmission efficiency between the *P* and F_1* generations, testing the hypothesis that patrilineal transmission to sons (left) is equal to that to daughters (right; $P = 0.79$). The middle panel compares transmission efficiency from the F_1* to the F_2, specifically between sons (left) and daughters (right) of infected sires from the field; transmission is higher from F_1* daughters than F_1* sons ($P = 0.032$). Finally, the right-hand panel represents the decrease in transmission efficiency (by males, to male and female offspring combined) from the first to the second generation (i.e. *P* to F_1* relative to male F_1* to F_2; $P < 0.0001$).

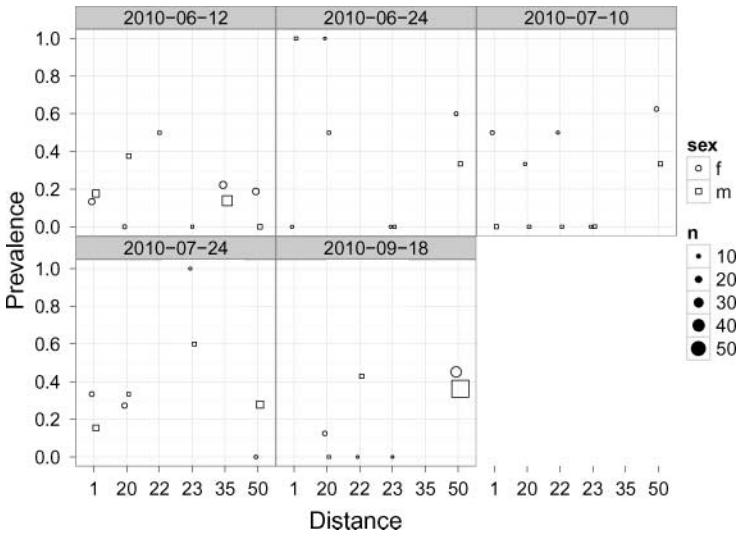

Fig. 4. Prevalence of sigma virus in north-central Georgia. The proportion of infected *D. melanogaster* is shown. Each panel represents a particular date/trip, and each column in a given panel indicates the collection site (distances not proportional). Females and males are represented by circles and squares, respectively; the size of the symbol indicates the sample size. The mean proportion of flies infected with sigma virus was 0.28 ± 0.1.

Prevalence

Prevalence was estimated five times in the summer of 2009, for flies collected from six sites (see Fig. 4). Females had slightly higher prevalence than males (31.1% vs. 27.7%), and while perhaps not biologically significant, this difference was statistically significant at $P = 0.041$ ($F_{1,17} = 4.89$). No components of variance among trip/site combinations, or among sites, were detectable; the among-trip and residual variances were 6.8×10^{-4} and 0.031 respectively (standard deviations of 0.026 and 0.177, or 2% and 98% of variance). There were no obvious trends predicting prevalence (see Fig. 4).

Fecundity cost/virulence

Four infected and four uninfected independent isofemale lines (lines originated by a single inseminated wild-caught female) were scored for fecundity. Models that included a block × infection interaction overfitted the data (i.e. perfect positive correlation between uninfected and infected random effects by block), so we dropped this term from the model. Infection lowered fecundity substantially (see Fig. 5), decreasing it by 54.2 (S.E. = 35.01) eggs below the average uninfected fecundity of 169.2 (S.E. = 31.0) eggs, but the parametric bootstrap showed that this effect (although biologically significant) had an estimated *P*-value of 0.069 (the simulated null distribution of *t*-statistics approximately matched a *t*-distribution with 15 degrees of freedom). Among-line and among-block variation was substantial, with standard deviations of 38.3 for line and 26.2 for block, compared with a residual standard deviation of 37.8 (variances: 1466 for line, 685 for block, 1425 for residual, or proportions of 41% for line, 19% for block, and 40% for residual).

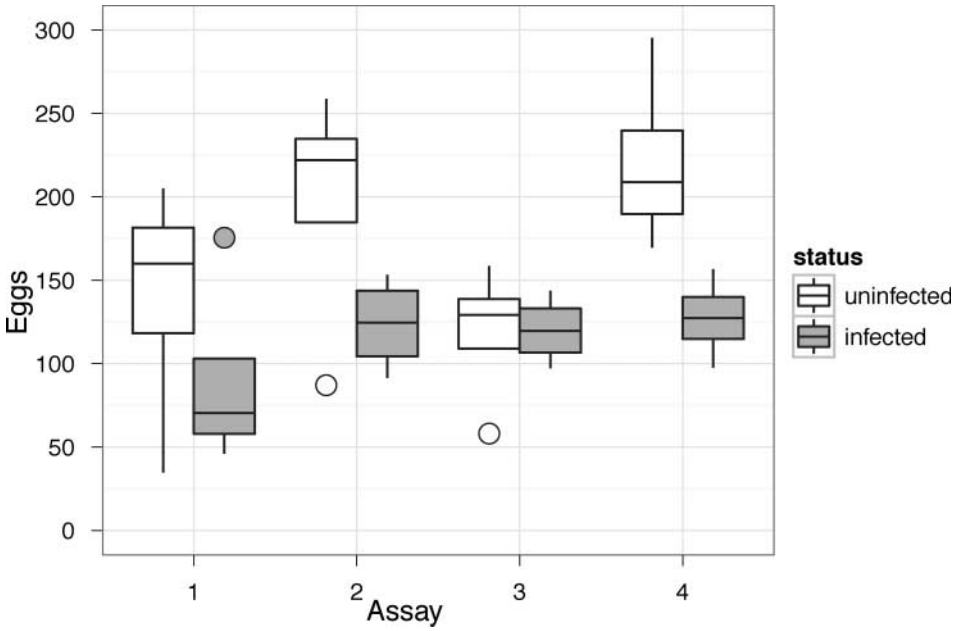

Fig. 5. Effect of infection status on fecundity. Boxes represent the mean female fecundity of lines within treatments (i.e. uninfected, white boxes vs. infected, grey boxes) for each of the four blocks (i.e. independent, temporally discrete replicates of the experiment). Error bars represent two standard deviations from the mean of each group of four lines. Circles represent outlier lines within treatments (i.e. lines more than 1.5 times the group inter-quartile range), and are shaded to indicate infection status as described above. Fecundity is generally higher in uninfected lines than in infected lines, except in the third block.

Model analysis

Condition for increase of the virus when rare

When the virus is rare, $p_t \approx 0$. With this approximation, we can ignore second-order terms in p_t in the numerator, and first-order terms in the denominator, of equation (1), which then becomes

$$p_{t+1} \approx p_t(qe_F + e_M) = p_t R, \tag{2}$$

where

$$R = qe_F + e_M \tag{3}$$

is the finite growth rate of the virus over one generation at low prevalence. Alternatively, equation (2) may be obtained by taking a Taylor series about zero, and retaining only the constant (which is 0) and first-order terms. Higher virulence corresponds to lower q, and clearly reduces parasite as well as host fitness.

For the virus to increase when rare, R must be greater than 1, which implies that

$$q > \frac{1 - e_M}{e_F}. \tag{4}$$

Thus we have a relationship between transmission efficiencies and virulence needed for an increase of the virus when rare. If there were no male transmission ($e_M = 0$), then the virus could invade only if $qe_F > 1$. Even if female transmission were perfect, invasion would require q to be greater than 1 (i.e. infected females would need to have higher fecundity than uninfected females). In other words, our model reproduces the result that invasion is precluded if infection imposes a cost on host fecundity, given female-only transmission: only mutualists ($q > 1$) can invade with uniparental inheritance. By contrast, adding male transmission permits the persistence of virulent vertically transmitted parasites. The condition for invasion is $qe_F + e_M > 1$: male transmission actually has a greater effect than female transmission, especially if virulence is high. This asymmetry occurs because female transmission requires the female parent to be infected, which lowers fecundity. Male infection, and therefore male transmission, does not lower male reproductive success (by assumption).

Equilibria

The equilibria of model (1) are either virus absence ($p^* = 0$) or prevalence at

$$p^* = \frac{qe_F + e_M - 1}{qe_F + e_M - 1 + q(1 - e_B)} = \frac{R - 1}{R - 1 + q(1 - e_B)}. \tag{5}$$

For a one-variable discrete system such as that described by (1), an equilibrium is locally stable if and only if the slope of p_{t+1} versus p_t has a magnitude <1 at the equilibrium. At $p_t = 0$, this slope is R, so if $R > 1$, the zero equilibrium is unstable, and the prevalence will increase when very low, while if $R < 1$, the 0 equilibrium is stable, and an initially low prevalence will drop.

The non-zero equilibrium (equation 5) is locally stable for $R > 1$. To gauge local stability, we take the derivative of (1) with respect to p_t and evaluate it at the equilibrium, leading to

$$\left.\frac{dp_{t+1}}{dp_t}\right|_{p^*} = \frac{q[(R-1)e_B + (1 - e_B)] - (R-1)^2}{q(R - e_B)}. \tag{6}$$

First consider $R > 1$. The quantity in (6) is less than $[(R-1)e_B + (1 - e_B)]/(R - e_B)$, obtained by removing the last negative term in the numerator and then cancelling the common factor q. The condition for this quantity to exceed 1 can easily be shown to be $e_B > 1$, which is not possible (because this is a probability), so the derivative in (6) is <1. Stability requires that it also be greater than -1. The denominator of (6) is positive when $R > 1$, so the sign of (6) is the sign of the numerator, which is an increasing function of q. If $q = R - 1$, the numerator is $(1 - e_B)(R - 1)(2 - R)$, which is non-negative since R cannot exceed 2 (from equation 3, given that all the quantities in that expression must be ≤ 1). Therefore, (6) can be negative only if $q < R - 1 = qe_F + e_M - 1$, which requires $q(1 - e_F) < -(1 - e_M)$. This is impossible, since both terms in parentheses (and q) are non-negative. Therefore, the slope in (6) is non-negative and <1, so the equilibrium is stable (assuming $R > 1$).

If $R < 1$, the only feasible non-zero equilibrium is at a prevalence of 1, which occurs for $e_B = 1$ (if $R < 1$ and $e_B < 1$, then the calculated p^* can be positive, but if so it is >1, which is not feasible for a prevalence). The derivative in (6) for $e_B = 1$ is equal to $1 + (1 - R)/q$. If $R < 1$, the derivative exceeds 1, and the non-zero equilibrium is unstable. Starting at a prevalence just below 1 leads to a monotonic drop to 0 in this case. Therefore, the virus increases when rare, and persists at a stable equilibrium, if and only if $R > 1$.

The increase of the virus when rare does not depend on e_B, because if the virus is rare, it is very unlikely that both parents are infected. However, if it can increase when rare, it has a stable equilibrium given by (5) that does depend on e_B. Specifically, as e_B approaches 1, the equilibrium prevalence approaches 1, since the numerator and denominator of (5) differ only by the last term in the denominator. Since we have high female transmission, e_B is likely to be high, so the predicted prevalence will be high unless R is not much above 1, in which case the prevalence would be quite sensitive to the values of the parameters determining R. If e_B is near 0, the prevalence depends on the virulence (q) relative to the amount by which R exceeds 1.

If male and female transmission are independent, then $e_B = e_F + e_M - e_F e_M$. Biparental transmission is higher than this quantity if there is a synergistic effect of both parents being infected (for example, successful infection might depend on the total viral load of the fertilized egg exceeding a threshold, in which case e_B could even exceed $e_F + e_M$ in some cases). It is lower if there is interference (for example, male infection might be irrelevant if the egg is infected, in which case $e_B = e_F$). Equation (5) also indicates that a high prevalence could be produced by a low q or a high R (as possible alternatives to a high e_B). However, a low q (high virulence) would make virus persistence unlikely (unless female and especially male transmission are high, in which case e_B is probably also high), and R cannot exceed 2 unless there is mutualism; therefore, with a high prevalence there is likely a high e_B.

Expected prevalence based on empirical estimates of parameters

We can now compare the equilibrium prevalence estimated from the model to that observed in the field. The point estimate of observed prevalence based on the beta distribution was 0.25 (95% CI {0.19, 0.34}). The point estimate of male transmission (e_M) was 0.50 (95% CI {0.36, 0.64}), and female transmission (e_F) was estimated as 0.92 (95% CI {0.87, 0.95}). Fecundity of infected females was 115.0 (95% CI {30, 254}) and that of uninfected females 169.2 (95% CI {45, 372}), making $q = 0.68$.

Substituting the point estimates of the parameters in (3) and (5) gives $R = 1.13$ and $p^* = 0.82$ (we assumed independent female and male transmission to calculate e_B). Non-parametric bootstrapping leads to 95% confidence intervals for R of 0.53 to 5.53 and for p^* of 0 to 0.97. [The upper bound on R was greater than 2 because the variability in fecundity among flies in both treatments was so large that we could not rule out values of $q > 1$ empirically, although on the basis of previous information about sigma virus, reviewed in Fleuriet (1996), such values are extremely unlikely.] Considering persistence, our point estimate for R suggests that the virus should be able to increase when rare.

Figure 6 shows the results of the combined uncertainty analysis. With the current level of precision in the parameters, we are unable to bound the expected equilibrium prevalence very precisely [CI (p^*) = {0, 0.97}, as described above, using the non-parametric approach]. Overall, it would appear that the prevalence one would predict based on the laboratory transmission data is greater than that generally observed in the field.

Model with transmission efficiencies dependent on sex of parent from which infection derived

Our results indicate that transmission of infection by a fly of either sex is lower if that fly was infected by its male parent (sire) than its female parent (dam). Therefore, the previous model is modified to include four transmission parameters, with different female and male transmission rates, and different rates for individuals infected by a dam or a sire. (We will

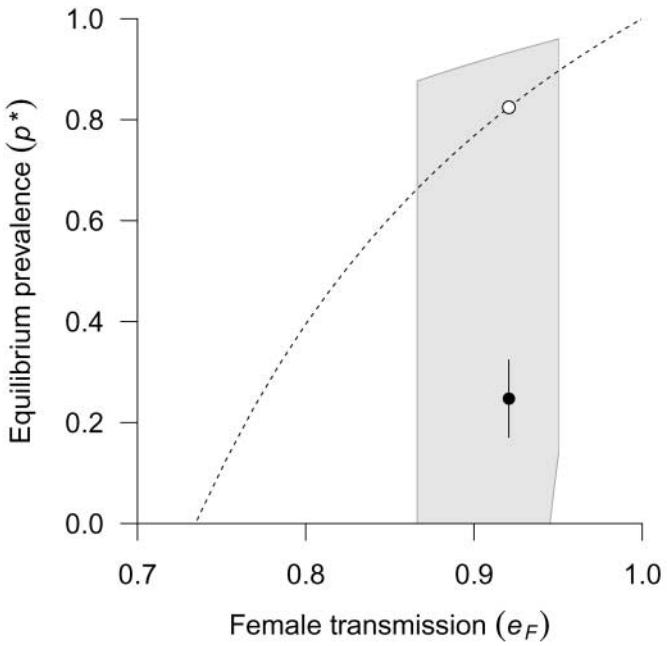

Fig. 6. Equilibrium prevalence of sigma virus as a function of female transmission efficiency (abscissa) and male transmission efficiency (dashed curve). The open circle is the prevalence predicted using the point estimates of the parameters and equation (5). The solid circle represents the observed mean prevalence and mean female transmission efficiency; error bars represent the 95% binomial confidence intervals at the observed prevalence. The dashed curve shows the expected prevalence as a function of female transmission (e_F, point estimate 0.92), given that male transmission (e_M) is equal to its point estimate (0.50). The grey shaded area shows the region of possible prevalence as a function of e_F, given that e_M is restricted to be within in its 95% confidence interval. The point estimate for virulence (q) was 0.68.

use male and female to refer to flies mating in the current generation, and dam and sire to refer to sexes of the flies in the previous generation.) Let e_{ij} be the transmission efficiency of a fly of sex i (F or M) that was infected by a fly of sex j (D for dam and S for sire).

To construct the model, we also need an assumption about what happens when mating flies are both infected. Let y_{ij} be the transmission by a female when she was infected by her parent of sex i, and her mate was infected by his parent of sex j, and x_{ij} be the corresponding probabilities for male transmission (i and j are D or S). We shall assume here that when both flies are infected, the female transmits at the same efficiency she would if the male was uninfected, while the male can transmit only to offspring not infected by the female. Thus $y_{ij} = e_{Fi}$ and $x_{ij} = (1 - e_{Fi})e_{Mj}$.

Transmission of infection does not depend on the sex of the offspring, so males and females should be infected at the same rate in each mating, and thus have the same prevalence, which again is p_t in generation t. Since the transmission efficiency of a fly depends (in addition to its own sex) on the sex of the parent from which it received the infection, we also need to keep track of the fraction of all offspring infected by a female, which we call r_t. There are in this case nine different types of mating, since the female can be

uninfected, infected by her dam, or infected by her sire. The probabilities of these are $1 - p_t$, $r_t p_t$, and $(1 - r_t)p_t$, respectively. There are also the same three possibilities for the male. The probability of each type of mating is the product of the probabilities for the female and the male.

The total offspring production does not depend on the transmission efficiencies, and so is the same as the simpler model, which (when normalized by the fecundity of an uninfected female) is $1 - p_t(1 - q)$. We now need an expression for the number of offspring infected by females, and one for the number infected by males. Each is found as in the previous model, with all infected offspring of an infected fly mating with an uninfected fly obviously assigned to the sex of the infected parent, but the sex-specific transmission rates y_{ij} (for female transmission) and x_{ij} (for male transmission) used for matings of two infected flies. (For a detailed explanation of model derivation, see the Appendix.) The number of offspring infected by females can be simplified when $y_{ij} = e_{Fi}$ (i.e. female transmission dominates such that male infection has no effect if the female is infected) to

$$p_t q[r_t e_{FD} + (1 - r_t)e_{FS}] = p_t q\bar{e}_F, \tag{7}$$

where $\bar{e}_F = r_t e_{FD} + (1 - r_t)e_{FS}$ is the average female transmission efficiency (this is not a fixed parameter, since it varies with the dynamic variable r_t).

The number infected by males is similar, except that the male transmission efficiencies are used, which can be simplified when $y_{ij} = e_{Fi}$ and $x_{ij} = (1 - e_{Fi})e_{Mj}$ (i.e. when both parents are infected, the male can transmit only to offspring which are not infected by the female) to

$$p_t(1 - p_t)\bar{e}_M + p_t^2 q\bar{e}_M(1 - \bar{e}_F), \tag{8}$$

where $\bar{e}_M = r_t e_{MD} + (1 - r_t)e_{MS}$ is the average male transmission efficiency.

The new prevalence is the total infected progeny (i.e. the sum of 7 and 8), divided by the total offspring $1 - p_t(1 - q)$, while the new fraction infected by females is the number infected by females (7) divided by the total infected progeny. The recursion for prevalence is, therefore,

$$p_{t+1} = \frac{p_t q\bar{e}_F + p_t(1 - p_t)\bar{e}_M + p_t^2 q\bar{e}_M(1 - \bar{e}_F)}{1 - p_t(1 - q)} = \frac{p_t(1 - p_t)(\bar{e}_M + q\bar{e}_F) + p_t^2 q\bar{e}_B}{1 - p_t(1 - q)}, \tag{9}$$

where $\bar{e}_B = \bar{e}_F + \bar{e}_M - \bar{e}_F\bar{e}_M$. This is of the same form as equation (1). The recursion for the fraction of the population infected by females is

$$r_{t+1} = \frac{q\bar{e}_F}{q\bar{e}_F + (1 - p_t)\bar{e}_M + p_t\, q\bar{e}_M(1 - \bar{e}_F)} = \frac{q\bar{e}_F}{(1 - p_t)(\bar{e}_M + q\bar{e}_F) + p_t q\bar{e}_B}. \tag{10}$$

Equations (9) and (10) were solved numerically using parameters from this experiment for transmission when a parent was infected by its sire, and from the literature when it was infected by its dam. The parameters used were $e_{FS} = 0.32$, $e_{MS} = 0.18$, $e_{FD} = 0.98$, and $e_{MD} = 0.45$. To keep the virus from going extinct, we used $q = 0.9$ (in contrast to the previous model, where persistence was possible using the empirically derived estimate of $q = 0.68$). This gave an equilibrium prevalence of 83.5%, with 89% of infected individuals infected by females. This made the average female and male transmission efficiencies equal to 0.91 and 0.42, respectively.

DISCUSSION

Our point estimate for prevalence, based on laboratory estimates of sex-specific transmission and cost of infection on fecundity, is consistent with prevalence observed in the field; however, laboratory-estimated parameters also produce very wide confidence intervals, and the equilibrial prevalence they predict tends to exceed observed field values. One possible explanation for this is that we underestimated the field prevalence for sigma because of false negatives from the CO_2 assay for infection. While the false positive rate is expected to be negligible, the false negative rate is unknown (see Methods for details). Regardless, the overlap suggests that our model provides a sensible springboard for more detailed investigations of virulence and transmission in the sigma virus–*Drosophila* system, including a wider range of known and suspected biological factors.

Our estimates of prevalence and transmission efficiency were obtained from the same population of flies within a single season, and our estimates of virulence were obtained from the same population (albeit 2 years earlier). We found that sigma infects a mean of 28% of *D. melanogaster* individuals in north-central Georgia (approximate latitude 33.9°N), with little difference in prevalence between males and females. The prevalence of sigma virus in 2009 (when flies were collected for our laboratory study) was higher than in 2005, when it infected only 6.2% of the population of *D. melanogaster* in Athens, Georgia (Carpenter *et al.*, 2007), or in 2006, when it infected 7% of the population (M.L. Wayne, unpublished data). However, in 2011, we estimate prevalence at 24% [$N = 355$ (M.L. Wayne, unpublished data)]. Thus, prevalence in these natural populations varies greatly from season to season. Prevalence and transmission efficiencies were obtained from the same population for the same season, 2009 (though virulence estimates are from lines collected in 2007), so the data are at least self-consistent. Our estimate of virulence (32%), while high, is not grossly dissimilar from previous estimates of virulence in terms of fecundity. For unstabilized females, which transmit the virus at relatively low frequencies, the decrease in fecundity has been estimated at 19% (±4.6%), and for stabilized females, which transmit the virus at close to 100%, at 10% (±2.4%) (Fleuriet, 1981).

It is interesting to place our field estimates of prevalence into a broader geographical context. The prevalence of sigma virus in our study area, which is among the southernmost sites studied to date (33.9°N), is also among the highest yet recorded, at about 28%, though again prevalence varies among years: 6.2% in 2005 (Carpenter *et al.*, 2007), 7% in 2006 (M.L. Wayne, unpublished data), but 24% in 2011 (M.L. Wayne, unpublished data). In Athens, Greece (37.9°N), sigma was found in 14.9% of the population (Carpenter *et al.*, 2007); in Galicia, Spain (42.66°N), prevalence was 4.3% (Carpenter *et al.*, 2007); in Languedoc, France (43.7°N), prevalence ranged from 10 to 20% (Fleuriet, 1976, 1980, 1996); in Ithaca, New York (47.4°N), prevalence was a mere 1.7% (Yampolsky *et al.*, 1999); and finally in Essex, UK (51.79°N), prevalence was 7.0%, though prevalence was zero in nearby Kent, UK (Carpenter *et al.*, 2007). Latitude is, of course, an imperfect proxy for climatic variables that presumably can exert an influence on prevalence, but inspecting this suite of studies suggests to us the possibility of a latitudinal or climatic gradient in prevalence of sigma virus. (Note that Galicia is on average considerably wetter and cooler than both Languedoc and lowland Greece.)

One published outlier to this trend comes from Clermont, Florida (Apshawa Road; 28.61°N), the southernmost site, where prevalence was estimated at 1.5% (Carpenter *et al.*, 2007). There are reasons to believe that prevalence may be low for several reasons distinct to that study. First, it was estimated early in the season (March 2005); second, *D. melanogaster* is

locally rare in the area, such that sample sizes were low; and third, the viral isolate was quite distinct from all other samples sequenced (Carpenter *et al.*, 2007; Longdon *et al.*, 2010), and so might well have distinct epidemiological properties.

Our analysis of the models assumed that a population had reached equilibrium, and that fitness costs of parasitism are fixed. Natural populations are likely to be strongly disequilibrial. Fly populations are likely to fluctuate greatly in numbers, in response to variation in climate, fluctuations in resource availability, and other factors, and may go extinct and become replenished by re-colonization. If sigma virus is lost by chance from a population at low numbers, when it re-colonizes there will be a lag before it reaches equilibrium, and during this lag prevalence will be less than the local equilibrium. We measured fitness costs of parasitism to female fecundity in the laboratory, but it is plausible that additional costs could be incurred in natural conditions when females are exposed to a wide range of stressors. This, too, would tend to depress viral prevalence. For example, variation in prevalence in Mexico City might be explained by decreased overwintering survival of infected relative to uninfected flies, and thus reduced frequency of infection of founders of the following spring population (Félix *et al.*, 1971a), combined with low dispersal among populations. Likewise, male mating success might be more strongly affected by infection in the field than in the laboratory, and this could lead to low infection in sparse populations (as in the Clermont, Florida population noted above). We note also that if there is spatial or temporal variation in R (or q), and local populations equilibrate in prevalence rapidly in response to such variation, then by Jensen's inequality from the concave-down shape of (5) where $R > 1$, prevalence averaged across sites will be less than the prevalence estimated from averaged values of the parameters (Inouye, 2005).

Without empirical estimates for his model parameters, L'Héritier (1970) sensibly examined a broad range of plausible values. He did, however, include 'stability' in his model, where stability is defined as a persistent level of infection within female hosts, including near-certainty of transmission to offspring. In contrast, we chose to model sex-specific transmission efficiencies and associated errors, as informed by our empirical data, rather than including stabilization status *per se*. Interestingly, we rarely observed female transmission efficiency of 100%. Similar to the models of L'Héritier (1970) and Yampolsky *et al.* (1999), our model shows that if transmission by one parent is less than 100%, at least some transmission by the other parent is required for persistence (equation 3). Our estimates of transmission efficiency are similar to those of Yampolsky *et al.* (1999), who inferred that the transmission efficiency for the two sexes must be around 0.67 based on the rate of the spread of infection within their experimental populations. Our estimates (see above) range from just under 0.5 (for males) to 0.92 (for females), and so bracket this value.

As previously noted (L'Héritier, 1970), sires transmit virus less efficiently than dams (Fig. 2). Although it has been previously stated that the sons of infected males do not transmit the virus (L'Héritier, 1970; Fleuriet, 1981), we have demonstrated that multiple generations of patrilineal transmission are possible, at least for virus and flies from the Athens, Georgia population. However, transmission frequency is lower from the second to third generation of patrilineal transmission than from the first to the second (see Fig. 3). The more complex model above (equations 9 and 10) was developed to incorporate this intriguing trans-generational effect on transmission. This model required a lower virulence (higher q) for the virus to persist (because the transmission efficiencies when infection was from the sire were low). With the decreased virulence, the predicted equilibrium prevalence was not changed significantly from the simpler model, because both predict a fairly high prevalence due to high trans-

mission when both parents are infected. More complicated models would be needed to incorporate other factors, such as the distinction among classes of hosts with different patterns of within-host dynamics first explored by L'Héritier (1970). Alternatively, incorporating a more complex virulence function could be explored, such that virulence is dependent on whether the transmitting parent is male or female. For example, there is some speculation that sigma may actually increase male reproductive success (Fleuriet, 1981). A consideration of distinct patterns of within-host dynamics may be needed to fully explain our results.

The persistence of paternally acquired virus is particularly important because, as reiterated by our model results and previously demonstrated by others, biparental inheritance is required for persistence of vertically transmitted parasites (Fine, 1975). One potentially interesting consequence of biparental transmission could be heteroplasmy of sigma virus within individuals (i.e. different viruses within an individual due to inheritance of different strains from each parent). This is particularly interesting because within-host dynamics could drive parasite virulence (Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995; Miralles *et al.*, 2001; Alizon and van Baalen, 2008). We feel this is unlikely in the case of sigma virus, however, because viral variation even between isolates from distant host populations is quite low, so low in fact that a very recent origin for the virus has been postulated (Carpenter *et al.*, 2007). Thus, it is not clear that virus acquired from both sire and dam would actually be genetically distant enough to result in competition between strains.

Another biological consequence of such male transmission is that given occasional interspecific hybridization, the virus could be transmitted across species boundaries. As noted above, sigma virus is currently known to infect several species of *Drosophila* (Williamson, 1961; Félix *et al.*, 1971b; Longdon *et al.*, 2010). It would be informative to have a fuller understanding of the phylogenetic scope of sigma virus among closely related species of flies, coupled with a deeper understanding of patterns of genetic variation in the virus within single host species. Future studies should incorporate population-genetic components that include genetic variation of both the host and the parasite. Together with the ecological factors sketched above, genetic differences could play a strong role in explaining differences among sites in sigma virus prevalence. For example, viral variation that causes detectable, functional differences in transmission and/or infectivity is well known (Goldstein, 1949; Brun and Plus, 1980; Wilfert and Jiggins, 2010a). Host genetic variation for male transmission and for resistance is also well documented, both by classical Mendelian genetic approaches and by QTL mapping (L'Héritier, 1970; Gay, 1978; Brun and Plus, 1980; Fleuriet, 1996; Wayne *et al.*, 1996; Bangham *et al.*, 2007, 2008; Carpenter *et al.*, 2009; Wilfert and Jiggins, 2010b). However, data on allele frequencies in virus and host, as well as potential host–parasite interactions [or lack thereof (Wilfert and Jiggins, 2010b)], are required to make meaningful progress in this direction.

ACKNOWLEDGEMENTS

We thank Associate Editor, Lev Yamopolsky, and an anonymous reviewer for helpful comments on the manuscript. We also thank Cuong Nguyen and Luis Matos for assistance with 2006 collections, and Kelly Dyer and Scott Paterson for assistance with 2011 collections. This work was funded by NIH grant GM083192, and (R.D.H. and M.B.) by the University of Florida Foundation, NSF DEB 0525751 and DEB 0515598. B.Y.B. was supported by NIH grant GM083192S1. M.E.B. was supported by NSF IGERT #0801544, QSE3: Quantitative spatial ecology, evolution and environment.

REFERENCES

Alizon, S. and van Baalen, M. 2005. Emergence of a convex trade-off between transmission and virulence. *Am. Nat.*, **165**: E155–E167.

Alizon, S. and van Baalen, M. 2008. Multiple infections, immune dynamics, and the evolution of virulence. *Am. Nat.*, **172**: E150–E168.

Bangham, J., Obbard, D.J., Kim, K.W., Haddrill, P.R. and Jiggins, F.M. 2007. The age and evolution of an antiviral resistance mutation in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B*, **274**: 2027–2034.

Bangham, J., Kim, K.W., Webster, C.L. and Jiggins, F.M. 2008. Genetic variation affecting host–parasite interactions: different genes affect different aspects of sigma virus replication and transmission in *Drosophila melanogaster*. *Genetics*, **178**: 2191–2199.

Bates, D., Maechler, M. and Bolker, B. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-40/r1308 (http://R-Forge.R-project.org/projects/lme4/).

Bjornstad, O.N., Finkenstadt, B.F. and Grenfell, B.T. 2002. Dynamics of measles epidemics: estimating scaling of transmission rates using a time series SIR model. *Ecol. Monogr.*, **72**: 169–184.

Bolker, B.M. 2008. *Ecological Models and Data in R*. Princeton, NJ: Princeton University Press.

Brun, G. and Plus, G. 1980. The viruses of *Drosophila*. In *The Genetics and Biology of Drosophila* (A. Ashburner and T.R.F. Wright, eds.), pp. 625–702. London: Academic Press.

Carpenter, J.A., Obbard, D.J., Maside, X. and Jiggins, F.M. 2007. The recent spread of a vertically transmitted virus through populations of *Drosophila melanogaster*. *Mol. Ecol.*, **16**: 3947–3954.

Carpenter, J., Hutter, S., Baines, J.F., Roller, J., Saminadin-Peter, S.S., Parsch, J. *et al.* 2009. The transcriptional response of *Drosophila melanogaster* to infection with the sigma virus (Rhabdoviridae). *PLoS ONE*, **4**: e6838.

Combes, C. 2001. *Parasitism: The Ecology and Evolution of Intimate Interactions*. Chicago, IL: University of Chicago Press.

Ebert, D. 1999. The evolution and expression of parasite virulence. In *Evolution in Health and Disease* (S.C. Stearns, ed.), pp. 161–172. New York: Oxford University Press.

Ebert, D. and Bull, J.J. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.*, **11**: 15–20.

Engelstädter, J. and Hurst, G.D.D. 2009. What use are male hosts? The dynamics of maternally inherited bacteria showing sexual transmission or male killing. *Am. Nat.*, **173**: E159–E170.

Félix, R., Guzmán, J. and de Garay Arellano, A. 1971a. Distribution of CO_2 sensitivity (sigma virus) in an urban population of *D. melanogaster* from Mexico City. II. Low dispersal, a factor which explains differences among locations. *Drosophila Information Service*, **47**: 105–109.

Félix, R., Gúzman, J. and de Garay Acellano, A. 1971b. Carbon dioxide sensitivity of Drosophilid flies from a location in the outskirts of Mexico City. *Drosophila Information Service*, **47**: 110–112.

Fine, P.E.M. 1975. Vectors and vertical transmission: an epidemiological perspective. *Ann. NY Acad. Sci.*, **266**: 173–194.

Fleuriet, A. 1976. Presence of the hereditary rhabdovirus sigma and polymorphism for a gene for resistance to this virus in natural populations of *Drosophila melanogaster*. *Evolution*, **30**: 735–739.

Fleuriet, A. 1980. Polymorphism of the hereditary sigma virus in natural populations of *Drosophila melanogaster*. *Genetics*, **95**: 459–465.

Fleuriet, A. 1981. Comparison of various physiological traits in flies (*Drosophila melanogaster*) of wild origin, infected or uninfected by the hereditary Rhabdovirus sigma. *Arch. Virol.*, **69**: 261–272.

Fleuriet, A. 1996. Polymorphism of the *Drosophila melanogaster*–sigma virus system. *J. Evol. Biol.*, **9**: 471–484.

Gay, P. 1978. Les genes de la drosophile qui interviennent dans la multiplication du virus sigma. *Mol. Gen. Genet.*, **159**: 269–283.

Goddard, L.B., Roth, A.E., Reisen, W.K. and Scott, T.W. 2003. Vertical transmission of West Nile virus by three California Culex (Diptera: Culicidae) species. *J. Med. Entomol.*, **40**: 743–746.

Goldstein, L. 1949. A mutant genoid in *Drosophila* sensitive to carbon dioxide. *Nature*, **164**: 369.

Inouye, B.D. 2005. The importance of the variance around the mean effect size of ecological processes: Comment. *Ecology*, **86**: 262–265.

L'Héritier, P. 1958. The hereditary virus of *Drosophila*. *Adv. Virus Res.*, **5**: 195–245.

L'Héritier, P. 1970. *Drosophila* viruses and their role as evolutionary factors. *Evol. Biol.*, **4**: 185–209.

L'Héritier, P. and Teissier, G. 1945. Transmission héréditaire de la sensibilité au gaz carbonique chez la Drosophile. *Publ. Lab. ENS Biol.*, **1**: 35–76.

Longdon, B., Obbard, D.J. and Jiggins, F.M. 2010. Sigma viruses from three species of *Drosophila* form a major new clade in the rhabdovirus phylogeny. *Proc. R. Soc. Lond. B*, **277**: 35–44.

Mangin, K.L., Lipsitch, M. and Ebert, D. 1995. Virulence and transmission modes of 2 microsporidia in *Daphnia magna*. *Parasitology*, **111**: 133–142.

May, R.M. and Nowak, M.A. 1995. Coinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B*, **261**: 209–215.

Miralles, R., Ferrer, R., Sole, R.V., Moya, A. and Elena, S.F. 2001. Multiple infection dynamics has pronounced effects on the fitness of RNA viruses. *J. Evol. Biol.*, **14**: 654–662.

Mishra, A.C. and Mourya, D.T. 2001. Transovarial transmission of West Nile virus in *Culex vishnui* mosquito. *Indian J. Med. Res.*, **114**: 212–214.

Morris, W.F. 1997. Disentangling effects of induced plant defenses and food quantity on herbivores by fitting nonlinear models. *Am. Nat.*, **150**: 299–327.

Murray, K.O., Mertens, E. and Despres, P. 2010. West Nile virus and its emergence in the United States of America. *Vet. Res.*, **41**: 67.

Nowak, M.A. and May, R.M. 1994. Superinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B*, **255**: 81–89.

R Development Core Team. 2011. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing (http://www.R-project.org/).

Sall, A.A., Faye, O., Diallo, M., Firth, C., Kitchen, A. and Holmes, E.C. 2010. Yellow fever virus exhibits slower evolutionary dynamics than dengue virus. *J. Virol.*, **84**: 765–772.

Scheipl, F. 2011. RLRsim: Exact (Restricted) Likelihood Ratio tests for mixed and additive models. R package version 2.0-7 (http://CRAN.R-project.org/package=RLRsim).

Serbus, L.R., Casper-Lindley, C., Landmann, F. and Sullivan, W. 2008. The genetics and cell biology of *Wolbachia*–host interactions. *Annu. Rev. Gen.*, **42**: 683–707.

van Baalen, M. and Sabelis, M.W. 1995. The dynamics of multiple infection and the evolution of virulence. *Am. Nat.*, **146**: 881–910.

Wayne, M.L., Contamine, D. and Kreitman, M. 1996. Molecular population genetics of *ref(2)P*, a locus which confers viral resistance in *Drosophila*. *Mol. Biol. Evol.*, **13**: 191–199.

Wayne, M.L., Telonis-Scott, M., Bono, L.M., Harshman, L., Kopp, A., Nuzhdin, S.V. *et al.* 2007. Simpler mode of inheritance of transcriptional variation in male *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*, **104**: 18577–18582.

Wilfert, L. and Jiggins, F.M. 2010a. Disease association mapping in *Drosophila* can be replicated in the wild. *Biol. Lett.*, **6**: 666–668.

Wilfert, L. and Jiggins, F.M. 2010b. Host–parasite coevolution: genetic variation in a virus population and the interaction with a host gene. *J. Evol. Biol.*, **23**: 1447–1455.

Williamson, D.L. 1961. Carbon dioxide sensitivity in *Drosophila affinis* and *D. athabasca*. *Genetics*, **46**: 1053–1060.

Yamauchi, A., Telschow, A. and Kobayashi, Y. 2010. Evolution of cytoplasmic sex ratio distorters: effect of paternal transmission. *J. Theor. Biol.*, **266**: 79–87.

Yampolsky, L.Y., Webb, C.T., Shabalina, S.A. and Kondrashov, A.S. 1999. Rapid accumulation of a vertically transmitted parasite triggered by relaxation of natural selection among hosts. *Evol. Ecol. Res.*, **1**: 581–589.

APPENDIX

Detailed explanation of second model, with transmission rates dependent on transmitting parent's source of infection (sire vs. dam) and sex of transmitting parent

As mentioned in the main text, we let e_{ij} be the transmission efficiency of a fly of sex i (F or M) that was infected by a fly of sex j (D for dam and S for sire), y_{ij} be the transmission by a female when she was infected by her parent of sex i and her mate was infected by his parent of sex j, and x_{ij} be the corresponding probabilities for male transmission (i and j are D or S).

The prevalence for both sexes is p_t in generation t, and the fraction of all offspring infected by a female is r_t. In this case, there are nine different types of mating, since the female can be uninfected, infected by her dam, or infected by her sire. The probabilities of these are $1 - p_t$, $r_t p_t$, and $(1 - r_t)p_t$, respectively. There are also the same three possibilities for the male. The probability of each type of mating is the product of the probabilities for the female and the male. Table A1 gives the types of mating pairs, their probabilities, and the corresponding fecundities and transmission efficiencies from the female and the male.

The total offspring production is the same as in our simple model, $1 - p_t(1 - q)$. We now need an expression for the number of offspring infected by females, and another for the number infected by males. Each is found as in the simple model, by finding sums of products of probabilities, fecundities, and transmission efficiencies for each mating type (see Table A1). For each infected offspring, we also need to assign the sex of the parent from which it was obtained. All infected offspring of an infected fly mating with an uninfected fly are obviously assigned to the sex of the infected parent, but the sex-specific transmission rates y_{ij} (for female transmission) and x_{ij} (for male) are used for matings of two infected

Table A1. Types of mating pairs, the probability of each (as a function of the prevalence p_t and fraction of infected offspring infected by the dam r_t), and corresponding parameters for relative fecundities and female and male transmission efficiencies

			Transmission	
Mating pair infection status	Probability	Fecundity	Female	Male
Both uninfected	$(1 - p_t)^2$	1	0	0
F infected by D	$r_t p_t(1 - p_t)$	q	e_{FD}	0
F infected by S	$(1 - r_t)p_t(1 - p_t)$	q	e_{FS}	0
M infected by D	$r_t p_t(1 - p_t)$	1	0	e_{MD}
M infected by S	$(1 - r_t)p_t(1 - p_t)$	1	0	e_{MS}
Both infected by D	$r_t^2 p_t^2$	q	y_{DD}	x_{DD}
Both infected by S	$(1 - r_t)^2 p_t^2$	q	y_{SS}	x_{SS}
F infected by D, M by S	$r_t(1 - r_t)p_t^2$	q	y_{DS}	x_{DS}
F infected by S, M by D	$r_t(1 - r_t)p_t^2$	q	y_{SD}	x_{SD}

Note: Only infected parents are specified in the first column, thus 'F infected by D' indicates the mating of a female infected by a dam with an uninfected male.

flies. The number of offspring infected by females is the sum of products of the probabilities, fecundities, and female transmission efficiencies, which is

$$o_F = \{p_t(1-p_t)q[r_t e_{FD} + (1-r_t)e_{FS}]\} + \{p_t^2 q[y_{DD} r_t^2 + y_{SS}(1-r_t)^2 + (y_{DS}+y_{SD})r_t(1-r_t)]\}, \quad \text{(A1)}$$

where the first term in curly brackets is for infected females mating with uninfected males [probability of this type of mating is $p_t(1-p_t)$ and fecundity q; transmission efficiencies are e_{FD} if the female was infected by the dam and e_{FS} if by the sire, and each is weighted by the corresponding probability]. The second term in curly brackets is for matings of two infected flies (all with probabilities p_t^2 and fecundity q; there are four different types of these matings and therefore four terms in the square brackets).

The number of offspring infected by males is similar, except that the male transmission efficiencies are used, which gives

$$o_M = p_t(1-p_t)[r_t e_{MD} + (1-r_t)e_{MS}] + p_t^2 q[x_{DD} r_t^2 + x_{SS}(1-r_t)^2 + (x_{DS}+x_{SD})r_t(1-r_t)]. \quad \text{(A2)}$$

The total number of infected offspring is the sum of the female- and male-infected offspring, $o_F + o_M$. The new prevalence is this divided by the total offspring $1 - p_t(1-q)$, while the new fraction infected by females is the number infected by females (o_F) divided by the total infected offspring ($o_F + o_M$). The recursions are therefore

$$p_{t+1} = \frac{o_F + o_M}{1 - p_t(1-q)} = p_t \frac{(1-p_t)R_{eff} + p_t q e_{B,eff}}{1 - p_t(1-q)} \quad \text{(A3)}$$

and

$$r_{t+1} = \frac{o_F}{o_F + o_M} = \frac{(1-p_t)q[r_t e_{FD} + (1-r_t)e_{FS}] + p_t q[y_{DD} r_t^2 + y_{SS}(1-r_t)^2 + (y_{DS}+y_{SD})r_t(1-r_t)]}{(1-p_t)R_{eff} + p_t q e_{B,eff}}, \quad \text{(A4)}$$

where

$$R_{eff} = q[r_t e_{FD} + (1-r_t)e_{FS}] + r_t e_{MD} + (1-r_t)e_{MS}$$

and

$$e_{B,eff} = (y_{DD} + x_{DD})r_t^2 + (y_{SS} + x_{SS})(1-r_t)^2 + (y_{DS} + y_{SD} + x_{DS} + x_{SD})r_t(1-r_t).$$

(These are analogous to the R and e_B parameters in the previous model, but there is averaging over the sex of the parent transmitting the infection.) Equations (A3) and (A4) reduce to (9) and (10) in the main text, where we assumed that when both flies are infected, the female transmits at the same efficiency she would if the male was uninfected, while the male can transmit only to offspring not infected by the female, so that $y_{ij} = e_{Fi}$ and $x_{ij} = (1 - e_{Fi})e_{Mj}$.