JOURNAL OF Evolutionary Biology

doi:10.1111/j.1420-9101.2010.02002.x

Host–parasite coevolution: genetic variation in a virus population and the interaction with a host gene

L. WILFERT & F. M. JIGGINS

Department of Genetics, University of Cambridge, Cambridge, UK

Keywords:

genetic interaction;
Host–parasite coevolution;
maintenance of genetic variation;
MCMCglmm;
paternal transmission;
QTL.

Abstract

Host–parasite coevolution is considered to be an important factor in maintaining genetic variation in resistance to pathogens. *Drosophila melanogaster* is naturally infected by the sigma virus, a vertically transmitted and host-specific pathogen. In fly populations, there is a large amount of genetic variation in the transmission rate from parent to offspring, much of which is caused by major-effect resistance polymorphisms. We have found that there are similarly high levels of genetic variation in the rate of paternal transmission among 95 different isolates of the virus as in the host. However, when we examined a transmission-blocking gene in the host, we found that it was effective across virus isolates. Therefore, the high levels of genetic variation observed in this system do not appear to be maintained because of coevolution resulting from interactions between this host gene and parasite genes.

Introduction

Hosts and their parasites are bound up in an intimate and perpetual arms race. Hosts are under selection to develop resistance towards costly parasite infections. A parasite's fitness in turn rests entirely on it being able to overcome any such resistance to reproduce in its host and to be transmitted to new hosts. Despite the strong selection for hosts to become resistant to parasites and for parasites to be universally infectious, which can lead to a reduction in genetic variation, genetic variance for disease resistance is widely found in nature (e.g. Carius *et al.*, 2001; Holub, 2001; Riehle *et al.*, 2006). One explanation as to why high genetic variation is so common is that, under certain circumstances, coevolution between hosts and parasites may itself be responsible for maintaining genetic variation within populations.

Coevolution can maintain genetic variation in host resistance because of parasites adapting to common host genotypes, which can result in an advantage to rare host genotypes and therefore negatively frequency-dependent selection. Models of coevolution typically rely on genotype–genotype interactions, where the outcome of an infection is determined by specific genotypic interactions and therefore the fitness of both players depends on the particular combination of host and parasite genotypes (Clay & Kover, 1996; Lively, 1996; Schmid-Hempel & Ebert, 2003). Coevolution can maintain polymorphisms under a wide range of conditions when the selective advantage of an allele declines as a direct consequence of its own frequency increasing (Tellier & Brown, 2007).

To predict whether host–parasite coevolution will lead to the eventual fixation of alleles in selective sweeps or whether genetic variation will be maintained as stable polymorphisms, we need to understand the genetics of both host resistance and parasite infectivity and transmission (Grech *et al.*, 2006). In many systems ranging from plant R genes (Ellis *et al.*, 2000) to the vertebrates' MHC complex (Piertney & Oliver, 2006), the molecular and evolutionary biology of host-resistance genes is well understood. Yet although we have detailed knowledge of genetic variation in the hosts, including in nonmodel invertebrate systems (e.g. Timmann *et al.*, 2007; Vorburger *et al.*, 2009), we lack this level of understanding in the corresponding parasites (Salvaudon *et al.*, 2008). This is because studies of host–parasite interactions either focus solely on the host (Kover, 2002; Tinsley *et al.*, 2006) or use few parasite isolates (e.g. Vale & Little,

Correspondence: Lena Wilfert, Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK.
Tel.: +44 (0)1223 333945; fax: +44 (0)1223 333999;
e-mail: lb445@cam.ac.uk

2009). Here, we use a tractable host–parasite system to study both sides of the interaction, by analysing genetic variation in the parasite and by testing whether there is an interaction between a known host-resistance gene and the parasite.

The *Drosophila melanogaster* – sigma virus interaction

The sigma virus (DmelSV) is a vertically transmitted pathogen of *D. melanogaster* that on average infects about 4% of flies in natural populations (Carpenter *et al.*, 2007). The virus is transmitted exclusively from parent to offspring via both eggs and sperm. *Drosophila melanogaster* is expected to be under selection to evolve resistance and reduce transmission from parent to offspring, as sigma virus infection has been shown to be costly, reducing egg viability (Fleuriet, 1981a) and increasing larval development time (Seecof, 1964). Further evidence that the sigma virus harms infected flies comes from population level studies, that have found that sigma prevalence is reduced under stressful conditions, such as overwintering (Fleuriet, 1981b) or resource competition (Yampolsky *et al.*, 1999).

As sigma is the only known naturally occurring and species-specific pathogen of *D. melanogaster*, it is a prime model for studies on host–parasite evolution. This system is also particularly tractable. Transmission from parent to offspring is under natural selection in both the host and the parasite (Bangham *et al.*, 2007; Fleuriet, 1980). By measuring vertical transmission, we can therefore estimate the genetic variance for the trait under selection in both the host and parasite. Sigma infections can be easily diagnosed as infected flies become permanently paralysed when exposed to high doses of CO_2 (Brun & Plus, 1980), which allows for large numbers of flies to be assayed for their infection status, providing statistical power for detailed quantitative genetic analyses. Finally, there is no recombination between sigma viruses (Carpenter *et al.*, 2007), so from a population genetic perspective, the entire genome can be viewed as a single gene. This simplifies any attempt to distinguish between alternative genetic models such as gene for gene and matching allele models.

The genetic variation – the raw material for adaptation – controlling sigma virus transmission in the host *D. melanogaster* is well understood. Variation for transmission through eggs is bimodal, with a polymorphism in the resistance gene *ref(2)p* explaining all of the phenotypic variation (Contamine *et al.*, 1989; Bangham *et al.*, 2008a). Paternal transmission through sperm on the other hand exhibits high quantitative genetic variation (Bangham *et al.*, 2008a). Some of this variation is caused by a major effect quantitative trait locus (QTL) on the second chromosome that causes a ~50% reduction in the proportion of infected offspring in lines carrying the homozygous resistant allele (Bangham *et al.*, 2008b).

In this paper, we estimate the genetic variance in the rate of paternal transmission in populations of the sigma virus, and ask whether there is a genetic interaction between host and parasite genes. We show that the genetic variation is high in both host and pathogen. This variation is unlikely to be maintained by the coevolution of interacting host and parasite genes, as a major-effect gene blocking transmission in the host is effective across viral isolates.

Materials and methods

Virus isolates

Wild isolates of sigma were collected from 10 individual populations across Europe and the United States in 2005 and 2007 (Table 1; the samples from 2005 have been previously described by Carpenter *et al.*, 2007). Each population was sampled from multiple fruit baits placed in one site no larger than 1 km across. We collected individual females and allowed them to lay eggs on yeasted standard *Drosophila* medium or instant *Drosophila* medium (Carolina Biological Supplies Co., Burlington, NC, USA). Additionally, we collected virus isolates from wild males from Athens, Greece. The protocol was identical as for the females, but we allowed individual field-collected males to mate with uninfected lab-reared sympatric virgin females. As we found no significant difference in paternal transmission for viruses isolated from males and females, we have pooled the data in all further analyses. In the laboratory, we assayed the offspring of field-collected flies for sigma after having allowed them to lay eggs on fresh standard medium. Flies were exposed to CO_2 at 12 °C for 15 min. Uninfected flies quickly recover from this anaesthesia, but flies carrying sigma remain paralysed and die (Brun & Plus, 1980). Flies were scored for recovery 2 h post-treatment and uninfected lines were discarded.

Table 1 Virus isolates.

Population	Collected in 2007	Collected in 2005 (Carpenter *et al.*, 2007)
Germany – Tubingen	4	–
Germany – Fichtelgebirge	7	–
Greece – Athens	55 (21)*	5
Spain – Galicia	–	2
Sweden – Uppsala	4	–
UK – Derby	1	–
UK – Essex	9	5
UK – Kent	1	–
USA – North Carolina	1	–
USA – Florida	–	1

*no. of isolates collected from males.

The sigma virus' only mode of transmission is vertical, so to measure genetic variation among virus isolates we crossed all virus isolates into the same fly genetic background. Using the double balancer line *SM2/Pm; TM3/Sb; spa^{Pol}*, which has an isogenic IS4 X chromosome, we substituted the 2nd and 3rd wildtype chromosomes with the isogenic line w^{1118} (described by Parks *et al.*, 2004). For the first cross, sigma-infected wildtype females were crossed to double balancer males. From this cross, we collected infected females that carried the balancer chromosomes (*SM2/+; TM3/+*) and crossed them to homozygous w^{1118} males. We backcrossed infected females (*SM2/*w^{1118}*; TM3/*w^{1118}) from this cross to w^{1118} males. We then performed an additional round of backcrossing to w^{1118} males and selected for white-eyed females. This should have reduced the wildtype and balancer background on the X-chromosome to ~12.5%. We ignored the 4th chromosome, as it contains only ~1% of the genome (Adams *et al.*, 2000). Through this crossing scheme, the genetic background that virus isolates are maintained in is expected to be > 95% from the w^{1118} stock. In the generation in which genetic variation for paternal transmission was measured, the virus isolates are expected to be in ~99% isogenic backgrounds because of an additional cross to an isogenic line. In a separate experiment, we sequenced a region of the viral genome from all these isolates (L. Wilfert, unpublished data). Three isolates were heteroplasmic (more than one sequence was present), and these isolates were removed from the experiment (AT029, B43, J028).

Measuring viral transmission

The paternal transmission rate of sigma is affected by a codominant QTL on the 2nd chromosome of *D. melanogaster* (Bangham *et al.*, 2008b). We measured the paternal transmission rate of our viral isolates in two fly lines that carried either the susceptible or the resistant allele of this QTL, but were otherwise genetically similar. As donors for the two QTL alleles, we used the 11B (resistant) and 2G (susceptible) stocks that were used to identify the QTL (cross 1 in Bangham *et al.*, 2008b). These lines are isogenic and genetically identical except for their 2nd chromosomes, which were sampled from a population in Pennsylvania (USA) (Lazzaro *et al.*, 2004).

For each virus isolate, two sigma-infected w^{1118} females were crossed to 11B (resistant) or 2G (susceptible) males. These crosses produced sons that were heterozygous for the entire second chromosome, carrying either the resistant or the susceptible allele over the w^{1118} background. It should be noted that, while w^{1118} flies are phenotypically susceptible to male transmission, whether the susceptible/w^{1118} individuals are homozygous or heterozygous for the resistance QTL cannot be determined, as the underlying gene has as yet not been cloned. Paternal transmission was measured in these males (5 days old) by crossing them to females (4–6 days old) from P18, an isogenic line from Pennsylvania that was used in the original QTL analysis (Bangham *et al.*, 2008a). These P18 flies had been reared at constant density by collecting eggs from grape-juice plates and pipetting 13 μL of eggs in phosphate-buffered saline into bottles of *Drosophila* medium (Clancy & Kennington, 2001). For all crosses, virgin females were kept on standard *Drosophila* food with *ad libitum* live yeast. Crosses were then set up with two males and two virgin females each in vials without live yeast and allowed to lay eggs for 2 days. All flies were kept at 25 °C on a 12-h light/dark cycle. In both generations of this cross, we checked that the sigma-contributing parents were infected using the CO_2 assay. To ensure complete transmission before the experimental cross, any vial containing an uninfected female (first cross) or male (second cross) was removed from the experiment. Sigma infection was assayed in the progeny of the experimental crosses 15 days after the crosses were set up. We repeated the experiment on 3 days. On each day, we carried out 1–6 replicates of each viral isolate with each QTL allele. In total, we assayed 54 333 flies in 1456 vials for 95 viral genotypes.

Molecular confirmation of CO_2 assay for sigma

We checked that our CO_2 assay is diagnostic of the presence or absence of the virus by PCR. Following the aforementioned procedure, we crossed w^{1118} males infected with the virus isolates AP30, E220 and PF115 to P18 females. The offspring were exposed to CO_2 in the normal way, and we selected two daughters that were paralysed and two that recovered per cross. RNA was extracted from these flies using 50 μL Trizol per fly (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The RNA was resuspended in 20 μL RNA storage buffer (Ambion, Austin, TX, USA) and reverse transcribed into cDNA using random hexamers and the M-MLV reverse transcriptase (Invitrogen). To check whether the flies had been infected, we used the primers σ2-F 5′-AAGATTCCCCTCGCATGG-3′ and σ2-R 5′-ACCCTTTCGGAAGGTTTGAG-3′ that amplify a region of the sigma virus genome. As a control for successful RNA extraction, we used primers act-F 5′-TGCAGCG GATAACTAGAAACTACTC-3′ and act-R 5′-CAAAGGA GCCTCAGTAAGCAAG-3′, which amplify the *D. melanogaster* housekeeping gene *Actin 42A*.

Statistical analysis of genetic variation

We analysed genetic variation in paternal transmission of the sigma virus using a generalized linear mixed model (GLMM). Paternal transmission is measured as a ratio of infected and uninfected individuals, so we modelled transmission with a binomial error distribution using a logit link function. Unlike the more traditional arcsine

transformation, this method allows the proportion data to be weighted according to sample size. Variances and related statistics are therefore reported on the logit scale. The model parameters and their confidence intervals were estimated using a Bayesian Markov chain Monte Carlo technique implemented in the R package MCMCglmm (Hadfield, 2010). The use of this Bayesian approach avoids problems with maximum likelihood GLMMs, such as difficulties in selecting the number of degrees of freedom when assessing the significance of fixed effects and relying on approximations of the likelihood (Bolker *et al.*, 2009). Credibility intervals can also be calculated directly even for derived parameters such as heritability, as the MCMC routine samples from the posterior distributions of all the model parameters (Nakagawa & Schielzeth, in press). All statistical analyses were carried out using R (v. 2.8.1).

In this model, we have treated QTL allele (resistant or susceptible) and the day on which the cross was set up as fixed effects, and viral isolate as a random effect.

The genetic variance V_g and error variance V_e of viral transmission were estimated as separate parameters in the resistant and susceptible flies. The model was formulated as:

$$Y_{i,j,k} = \mu + A_i + D_j + (A|V)_{i,k} + \varepsilon_{i,j,k} \quad (1)$$

where μ is the grand mean of the proportion of infected offspring, A_i is the fixed effect of the ith QTL allele (i = susceptible, resistant), D_j is the fixed effect of day j = 1, …, 3, V_k is the random effect of viral isolate k = 1, …, 95 and $\epsilon_{i,j,k,l}$ is the residual variance. Note that the model includes separate residual variances in the resistant and susceptible flies on different days, which avoids the overestimation of genetic variation.

The posterior distributions of the model parameters were estimated via Markov chain Monte Carlo runs, with a chain length of 10^6 iterations, of which 1000 were sampled. The parameter estimates quoted are the mean of these 1000 samples, and the 95% credible interval (CI) is the region with the 95% highest posterior density. Significance is indicated by the 95% highest posterior density not overlapping 0.

Results

The paternal transmission of the sigma virus is under the host's genetic control, and a major effect QTL on the second chromosome is known to reduce the paternal transmission rate of the viral isolate AP30 (Bangham *et al.*, 2008b). We measured the rate at which 95 different viral isolates are transmitted by male flies carrying either the resistant or the susceptible allele of this gene. The resistant allele did indeed lead to a significant drop in transmission across virus isolates, with a mean reduction in paternal transmission of 18.8% (95% CI: 13.4–23.3%; Fig.1a).

Genetic variation

There was significant genetic variation in the rate of transmission (V_g) among the viral genotypes in both the resistant and susceptible hosts (Table 2), with the observed transmission rates of individual genotypes ranging from zero to near 100% (Fig. 1a). As sigma is nonrecombining and haploid (Carpenter *et al.*, 2007), the additive genetic variance (V_a) is equivalent to the total genetic variance (V_g). Although V_g was similar in both treatments, the residual variance (V_r) was significantly higher in the susceptible genetic background than in the

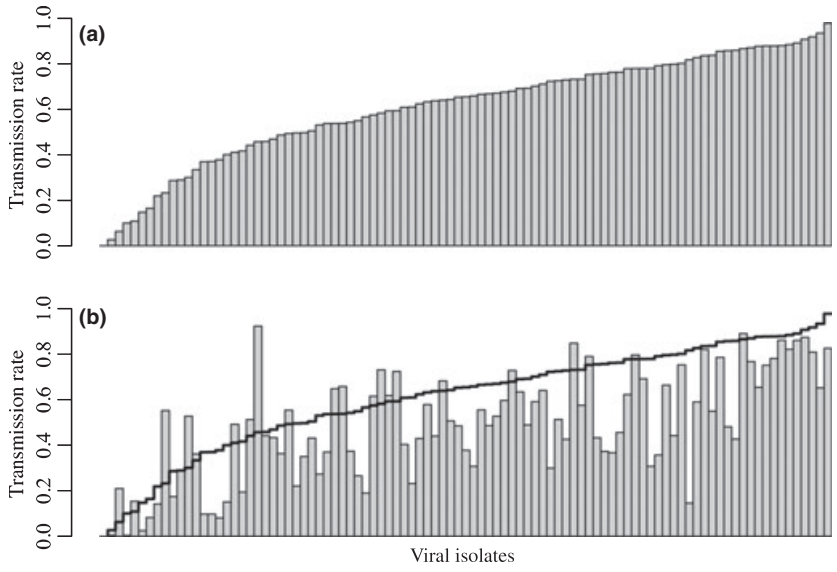

Fig. 1 The mean rate of transmission of different sigma virus isolates. Panel (a) shows the rate of paternal transmission in hosts carrying the susceptible allele of the transmission-blocking quantitative trait locus. Panel (b) shows the rate of paternal transmission in hosts carrying the resistant allele. The black line indicates the corresponding transmission rate in the susceptible hosts.

Table 2 Genetic variation in the paternal transmission of the sigma virus (values in brackets indicate 95% credible intervals). The analysis was performed using either all virus isolates (all populations, $n = 95$) or restricted to the Athens population ($n = 60$).

Fly allele	Population	Mean (%)*	V_g	V_r	h^2
Susceptible	All populations	63.06	2.11	1.21	0.32
		(55.78–69.47)	(1.43–2.78)	(1.03–1.40)	(0.24–0.38)
	Athens	68.28	1.61	1.49	0.22
		(59.73–76.15)	(1.25–2.12)	(1.28–1.77)	(0.15–0.31)
Resistant	All populations	44.33	2.44	0.85	0.37
		(37.27–53.31)	(1.72–3.22)	(0.72–0.97)	(0.30–0.45)
	Athens	49.4	1.96	1.88	0.18
		(41.8 1–57.97)	(1.51–2.61)	(1.55–2.35)	(0.13–0.25)

*Trait means (x) were backtransformed from the logit scale into percentages with the link function $\bar{X} = \frac{1}{1+\frac{1}{e^{\bar{x}}}}$.

flies carrying the resistant allele with $\Delta V_r = 0.36$ (95% CI = 0.15–0.61).

We calculated the heritability h^2 from V_g and the residual variance V_r as $h^2 = V_g/(V_v + V_r + \pi^2/3)$. The term $\pi^2/3$ is the distribution-specific variance of a logit-link model (Lee *et al.*, 2006), which is added to the residual variance V_r to calculate the heritability from a binomial model with additive overdispersion as implemented in MCMCglmm (Nakagawa & Schielzeth, in press). As V_g is equivalent to V_a, this is an estimate of narrow-sense heritability. The heritability of transmission was relatively high in both the susceptible and resistant hosts $h^2_{sus} = 0.32$, $h^2_{res} = 0.37$), indicating that this trait has the potential to respond rapidly to selection (Table 2).

Our experiments included many viral isolates to allow us to estimate genetic variances, meaning that trait means for individual viruses are poorly estimated. Nonetheless, it is interesting to ask whether the genetic variation that we have observed is caused by a smaller number of different viral types in the populations or by continuous genetic variation. Therefore, we tested for significant differences between all virus isolates in both host genotypes using pair-wise general linear models with virus and cohort as fixed effects and a quasi-binomial error structure. We corrected for multiple testing (number of tests per host background $m = 4465$) by using False Discovery Rates according to Benjamini & Hochberg (1995) and identified distinct viral types by their pattern of significance using multidimensional scaling. This approach confirmed the high level of continuous genetic variation between viral isolates with no clusters of identical viruses. Within the resistant host background, all virus isolates were phenotypically distinguishable, whereas in the susceptible background we found 91 distinct viral types with four pairs of viruses whose phenotypes were indistinguishable.

Genetic covariance

Are all viruses sensitive to the host's resistance allele? As Fig. 1a shows, most virus isolates showed reduced transmission rates in the resistant host line, although there was a large amount of variation. The genetic correlation between transmission in resistant and susceptible flies (C) was 86.6% (95% CI = 79.8–93.8%). This strong correlation indicates that the resistance QTL affects most of the virus genotypes similarly, even though the interaction itself was significant. The deviance information criterion (a generalization of the AIC and BIC for Markov Chain Monte Carlo Bayesian models (Spiegelhalter *et al.*, 2002)) was smaller for the model including the interaction (DIC = 54522.99) then for the model without an interaction (DIC = 55627.41).

Effect of population structure

Measures of genetic variance and heritability can be affected by population structure. To test whether our estimate of genetic variance for paternal transmission was inflated by underlying population structure, we included the population of origin as a fixed effect in the model. As in the simple model described earlier, the QTL allele significantly affected paternal transmission, with transmission being on average 14.14% lower in the resistant genetic background (CI 3.4–21.37%). The genetic variance V_g was very similar in the simple model and in the model accounting for population structure ($V_{g\_sus} = 2.11$ and $V_{g\_sus\_pop} = 1.8$ (CI 1.37–4.3) and $V_{g\_res} = 2.44$ and $V_{g\_res\_pop} = 2.4$ (CI 1.6–8.31) in the two models, respectively). We thus found no evidence for population structure leading to an overestimation of genetic variance.

As 60 of our 95 viral isolates (Table 1) originated from a particularly well-sampled field site in Athens (Greece), we repeated the analysis for this population on its own. While we found that, in the resistant host background, the residual variance V_r and the heritability h^2 were significantly lower in the Athens population than in the entire sample, there was no difference in the genetic variance V_g (see Table 2). We thus conclude that selection for increased transmission can act within populations of the sigma virus.

Maternal transmission

The sigma virus can be transmitted to offspring by both the father and the mother. While we found a large amount of genetic variation for paternal transmission, maternal transmission was nearly perfect across all lines. We measured maternal transmission in the cross providing the experimental males. We found very little variation in maternal transmission across virus lines, with all but two of 95 lines showing perfect transmission.

Molecular detection of sigma

We have verified that offspring diagnosed as uninfected based on CO_2 sensitivity are indeed free of sigma using PCR detection of the viral RNA. As shown in Fig. 2, flies that recovered from CO_2 exposure showed no or a very weak band for sigma, when compared to the very strong band in their siblings that were paralysed. Therefore, the CO_2 assay is indeed diagnostic of sigma infection in studies of vertical transmission, the natural way of infection in this host–parasite system.

Discussion

We found considerable genetic variation among isolates of the sigma virus in the rate at which they are transmitted from infected males to offspring. As a vertically transmitted parasite, the fitness of the sigma virus is equal to the number of infected offspring produced by an infected fly. Therefore, there will be strong selection on the sigma virus to increase the rate of vertical transmission. The high genetic variation that we have observed shows that populations of the virus are able to rapidly respond to this selection pressure.

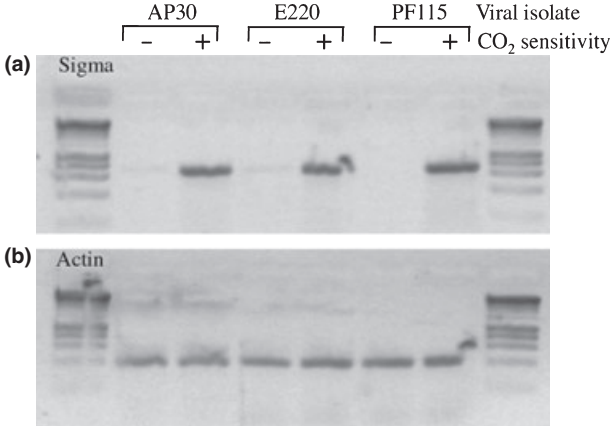

Fig. 2 Molecular detection in of the sigma virus in CO_2 sensitive (+) and insensitive (−) offspring of males infected with three different viral isolates. The upper panel shows a PCR product amplified from the sigma virus, and the lower panel is a product of *Drosophila melanogaster* actin mRNA.

The pattern of genetic variation that we observed in the virus population mirrors what we have found in the host population, where there is considerable genetic variation among fly genotypes in the rate at which infected males transmit the virus to their offspring (Bangham *et al.*, 2007). In flies, selection will act to reduce the rate of transmission and the high genetic variation in transmission rates means that they can rapidly respond to this selection pressure, even though the strength of selection is presumably lower in the host than the virus, as sigma only has a small effect on fitness (e.g. Fleuriet, 1981a) and most flies are uninfected (Carpenter *et al.*, 2007). The fitness of the virus in contrast hinges exclusively on its transmission to offspring.

How are sigma virus genotypes with low transmission maintained in the population? This genetic variation may be maintained if the genes that control transmission in the host and the virus interact. In the system we describe here, both the variation in the viral population and the resistant and susceptible alleles of the transmission-blocking QTL (Bangham *et al.*, 2008b) could be maintained if the effect of the QTL is reversed when confronted with different parasite genotypes. We can clearly reject this extreme scenario, as there is a strong positive genetic correlation between transmission in resistant and susceptible hosts. This shows that selection to increase transmission in resistant flies will increase transmission in susceptible flies and *vice versa*. Therefore, viruses do not face a trade-off between transmission in resistant and susceptible hosts.

While the genetic correlation was high, we also found a significant interaction between transmission in resistant and susceptible hosts. In combination, this shows that only a small proportion of the viral genetic variance interacts with the resistance locus. We can therefore not rule out that this genetic interaction plays a role in maintaining genetic variation for paternal transmission in the host and its parasite, although it is unlikely to be the main factor. Alternative explanations for the maintenance of susceptible hosts and low transmission parasites in this system include the presence of trade-offs with other host genes or genotype-environment interactions (Gillespie & Turelli, 1989). In this system, where the virus is vertically transmitted by both parents, trade-offs between fitness and transmission in the two sexes may be important for the maintenance of genetic variation.

Alternatively, this variation may not be stably maintained. It could result either from mutation-selection balance (Zhang & Hill, 2005), where deleterious mutations introduce genetic variation and purifying selection removes it. It is however unclear whether the observed magnitude of genetic variation in such a primary component of viral fitness could be maintained under this scenario. On the other hand, many viral genotypes may not be at their adaptive equilibrium, and the genetic variation could be because of transient polymorphisms that occur as new advantageous mutations and sweep

through the host and parasite populations. Such polymorphisms explain much of the genetic variation in the resistance of flies to the sigma virus, as is exemplified a recent selective sweep in the *ref(2)P* gene (Bangham *et al.*, 2007). A third relevant factor may be the strong population structure seen in the sigma virus population (Carpenter *et al.*, 2007). Population structure can maintain genetic variation across populations, by allowing local adaptation or by reducing genetic drift across the global population (Felsenstein, 1976). However, our analysis suggests that population structure does not play a major role in this system, as we found no evidence for genetic variation being partitioned between populations.

In contrast to the quantitative variation found for paternal transmission, genetic variation for maternal transmission has very simple genetics in both the host and the virus. In the host, all of the genetic variation in maternal transmission is explained by a single polymorphism in the *ref(2)P* gene (Contamine *et al.*, 1989; Bangham *et al.*, 2008a; Dru *et al.*, 1993; Wayne *et al.*, 1996). The virus also occurs in two forms in nature, an avirulent type that is sensitive to the resistant *ref(2)P* allele and a virulent type that is not (Fleuriet, 1980). Therefore, unlike the gene we have studied, the genetics of this interaction clearly fits the gene-for-gene model. As all the flies we used carried the susceptible allele of *ref(2)P,* we did not see any genetic variation in maternal transmission. Both the resistant *ref(2)p* allele (Bangham *et al.*, 2007) and the virulent virus type (Fleuriet & Periquet, 1993; Fleuriet & Sperlich, 1992) have recently increased in frequency in natural populations. This suggests that the host-resistance gene and the viral counter-adaptation either may be sweeping to fixation or they may cycle in frequency as suggested by many models of coevolution.

In this study, we found that a transmission-blocking QTL that was identified using a single viral isolate (Bangham *et al.*, 2008b) is effective against a wide range of other natural isolates. QTL studies for disease resistance, including ones relevant for agriculture and medicine, frequently focus on a particular cross of a resistant and a susceptible host line and a single parasite isolate. Yet QTLs found with this straightforward design are often specific to the particular host–parasite genotype combinations and may not be a general characteristic of that system (Wilfert & Schmid-Hempel, 2008). For example, Denby *et al.* (2004) report entirely different QTLs for resistance to two isolates of the fungus *Botrytis cinerea* in *Arabidopsis thaliana* in the same study. Similar patterns were found for resistance to dengue virus infection in the mosquito *Aedes aegypti*. Different QTLs were found for increasing the midgut infection barrier in a cross between lines recently isolated from the field and tested with a virus isolate from Puerto Rico (Bosio *et al.*, 2000), and crosses of artificially selected laboratory lines tested with another isolate (Gomez-Machorro *et al.*, 2004). In contrast, in a study of the paternal transmission of the sigma virus in *D. melanogaster*, Bangham *et al.* (2008b) used three independent crosses of fly lines with low or high transmission rates, and found the same QTL in all the three crosses. We have completed this by showing that this QTL conveys resistance to paternal transmission in a large panel of wild virus isolates. Thus, we can conclude that this locus is an important fixture in the evolution of resistance to sigma in *D. melanogaster* in nature.

The relative lack of host genotype × parasite genotype interactions that we found contrasts with many other invertebrate–parasite systems where there are strong interactions (e.g. Lambrechts *et al.*, 2005; Little *et al.*, 2006). Like many other negative sense RNA viruses, the sigma virus does not recombine (Carpenter *et al.*, 2007; Chare *et al.*, 2003). This could reduce the number of genetic interactions, as whenever natural selection fixes an advantageous mutation in the viral population, it will remove variation at all other genes in the genome. In the sigma virus, it is likely that a virulent strain that is not affected by the *ref(2)P* resistance mutation is currently sweeping through the population. This may have removed genetic variation in the viral population that interacts with other resistance genes in the host. Therefore, the maintenance of balanced polymorphisms may be less likely in entirely clonal parasites, than in parasites that are sexual or have other means of genetic exchange.

Conclusions

How genetic variation in the fitness of hosts and their parasites is maintained is a hotly debated topic in evolutionary biology. In a tightly coevolving system, we have found that both the host and parasite populations have high levels of genetic variation affecting the parasite's rate of vertical transmission through sperm. Therefore, the host can rapidly respond to selection to reduce the transmission rate, and the parasite can rapidly evolve a higher transmission rate. Theoretical models predict that this variation could be maintained if there is an interaction between the two partners, so that the rate of transmission depends on the combination of host-resistance genes and parasite genes. However, we found no evidence for a strong genetic interaction between a major-effect host gene and parasite genes. Therefore, we conclude that this genetic variation for paternal transmission is probably not stably maintained because of interactions between host and parasite genotypes.

Acknowledgments

We thank Claire Webster, Jenny Bangham and Mike Magwire for assistance in the laboratory. Jarrod Hadfield and Shinichi Nakagawa provided invaluable statistical advice. We are grateful to the people who helped collecting viral isolates in the field: Natasa Fytrou & family, Sandra South, Rebecca Schulte, Florian Bayer &

family, Ben Longdon and Darren Obbard. This work was financially supported by a Leverhulme Trust grant and a Royal Society University Research Fellowship to F.J.

References

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., George, R.A., Lewis, S.E., Richards, S., Ashburner, M., Henderson, S.N., Sutton, G.G., Wortman, J.R., Yandell, M.D., Zhang, Q., Chen, L.X., Brandon, R.C., Rogers, Y.H.C., Blazej, R.G., Champe, M., Pfeiffer, B.D., Wan, K.H., Doyle, C., Baxter, E.G., Helt, G., Nelson, C.R., Miklos, G.L.G., Abril, J.F., Agbayani, A., An, H.J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R.M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E.M., Beeson, K.Y., Benos, P.V., Berman, B.P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M.R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K.C., Busam, D.A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J.M., Cawley, S., Dahlke, C., Davenport, L.B., Davies, A., de Pablos, B., Delcher, A., Deng, Z.M., Mays, A.D., Dew, I., Dietz, S.M., Dodson, K., Doup, L.E., Downes, M., Dugan-Rocha, S., Dunkov, B.C., Dunn, P., Durbin, K.J., Evangelista, C.C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A.E., Garg, N.S., Gelbart, W.M., Glasser, K., Glodek, A., Gong, F.C., Gorrell, J.H., Gu, Z.P., Guan, P., Harris, M., Harris, N.L., Harvey, D., Heiman, T.J., Hernandez, J.R., Houck, J., Hostin, D., Houston, D.A., Howland, T.J., Wei, M.H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G.H., Ke, Z.X., Kennison, J.A., Ketchum, K.A., Kimmel, B.E., Kodira, C.D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z.W., Lasko, P., Lei, Y.D., Levitsky, A.A., Li, J.Y., Li, Z.Y., Liang, Y., Lin, X.Y., Liu, X.J., Mattei, B., McIntosh, T.C., McLeod, M.P., McPherson, D., Merkulov, G., Milshina, N.V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S.M., Moy, M., Murphy, B., Murphy, L., Muzny, D.M., Nelson, D.L., Nelson, D.R., Nelson, K.A., Nixon, K., Nusskern, D.R., Pacleb, J.M., Palazzolo, M., Pittman, G.S., Pan, S., Pollard, J., Puri, V., Reese, M.G., Reinert, K., Remington, K., Saunders, R.D.C., Scheeler, F., Shen, H., Shue, B.C., Siden-Kiamos, I., Simpson, M., Skupski, M.P., Smith, T., Spier, E., Spradling, A.C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A.H.H., Wang, X., Wang, Z.Y., Wassarman, D.A., Weinstock, G.M., Weissenbach, J., Williams, S.M., Woodage, T., Worley, K.C., Wu, D., Yang, S., Yao, Q.A., Ye, J., Yeh, R.F., Zaveri, J.S., Zhan, M., Zhang, G.G., Zhao, Q., Zheng, L.S., Zheng, X.Q.H., Zhong, F.N., Zhong, W.Y., Zhou, X.J., Zhu, S.P., Zhu, X.H., Smith, H.O., Gibbs, R.A., Myers, E.W., Rubin, G.M. & Venter, J.C. 2000. The genome sequence of *Drosophila melanogaster*. *Science* **287**: 2185–2195.

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

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

Bangham, J., Knott, S.A., Kim, K.W., Young, R.S. & Jiggins, F.M. 2008b. Genetic variation affecting host-parasite interactions: major-effect quantitative trait loci affect the transmission of sigma virus in *Drosophila melanogaster*. *Mol. Ecol.* **17**: 3800–3807.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B Met.* **57**: 289–300.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**: 127–135.

Bosio, C.F., Fulton, R.E., Salasek, M.L., Beaty, B.J. & Black, W.C. 2000. Quantitative trait loci that control vector competence for dengue-2 virus in the mosquito *Aedes aegypti*. *Genetics* **156**: 687–698.

Brun, P. & Plus, N. (1980) The viruses of *Drosophila*. In: *The Genetics and Biology of Drosophila*, **Vol. 2d** (M. Ashburner & T.R.F. Wright, eds), pp. 625–702. Academic Press, London.

Carius, H.J., Little, T.J. & Ebert, D. 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. *Evolution* **55**: 1136–1145.

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

Chare, E.R., Gould, E.A. & Holmes, E.C. 2003. Phylogenetic analysis reveals a low rate of homologous recombination in negative-sense RNA viruses. *J. Gen. Virol.* **84**: 2691–2703.

Clancy, D.J. & Kennington, W.J. 2001. A simple method to achieve consistent larval density in bottle cultures. *Drosoph. Inf. Serv.* **84**: 168–169.

Clay, K. & Kover, P.X. 1996. The Red Queen Hypothesis and plant/pathogen interactions. *Annu. Rev. Phytopathol.* **34**: 29–50.

Contamine, D., Petitjean, A.M. & Ashburner, M. 1989. Genetic resistance to viral infection – the molecular cloning of a *Drosophila* gene that restricts infection by the rhabdovirus sigma. *Genetics* **123**: 525–533.

Denby, K.J., Kumar, P. & Kliebenstein, D.J. 2004. Identification of *Botrytis cinerea* susceptibility loci in *Arabidopsis thaliana*. *Plant J.* **38**: 473–486.

Dru, P., Bras, F., Dezelee, S., Gay, P., Petitjean, A.M., Pierredeneubourg, A., Teninges, D. & Contamine, D. 1993. Unusual variability of the *Drosophila melanogaster* Ref(2)P protein which controls the multiplication of sigma rhabdovirus. *Genetics* **133**: 943–954.

Ellis, J., Dodds, P. & Pryor, T. 2000. Structure, function and evolution of plant disease resistance genes. *Curr. Opin. Plant Biol.* **3**: 278–284.

Felsenstein, J. 1976. Theoretical population genetics of variable selection and migration. *Annu. Rev. Genet.* **10**: 253–280.

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

Fleuriet, A. 1981a. 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. 1981b. Effect of overwintering on the frequency of flies infected by the rhabdovirus sigma in experimental populations of *Drosophila melanogaster*. *Arch. Virol.* **69**: 253–260.

Fleuriet, A. & Periquet, G. 1993. Evolution of the *Drosophila melanogaster* sigma virus system in natural populations from Languedoc (Southern France). *Arch. Virol.* **129**: 131–143.

Fleuriet, A. & Sperlich, D. 1992. Evolution of the *Drosophila melanogaster* – sigma virus system in a natural population from Tubingen. *Theor. Appl. Genet.* **85**: 186–189.

Gillespie, J.H. & Turelli, M. 1989. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics* **121**: 129–138.

Gomez-Machorro, C., Bennett, K.E., Munoz, M.D. & Black, W.C. 2004. Quantitative trait loci affecting dengue midgut infection barriers in an advanced intercross line of *Aedes aegypti*. *Insect Mol. Biol.* **13**: 637–648.

Grech, K., Watt, K. & Read, A.F. 2006. Host-parasite interactions for virulence and resistance in a malaria model system. *J. Evol. Biol.* **19**: 1620–1630.

Hadfield, J.D. (2010) MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. *J. Stat. Software* **33**: 1–22.

Holub, E.B. 2001. The arms race is ancient history in *Arabidopsis*, the wildflower. *Nat. Rev. Genet.* **2**: 516–527.

Kover, P. 2002. Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. *Proc. Nat. Acad. Sci. USA* **99**: 11270–11274.

Lambrechts, L., Halbert, J., Durand, P., Gouagna, L.C. & Koella, J.C. 2005. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to *Plasmodium falciparum*. *Malar. J.* **4**: doi:10.1186/1475-2875-4-3.

Lazzaro, B.P., Sceurman, B.K. & Clark, A.G. 2004. Genetic basis of natural variation in *D. melanogaster* antibacterial immunity. *Science* **303**: 1873–1876.

Lee, Y., Nelder, J.A. & Pawitan, Y. 2006. *Generalized Linear Models with Random Effects: Unified Analysis via H-Likelihood*. Chapman & Hall/CRC, Boca Raton, FL.

Little, T.J., Watt, K. & Ebert, D. 2006. Parasite-host specificity: experimental studies on the basis of parasite adaptation. *Evolution* **60**: 31–38.

Lively, C.M. 1996. Host-parasite coevolution and sex – Do interactions between biological enemies maintain genetic variation and cross-fertilization? *Bioscience* **46**: 107–114.

Nakagawa, S. & Schielzeth, H. in press. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.*

Parks, A.L., Cook, K.R., Belvin, M., Dompe, N.A., Fawcett, R., Huppert, K., Tan, L.R., Winter, C.G., Bogart, K.P., Deal, J.E., Deal-Herr, M.E., Grant, D., Marcinko, M., Miyazaki, W.Y., Robertson, S., Shaw, K.J., Tabios, M., Vysotskaia, V., Zhao, L., Andrade, R.S., Edgar, K.A., Howie, E., Killpack, K., Milash, B., Norton, A., Thao, D., Whittaker, K., Winner, M.A., Friedman, L., Margolis, J., Singer, M.A., Kopczynski, C., Curtis, D., Kaufman, T.C., Plowman, G.D., Duyk, G. & Francis-Lang, H.L. 2004. Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome. *Nat. Genet.* **36**: 288–292.

Piertney, S.B. & Oliver, M.K. 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity* **96**: 7–21.

Riehle, M.M., Markianos, K., Niare, O., Xu, J.N., Li, J., Toure, A.M., Podiougou, B., Oduol, F., Diawara, S., Diallo, M., Coulibaly, B., Ouatara, A., Kruglyak, L., Traore, S.F. & Vernick, K.D. 2006. Natural malaria infection in *Anopheles gambiae* is regulated by a single genomic control region. *Science* **312**: 577–579.

Salvaudon, L., Giraud, T. & Shykoff, J.A. 2008. Genetic diversity in natural populations: a fundamental component of plant-microbe interactions. *Curr. Opin. Plant Biol.* **11**: 135–143.

Schmid-Hempel, P. & Ebert, D. 2003. On the evolutionary ecology of specific immune defence. *Trends Ecol. Evol.* **18**: 27–32.

Seecof, R.L. 1964. Deleterious effects on *Drosophila* development associated with the sigma virus infection. *Virology* **22**: 142–148.

Spiegelhalter, D.J., Best, N.G., Carlin, B.R. & van der Linde, A. 2002. Bayesian measures of model complexity and fit. *J. R. Stat. Soc. B Met* **64**: 583–616.

Tellier, A. & Brown, J.K.M. 2007. Stability of genetic polymorphism in host-parasite interactions. *Proc. R. Soc. Lond. B Biol. Sci.* **274**: 809–817.

Timmann, C., Evans, J.A., Konig, I.R., Kleensang, A., Ruschendorf, F., Lenzen, J., Sievertsen, J., Becker, C., Enuameh, Y., Kwakye, K.O., Opoku, E., Browne, E.N.L., Ziegler, A., Nurnberg, P. & Horstmann, R.D. 2007. Genome-wide linkage analysis of malaria infection intensity and mild disease. *Plos Genet.* **3**: 393–400.

Tinsley, M.C., Blanford, S. & Jiggins, F.M. 2006. Genetic variation in *Drosophila melanogaster* pathogen susceptibility. *Parasitology* **132**: 767–773.

Vale, P.F. & Little, T.J. 2009. Measuring parasite fitness under genetic and thermal variation. *Heredity* **103**: 102–109.

Vorburger, C., Sandrock, C., Gouskov, A., Castaneda, L.E. & Ferrari, J. 2009. Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction. *Evolution* **63**: 1439–1450.

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

Wilfert, L. & Schmid-Hempel, P. 2008. The genetic architecture of susceptibility to parasites. *BMC Evol. Biol.* **8**: doi:10.1186/1471-2148-8-187.

Yampolsky, L.Y., Webb, C.T., Shabalina, S.A. & 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.

Zhang, X.-S. & Hill, W.G. 2005. Genetic variability under mutation selection balance. *Trends Ecol. Evol.* **20**: 468–470.

Received 21 September 2009; revised 10 March 2010; accepted 26 March 2010