

SPECIFIC INTERACTIONS BETWEEN HOST AND PARASITE GENOTYPES DO NOT ACT AS A CONSTRAINT ON THE EVOLUTION OF ANTIVIRAL RESISTANCE IN *DROSOPHILA*

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Genetic correlations between parasite resistance and other traits can act as an evolutionary constraint and prevent a population from evolving increased resistance. For example, previous studies have found negative genetic correlations between host resistance and life-history traits. In invertebrates, the level of resistance often depends on the combination of the host and parasite genotypes, and in this study, we have investigated whether such specific resistance also acts as an evolutionary constraint. We measured the resistance of different genotypes of the fruit fly *Drosophila melanogaster* to different genotypes of a naturally occurring pathogen, the sigma virus. Using a multitrait analysis, we examine whether genetic covariances alter the potential to select for general resistance against all of the different viral genotypes. We found large amounts of heritable variation in resistance, and evidence for specific interactions between host and parasite, but these interactions resulted in little constraint on *Drosophila* evolving greater resistance.

KEY WORDS: *Drosophila melanogaster*, genetic variation, genetic variance–covariance matrix, host–parasite coevolution, sigma virus.

Background

If a parasite is common and harmful, it will impose selection for increased host resistance. To predict whether the population will respond to this selection pressure by evolving increased resistance, we need to understand what constrains the evolution of resistance. Genetic constraints exist when a trait's response to selection is limited by selection on another genetically correlated trait. These genetic correlations are thought to arise mainly because of pleiotropic effects—when a gene has an effect on two or more traits (Falconer and Mackay 1996)—and result in phenotypic change that is a consequence both of direct selection on a

trait and indirect selection on traits that genetically covary. And so, if traits are negatively genetically correlated and selected in the same direction, the overall response to selection will be reduced. Under certain circumstances, this has the potential to act as an absolute constraint, preventing any evolutionary change in either trait.

Genetic constraints on the ability to evolve resistance can be caused by trade-offs with other components of fitness (Boots and Begon 1993; Groeters et al. 1994; Kraaijeveld and Godfray 1997; Webster and Woolhouse 1999; Hosken 2001), or trade-offs in resistance against other pathogens (or pathogen genotypes). In

the search for the latter, there are a growing number of examples of specific host–parasite interactions, where host genotypes are resistant to one parasite genotype but susceptible to another (Gross et al. 1980; Siegel and Gross 1980; Gehad et al. 1999; Johnsen and Zuk 1999; Gill et al. 2000). However, in these studies, it is usually unclear if these specific interactions constrain the evolution of general resistance to all parasite genotypes. One of the difficulties in interpreting these studies is that often too few host genotypes have been sampled to accurately estimate genetic correlations between resistance to different pathogen genotypes.

Another reason that it is difficult to assess the importance of genetic constraints is that previous studies have tended to examine trade-offs between pairs of traits, and, by doing so, they may miss the role of constraints on the evolution of resistance in nature where selection acts simultaneously on many different traits. This is important for the evolution of host resistance, as there can be complex genetic correlations both between multiple aspects of the immune response and between the immune system and life-history traits (Cotter et al. 2004). When selection operates on multiple traits simultaneously, even small pairwise genetic correlations can result as absolute genetic constraints (Dickerson 1955; Charlesworth 1984; Blows and Hoffmann 2005). This is because there may be directions in multivariate trait space where no genetic variation is available to selection—even when individual traits display substantial genetic variation. And if selection acts in that direction, then evolutionary change will not be possible.

In this study, we examine whether trade-offs in resistance against different pathogen genotypes acts to constrain the evolution of increased resistance. To investigate this, we measured the variation in transmission of five different viral isolates in *Drosophila melanogaster* lines with different X, second, and third chromosomes. Our aim is threefold: First, to measure the amount of genetic variation affecting transmission rates for each chromosome separately, second, to determine what proportion of this variation affects transmission of all five viruses, and what proportion affects transmission of particular viruses; and third, to assess whether the evolution of general resistance is constrained by the specific interactions that we find.

To do this, we have used a multivariate statistical approach, based on Lande's **G**-matrix, to describe patterns of genetic variation in the rate that the virus is transmitted (Lande 1979). By calculating the genetic covariances between the rates at which different isolates of the virus are transmitted, it is possible to predict how selection for resistance to one virus will cause a change in resistance to the others. However, when more than two viruses are measured, it becomes difficult to interpret the increasing numbers of covariances. Therefore, we have used eigen analysis to analyze **G** matrices. This involves generating two new variables: eigenvectors, which are linear combinations of the original traits (i.e., the genetic variances and covariances); and eigenvalues, which

reflect the amount of genetic variance associated with each vector (see [McGuigan (2006)] for a review of this approach). This makes it simple to assess the relative importance of each of the eigenvectors, and therefore the proportion of the total genetic variation in the population that will increase resistance to all the viral strains.

Material and Methods

FLY STOCKS, VIRAL ISOLATES, AND GENERAL METHODS

The effect of the *D. melanogaster* X, second, and third chromosomes on sigma virus transmission was measured independently using three panels of chromosome substitution lines. Using different chromosomes lines allowed the virus to be crossed in with balancer chromosomes (see below) and allowed us to compare the contribution of different regions of the genomes to resistance. In these lines, a given chromosome taken from the wild was made homozygous and crossed into the same isogenic genetic background.

We used 69 X chromosome substitution lines that were created by Tony Long from a population in California (U.S.A.) in 1998. These lines were created by crossing isogenic $C(1)DXy^1 f^1; bw^1; st^1$ flies to the wild-type flies, and then backcrossing F1 males back to $C(1)DXy^1 f^1; bw^1; st^1$. The resulting $+; bw^1; st^1$ males (where + is the X chromosome from California) were maintained in a stock with $C(1)DXy^1 f^1; bw^1; st^1$ females. We used 77 second chromosome substitution lines that were created by (Lazzaro et al. 2004) from flies collected in Pennsylvania (U.S.A.) in 1998 and 1999. These lines have been previously genotyped for a polymorphism in a gene called *ref(2)P* that is known to affect the susceptibility of *D. melanogaster* to the sigma virus (Banghetam et al. 2007). We used 67 third chromosome substitution lines that we created ourselves from flies collected in North Carolina (U.S.A.) in 1997 by Trudy Mackay. To make these third chromosome substitution lines, we backcrossed the Mackay-collected flies to a $w^{1118}; TM3, Sb/TM6B, Tb$ stock that has the isogenic Canton-S B genetic background (Bellen et al. 2004; Harbison et al. 2004) for six generations, and then crossed siblings to obtain $w^{1118}; +$ flies (where + is the homozygous chromosome from North Carolina).

Because the sigma virus is transmitted vertically, we created fly stocks that were infected with the sigma virus, and used these to cross the virus into the chromosome substitution lines. We used five different viral isolates that were chosen to maximize phylogenetic and geographic diversity; the lines were collected in the United States (AP30), Spain (GC20), Greece (PF115), the United Kingdom (E27), and France (Hap23). Their collection is described in (Carpenter et al. 2007). The fly stocks that were infected were the same as those used to create the chromosome substitution

lines, and therefore have the same genetic background. To create the infected stocks, we took infected females from the five different wild-caught lines and backcrossed them for six generations to either *SM5/Pm; spa^{pol}* males (for the second chromosome experiment) or *w¹¹¹⁸; TM3, Sb/TM6B, Tb* males (for the third chromosome experiment). For the X chromosome experiment, we took infected males from the same five different wild-caught lines and crossed them to *C(1)DXY¹f¹; bw¹; st¹* females and then backcrossed F1 females to *bw¹; st¹* males. We checked that the backcrossed lines were infected with the virus every generation. To measure the transmission of the virus, we crossed infected males to a standard isogenic strain called P18 (Bangham et al. 2008). The methods we used to rear flies at constant density are described by (Bangham et al. 2008). The sigma virus makes infected flies die or become paralyzed after exposure to CO₂, so we tested flies for infection by exposing them to CO₂ for 15 min at 12°C (see Bangham et al. 2008).

SIGMA VIRUS TRANSMISSION IN SECOND AND THIRD CHROMOSOME SUBSTITUTION LINES

Here, we describe the experiment to measure transmission in the second chromosome substitution lines. However, this is identical to the experiment measuring transmission in the third chromosome substitution lines, except the balancer stock used to infect third chromosome substitution lines was *w¹¹¹⁸; TM3, Sb/TM6B, Tb*, not *SM5/Pm; spa^{pol}*.

Infected *SM5/Pm; spa^{pol}* virgin females were collected from the standard-density bottle cultures. After three days, pairs of females were placed in vials with pairs of males from each of the second chromosome substitution lines. Between two and four replicate crosses were set up for each second chromosome substitution line. After two days, the parents were removed from the vials and we checked that the female parent was infected; if either female was uninfected, the vial was discarded. The infected *SM5/+; spa^{pol}* female F1 offspring were aged for four days and then backcrossed to the chromosome substitution line *+/spa^{pol}*. Between one and four replicates were set up from each vial. As before, the flies were left to lay for two days and then the parents were removed and females were checked to ensure that they were infected, with vials being discarded if they were not. We collected F2 offspring that were homozygous for the wild-type second chromosome from this cross. These flies were genetically identical to the second chromosome substitution lines, but had been infected with the virus. Some males were put aside to be used to measure the rate of sigma virus transmission by infected males (see below). Fifteen days after the cross was set up, the remainder of the F2 flies were tested for infection. This provided an estimate of the effect of the wild-type second chromosome on transmission from a heterozygous female (*SM5/+; spa^{pol}*) to homozygous offspring (*+/spa^{pol}*) (see Fig. 1A). For simplicity,

we refer to this trait as female transmission. In total, we assayed 14,166 flies in 1021 vials.

Next, we measured the rate at which infected homozygous males transmit the sigma virus to their offspring. Five-day-old infected F2 males from the previous cross were mated to four-day-old P18 females. For each vial from the previous generation, between one and four replicates were set up. After two days, the parents were removed from the vials and we checked that the male parent was infected; if either male was uninfected, the vial was discarded. Fifteen days after the crosses were set up, the progeny were assayed for sigma infection. This provided an estimate of the effect of the wild-type second chromosome on transmission from a homozygous male (*+/spa^{pol}*) to heterozygous offspring (*+/P18; spa^{pol}*) (see Fig. 1B). For simplicity, we refer to this trait as male transmission. In total, we assayed 57,559 flies in 1703 vials.

In the experiment measuring female transmission in the third chromosome substitution lines (from a heterozygous *w¹¹¹⁸; TM3, Sb/+* female to homozygous *w¹¹¹⁸; +* offspring (see Fig. 1A), we assayed 7756 flies in 645 vials, and from a homozygous males (*w¹¹¹⁸; +*) to heterozygous offspring (*w¹¹¹⁸; +/P18*) (see Fig. 1B), we assayed 31,791 flies in 1092 vials.

SIGMA VIRUS TRANSMISSION IN X CHROMOSOME SUBSTITUTION LINES

Here, we describe the experiment to measure transmission in the X chromosome substitution lines. We used the infected attached-X stocks (*C(1)DXY¹f¹; bw¹; st¹*) to cross the virus into the X chromosome substitution lines.

Infected *C(1)DXY¹f¹; bw¹; st¹* virgin females were collected from standard-density bottle cultures and after four days, pairs of these females were placed in vials with pairs of males from each of the X chromosome substitution lines and allowed to lay for two days. Between one and four replicate crosses were set up for each X chromosome substitution line. After two days, the parents were removed from the vials and we checked that the female parent was infected; if either female was uninfected, the vial was discarded. We collected the male F1 offspring from this cross. Some males were put aside to be used to measure the rate of sigma virus transmission by infected males (see below). Fifteen days after the cross was set up, the remainder of the F1 flies were tested for infection. This provided an estimate of the effect of the wild-type X chromosome on the susceptibility of hemizygous male offspring (see Fig. 1C). It should be noted that this measure of female transmission is not directly equivalent to our measurements of female transmission in autosomes. This is because all the parental females are identical (*C(1)DXY¹f¹; bw¹; st¹*), and only the male progeny have a wild-collected X chromosome. Therefore, we are only measuring zygotic and not parental effects of the X chromosome on this trait in 1579 flies in 220 vials.

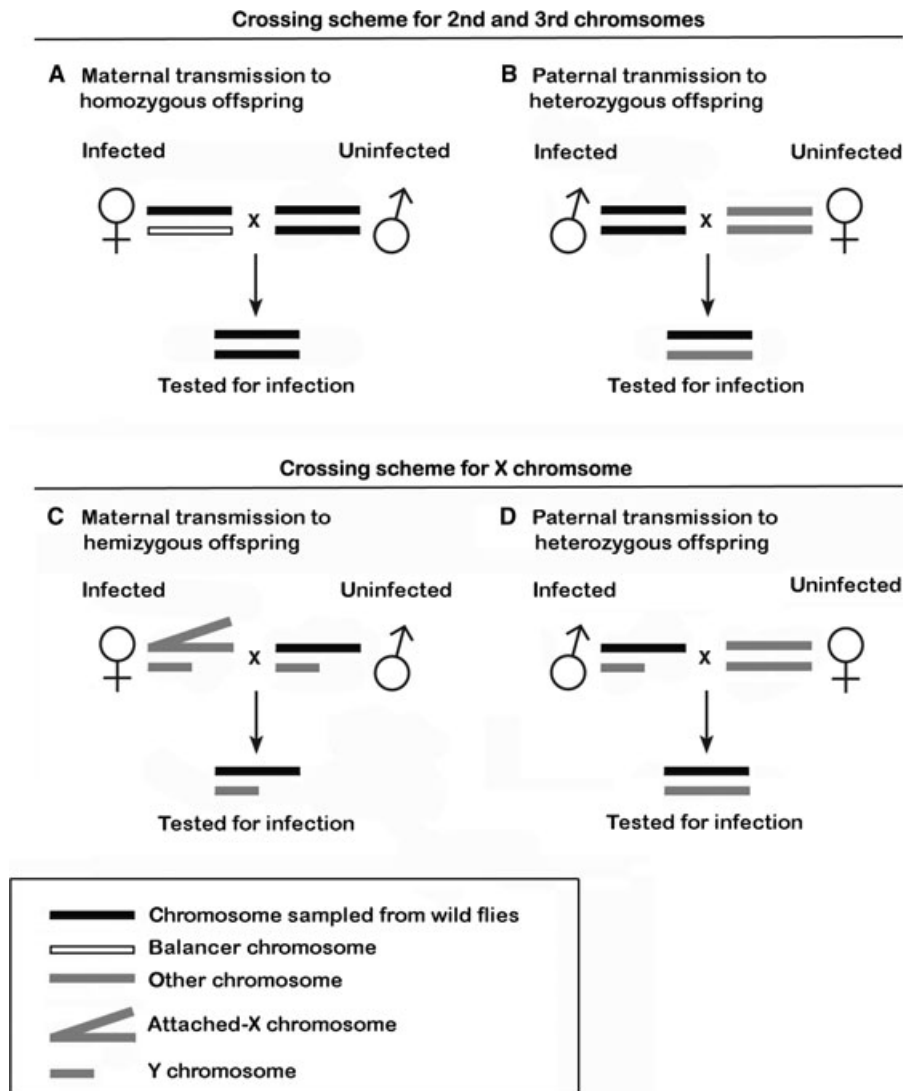


Figure 1. Schematic describing the four types of transmission experiment carried out.

Next, we measured the rate at which infected hemizygous males transmit the sigma virus to their offspring. Four-day-old infected F1 males from the previous cross were mated to five-day-old P18 females. For each vial from the previous generation, between one and eight replicates were set up. After two days, the parents were removed from the vials and we checked that the male parent was infected; if either male was uninfected, the vial was discarded. Seventeen days after the crosses were set up, the progeny were assayed for sigma infection. This provided an estimate of the effect of the wild-type first chromosome on transmission from a hemizygous male ($+;bw^1;st^1$) to heterozygous offspring ($+/P18; bw^1/P18; st^1/P18$) (see Fig. 1D). As with the autosomes, we refer to this trait as male transmission although it is not strictly analogous. In total, we assayed 71,440 flies in 1260 vials.

QUANTITATIVE GENETIC ANALYSIS

Because there was little genetic variation in maternal transmission, we analyzed the data on this trait using nonparametric statistics. There was more genetic variation in paternal transmission, and so we investigate the nature of this variation by estimating the matrix of genetic variances and covariances (the **G** matrix) with respect to the five different viruses for each chromosome separately. Our data consist of numbers of infected and uninfected flies which we treat as a binomial response in a generalized linear mixed model (GLMM).

For the X chromosome, the models were formulated as follows: let $v_{i,j,k}$ be the probability of flies in vial i from line j being susceptible to virus k .

$$v_{i,j,k} = \text{logit}^{-1}(\mathbf{x}_i^T \boldsymbol{\beta} + \alpha_{j,k} + \epsilon_{i,j,k}), \quad (1)$$

where β is a vector of fixed effects such as the effect of virus type and day, and x_i^\top is a row vector relating the fixed effects to vial i . $\alpha_{j,k}$ is the effect of virus k on line j . These effects were treated as random and were assumed to be multivariate normally distributed:

$$\begin{bmatrix} \alpha_{j,1} \\ \alpha_{j,2} \\ \alpha_{j,3} \\ \alpha_{j,4} \\ \alpha_{j,5} \end{bmatrix} \sim N \left(\begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_1^2 & \sigma_{1,2} & \sigma_{1,3} & \sigma_{1,4} & \sigma_{1,5} \\ \sigma_{2,1} & \sigma_2^2 & \sigma_{2,3} & \sigma_{2,4} & \sigma_{2,5} \\ \sigma_{3,1} & \sigma_{3,2} & \sigma_3^2 & \sigma_{3,4} & \sigma_{3,5} \\ \sigma_{4,1} & \sigma_{4,2} & \sigma_{4,3} & \sigma_4^2 & \sigma_{4,5} \\ \sigma_{5,1} & \sigma_{5,2} & \sigma_{5,3} & \sigma_{5,4} & \sigma_5^2 \end{bmatrix} \right) \quad (2)$$

where σ_m^2 is the between-line variance in susceptibility to virus m , and $\sigma_{m,n}$ is the between-line covariance in susceptibility to viruses m and n .

$\epsilon_{i,j,k}$ is a residual that captures over-dispersion within each vial due to unaccounted heterogeneity between vials in the probability of susceptibility. The residuals were assumed to be normally distributed with a separate variance estimated for each virus type.

The model for autosomes was identical to that of the X chromosome except f2rep (f2rep represents replication of chromosome substitution lines) was fitted as an additional random effect with a constant variance over virus types. For the second chromosome, we also ran an additional model where an interaction between the *ref(2)P* allele and the American virus—no other viral lines showed sensitivity to this allele—was fitted as a fixed effect.

The parameters of the model were estimated using the R library MCMCglmm (Hadfield 2010), which uses Bayesian Markov chain Monte Carlo (MCMC) techniques. Each model was run for 1.8 million iterations with a burn-in of 300,000 and a thinning interval of 100. Diffuse normal priors were used for the fixed effects with null mean vector and large variances (10^6) and improper flat priors were used for the variances and covariance matrices. Because inferences regarding (co)variances are known to be sensitive to putatively weak prior specifications (Gelman 2006), we also fitted the models with degree of belief parameters set to 6 and covariance matrices close to null matrices (diagonal matrices with 1^{-6} along the diagonal). This prior specification is approximately flat on the interval $[-1,1]$ for the correlation. Confidence limits on variance, covariances, and heritability were calculated from highest posterior density intervals.

Although systematic biases are not expected in eigenvector loadings (i.e., the orientation of \mathbf{G}), there is a well-known upward bias in the variance of the sample eigenvalues which increases with decreasing power (e.g., [Hill & Thompson 1978] in the context of genetic covariance matrices). The estimates of the leading eigenvalues are therefore, on average, larger than the true values, although the large sample sizes employed should minimize this bias.

Results

We measured the effect of the three major chromosomes of *Drosophila* on the transmission from an infected mother to her offspring. For the second chromosome, there is very low variation in the proportion of offspring infected for all viruses except for the U.S. isolate, which has a bimodal pattern of transmission—lines with the *ref(2)P* susceptible allele had a mean rate of transmission of 94%, whereas the subset of lines carrying the *ref(2)P* resistant allele had a mean transmission rate of 7% (Wilcoxon rank-sum test $W = 798.5$, $n_{\text{susceptible}} = 50$, $n_{\text{resistant}} = 16$, $P < 0.001$) (Fig. 2). This gene therefore makes flies specifically resistant to one of the five viral isolates. We think that the American isolate may be a remnant viral type that existed before a new viral-type spread in response to the increasing frequency of the resistant *ref(2)P* allele in *D. melanogaster* populations. In the last 30 years, European sigma populations have undergone replacement of one viral type that was sensitive to the resistant *ref(2)P* gene by another viral type that is not affected by this gene (Fleuriet 1990; Fleuriet and Sperlich 1992). In support of this idea, Carpenter et al. 2007 showed that the American viral isolate is phylogenetically distinct from the other viruses used in this study.

For the other two chromosomes, there is very low variation in the proportion of offspring infected across all viruses (a mean of 98% across all viruses, and for each of the viral strains, there was very low variation among chromosome lines [Fig. 2]).

GENETIC VARIANCE IN PATERNAL TRANSMISSION

Next, we examined how the five viruses were transmitted through sperm rather than eggs. As for maternal transmission, the resistant *ref(2)P* allele significantly reduces transmission of the American virus—lines with the *ref(2)P* susceptible allele had a mean rate of transmission of 23%, whereas the subset of lines carrying the *ref(2)P* resistant allele had a mean transmission rate of 8% (Wilcoxon rank-sum test $W = 385$, $n_{\text{susceptible}} = 50$, $n_{\text{resistant}} = 11$, $P < 0.04$) (Fig. 3).

However, in contrast to maternal transmission, there was substantial genetic variation in transmission rates caused by all three chromosomes against all the viruses (Fig. 3). This is reflected in the variances in Table 1, which shows that there is significant variation in susceptibility of flies to the different viruses (diagonals in matrices have 95% credible intervals considerably greater than 0). Why the discrepancy between the maternal and paternal transmission rates? One possibility might lie in the size differences between male and female gametes (Bangham et al. 2008). Sigma virus is found in the cytoplasm of cells and because male gametes deliver smaller quantities of cytoplasm to their offspring than females (Brun and Plus 1998), they transmit fewer viral particles. Therefore, genes that reduce viral titers could have larger effects when the virus is transmitted through sperm than eggs. There is

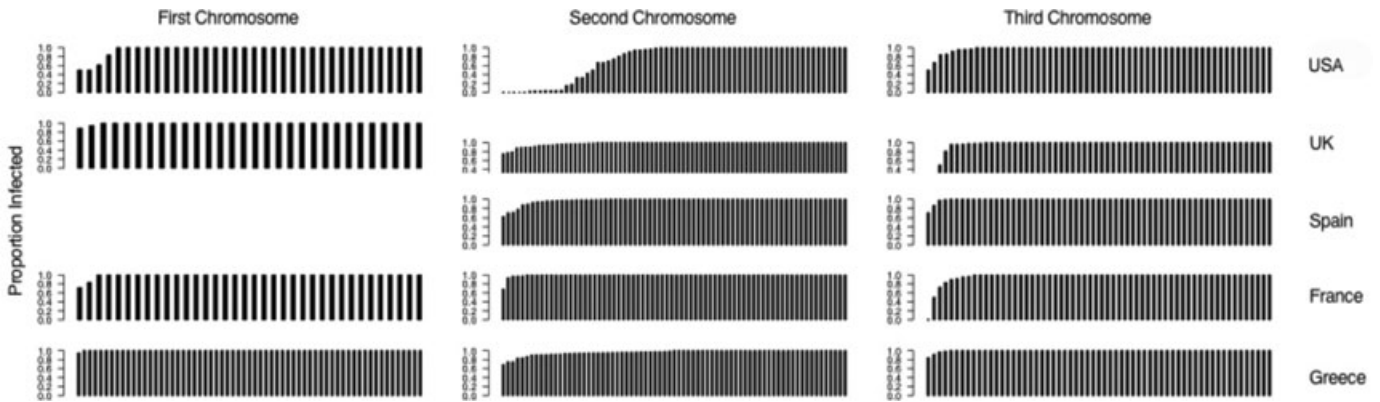


Figure 2. Sigma virus maternal-transmission rates across chromosome-extracted lines of *D. melanogaster* for five viruses. The bars show the means with equal weights to each vial.

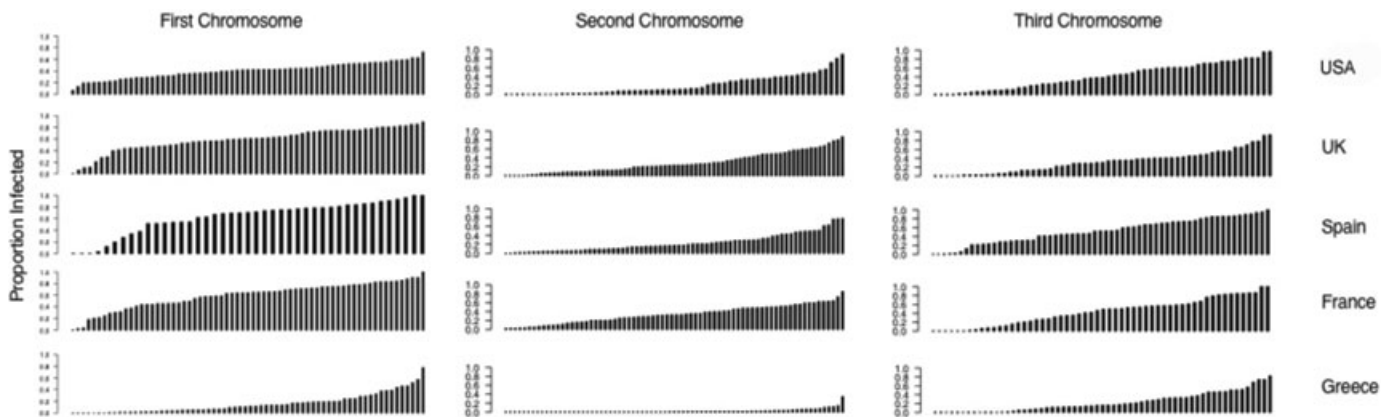


Figure 3. Sigma virus paternal-transmission rates across chromosome-extracted lines of *D. melanogaster* for five viruses. The bars show the means with equal weights to each vial.

greater variation in transmission rates for the second and third chromosomes compared to the X chromosome, which we expect, as the X is roughly half the size of the autosomes.

From the genetic variance (V_g) and residual variance (V_r), we estimated broad sense heritability on the latent scale (h^2) for the paternal transmission rate using the following equation: $h^2 = \frac{V_g}{V_g + V_r + \frac{\pi^2}{3}}$, where $\frac{\pi^2}{3}$ is the variance of a logistic distribution (the cumulative distribution function of the logistic distribution is the inverse logit function—the link function used in the model (Lee and Nelder 2001; Nakagawa and Schielzeth 2010), and V_g is half the between-line variance (because our lines are homozygous). We found that the heritability of viral transmission was similar for each of the five viruses and was relatively high, indicating that this trait has the potential to respond to selection (Table 3).

GENETIC COVARIANCE BETWEEN VIRUSES IN RATES OF TRANSMISSION

To investigate whether trade-offs in resistance against different viral isolates exist, we looked to see whether the genetic vari-

ation in transmission of the virus is general to all viruses or specific to particular viruses. To do this, we calculated eigenvectors and eigenvalues for each of the five principle components. We found, at least for the second and third chromosomes, that most of the variation in transmission is explained by the first eigenvector ($\sim 80\%$) (see Fig. 4), and that the viruses contribute more or less equally to this first vector (Table 4), indicating that most of the variation that we found is general to all five viruses.

In contrast, far less of the variation in transmission for the X chromosome is explained by the first eigenvector ($\sim 50\%$) (see Fig. 4), and the viruses are not contributing equally to each of the eigenvectors (Table 4). In fact, a single virus is contributing a significant amount of the variation to each of the five eigenvectors in turn, indicating that most of the variation is not general to all viruses, but is instead associated with a specific virus.

To allow comparison of the variation in transmission rates between chromosomes, we have used the first three eigenvectors

Table 1. G-matrices for X, second, and third chromosomes showing variances (**bold**) and correlations. Confidence limits (in brackets) are calculated from HPD intervals.

	[U.S.A.]	[U.K.]	[Spain]	[France]	[Greece]
X chromosome					
[U.S.A.]	0.324 (0.176, 0.558)				
[U.K.]	0.232 (-0.142, 0.590)	0.558 (0.289, 0.977)			
[Spain]	-0.021 (-0.519, 0.499)	-0.116 (-0.561, 0.465)	1.461 (0.427, 3.326)		
[France]	-0.023 (-0.404, 0.369)	0.072 (-0.298, 0.480)	0.001 (-0.494, 0.561)	1.029 (0.510, 1.753)	
[Greece]	0.125 (-0.233, 0.472)	0.197 (-0.153, 0.533)	0.093 (-0.333, 0.625)	0.148 (-0.200, 0.445)	3.029 (1.871, 4.563)
Second chromosome					
[U.S.A.]	3.873 (2.331, 6.240)				
[U.K.]	0.432 (0.189, 0.664)	2.068 (1.418, 3.047)			
[Spain]	0.416 (0.176, 0.651)	0.825 (0.725, 0.930)	1.657 (1.129, 2.420)		
[France]	0.741 (0.558, 0.876)	0.712 (0.543, 0.853)	0.660 (0.473, 0.820)	1.105 (0.686, 1.731)	
[Greece]	0.755 (0.591, 0.908)	0.708 (0.527, 0.867)	0.707 (0.531, 0.855)	0.795 (0.650, 0.930)	3.957 (2.099, 6.719)
Third chromosome					
[U.S.A.]	3.299 (1.800, 5.522)				
[U.K.]	0.875 (0.785, 0.968)	3.636 (2.076, 5.948)			
[Spain]	0.866 (0.734, 0.953)	0.874 (0.800, 0.971)	3.817 (2.242, 6.463)		
[France]	0.712 (0.512, 0.929)	0.790 (0.623, 0.942)	0.799 (0.640, 0.945)	4.157 (2.322, 6.949)	
[Greece]	0.788 (0.618, 0.939)	0.833 (0.682, 0.950)	0.847 (0.688, 0.953)	0.873 (0.759, 0.971)	5.150 (2.853, 8.984)

Note: HPD, highest probability interval.

Table 2. G-matrices for second chromosome calculated from paternal transmission rates with a model allowing an interaction between *ref(2)P* and the American viral isolate. Variances (**bold**) and correlations. Confidence limits (in brackets) are calculated from HPD intervals.

	[U.S.A.]	[U.K.]	[Spain]	[France]	[Greece]
Second chromosome					
[U.S.A.]	3.697 (2.238, 5.905)				
[U.K.]	0.418 (0.172, 0.647)	2.078 (1.378, 2.997)			
[Spain]	0.401 (0.148, 0.649)	0.833 (0.534, 0.862)	1.675 (1.083, 2.472)		
[France]	0.718 (0.543, 0.871)	0.714 (0.148, 0.649)	0.666 (0.539, 0.858)	1.107 (0.693, 1.692)	
[Greece]	0.761 (0.595, 0.903)	0.712 (0.722, 0.926)	0.695 (0.465, 0.827)	0.804 (0.647, 0.925)	4.170 (2.090, 7.502)

to define a subspace that contains most of the genetic variation associated with each chromosome. It is clear from Figure 5, where these subspaces are overlain to allow comparison between the different chromosomes, that the patterns of variation associated with the second and third chromosomes are very similar, whereas the X chromosome seems most different.

If we assume that the genetic variation is additive, we can sum all three chromosomes together to estimate the total genetic variation in transmission across the whole genome. We find that most of the variation in transmission is general (~70% of the genetic variation is explained by the first eigenvector and the viruses contribute more or less equally to it, see Fig. 4 and Table 4).

The importance of general resistance may be overestimated in these analyses, as there is an upward bias in the estimation

of the leading eigenvalues (e.g., [Hill & Thompson 1978] in the context of genetic covariance matrices). However, this bias only affects the magnitude and not the direction of the eigenvectors, and is greatest when the power is low (our sample sizes are not small). Therefore, it is unlikely to qualitatively affect our conclusions.

The use of homozygous lines may also have affected our results. Under an additive model, **G** from inbred lines should be the same as **G** arising in a randomly mating population (Robertson 1952). With nonadditive effects, the effect of inbreeding on **G** is likely to be complex, as is found following population bottlenecks (Lopez-Fanjul et al. 2004). In particular, if inbreeding depression primarily affects general resistance to all sigma virus strains rather than strain-specific resistance, it may cause us to overestimate the importance of general resistance (i.e., overestimate the genetic

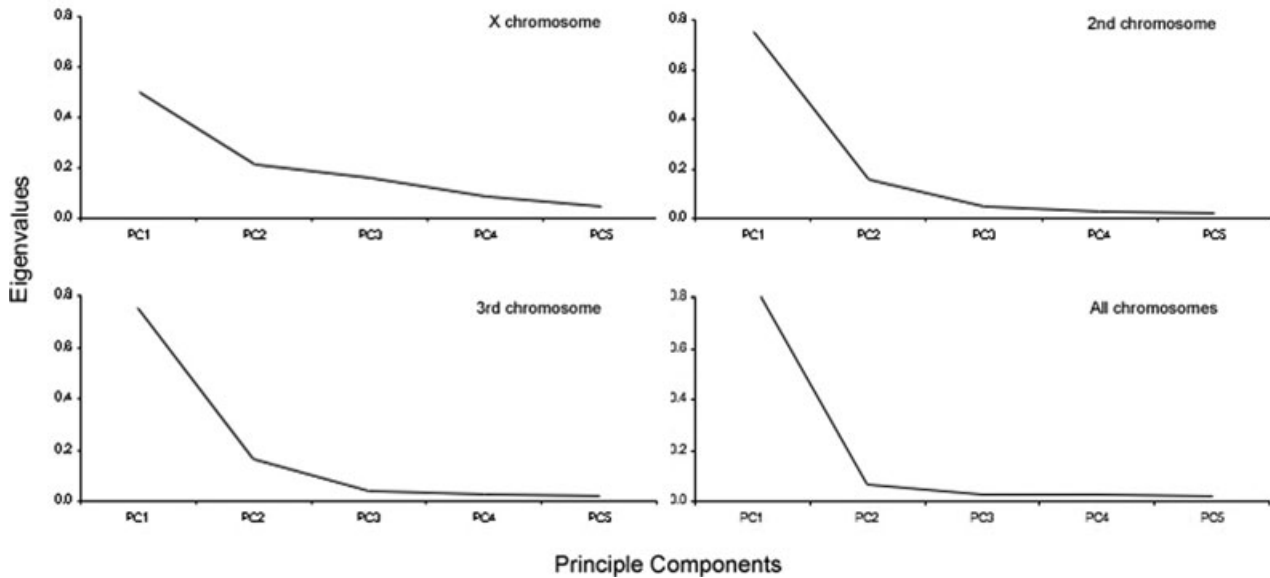


Figure 4. The scree plot displays the eigenvalues for each of the five principle components for the different chromosomes and all chromosomes together.

Table 3. Measures of the trait mean, and heritability (h^2) in viral transmission in *Drosophila melanogaster*, confidence limits (in brackets) are calculated from HPD intervals.

Virus	Mean transmission (%)*	h^2 **
U.S.A.	0.37 (0.38, 0.42)	0.553 (0.458, 0.634)
U.K.	0.61 (0.55, 0.67)	0.488 (0.404, 0.577)
Spain	0.71 (0.60, 0.80)	0.455 (0.338, 0.554)
France	0.60 (0.52, 0.67)	0.450 (0.352, 0.547)
Greece	0.05 (0.03, 0.07)	0.551 (0.459, 0.636)

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

**Assuming the genetic variance is additive, the total heritability is calculated by summing the genetic variance of the three chromosomes and using the mean of the residual variance.

correlations). However, empirical work on *Drosophila* wing shape in inbred lines found good agreement with theoretical predictions under the additive model. Whether these results are relevant to our data is unclear, as there is little inbreeding depression for wing shape (Whitlock and Fowler 1999) whereas inbreeding depression commonly affects parasite resistance (Gerloff et al. 2003; Bello-Bedoy and Núñez-Farfán 2011).

RESPONSE TO SELECTION ON EACH OF THE FIVE VIRUSES

We investigated how specific resistance affects the response to selection in our traits—the change in rate of transmission of each of the five viruses. This was done by exerting a selection pressure on any one virus and predicting the change in the transmission

rate of both the virus under selection, and the correlated change in the transmission rate of the other viruses. Ignoring *ref(2)P*, there is negligible genetic variation in maternal transmission, so the response to selection is determined solely by selection acting on males. Therefore, we can calculate the response to selection by applying the multivariate breeder’s equation, $\Delta z = 0.5G\beta$, where G is the additive genetic variance–covariance matrix (Table 1), β is the vector of selection; and Δz , the vector of predicted responses to selection. We performed this analysis on the combined G matrix from all three chromosomes.

Selection on each virus in turn indicates that no antagonism exists between resistance to each of the viruses as all of the viruses show a positive change in their trait mean, although the relative response varies across viruses (Table 5). However, in most cases, if we compare the response of a virus when it is being directly selected on to its response when another virus is being selected on, we can see that its correlated response is less than expected from the direct response.

We also examined the response to selection from a mixed population of all five viruses, as might occur in the wild. We found that there is roughly a 20% reduction in the response to selection from a mixed population of viruses compared to a single genotype (Table 5, comparing numbers in bold to the column entitled “Virus selected on simultaneously”).

THE EFFECT OF *ref(2)P* ON PATERNAL TRANSMISSION

Because the resistant *ref(2)P* allele was associated with a 14% drop in the rate of transmission of the American virus, we have examined whether the interaction between *ref(2)P* and the American isolate might account for the remaining variation on the second

Table 4. Eigenvectors, which are the linear combinations of the original traits (i.e., the variance and covariances) for each of the viruses for X, second, and third chromosomes separately and together. The columns are the first five principle components.

	[PC1]	[PC2]	[PC3]	[PC4]	[PC5]
X chromosome					
[U.S.A.]	-0.045	0.036	-0.042	0.339	0.938
[U.K.]	-0.098	0.158	0.019	0.921	-0.343
[Spain]	-0.096	-0.981	0.075	0.150	-0.018
[France]	-0.124	0.082	0.987	-0.031	0.046
[Greece]	-0.982	0.068	-0.132	-0.118	-0.013
Second chromosome					
[U.S.A.]	-0.541	0.692	0.384	0.157	0.236
[U.K.]	-0.367	-0.538	0.402	-0.495	0.410
[Spain]	-0.322	-0.480	0.153	0.794	-0.108
[France]	-0.304	-0.008	0.242	-0.294	-0.873
[Greece]	-0.614	-0.033	-0.780	-0.113	0.036
Third chromosome					
[U.S.A.]	-0.394	-0.551	0.045	0.170	0.714
[U.K.]	-0.429	-0.318	-0.261	0.541	-0.595
[Spain]	-0.444	-0.264	-0.072	-0.816	-0.250
[France]	-0.448	0.619	-0.588	0.015	0.264
[Greece]	-0.513	0.377	0.761	0.110	-0.066
All three chromosomes					
[U.S.A.]	-0.981	0.177	0.388	0.666	1.888
[U.K.]	-0.894	-0.698	0.160	0.967	-0.527
[Spain]	-0.862	-1.725	0.155	0.128	-0.376
[France]	-0.876	0.693	0.641	-0.309	-0.563
[Greece]	-2.108	0.412	-0.152	-0.122	-0.044

chromosome, which is unexplained by the first eigenvector. To investigate this, paternal transmission rates were re-analysed with a model allowing an interaction between *ref(2)P* and the American viral isolate and the estimated genetic variation in transmission rates was compared with those estimated from the former model that did not allow this interaction. We found little difference in estimates of the amount of genetic variation in transmission rates between these two models, indicating that this variation is not attributable to the *ref(2)P*–American isolate interaction (compare Table 2 with Table 1).

Discussion

DO GENETIC SPECIFICITIES CONSTRAIN THE EVOLUTION OF GENERAL RESISTANCE?

In many species, resistance to pathogens is specific, meaning that host genotypes are resistant to one parasite genotype and susceptible to another (Gross et al. 1980; Siegel and Gross 1980; Gehad et al. 1999; Johnsen and Zuk 1999; Gill et al. 2000; Cotter et al. 2004; Dybdahl et al. 2008). These specific interactions mean that

genetic correlations in resistance to different parasite genotypes may be low or negative. This means that although there is abundant genetic variation in resistance to any one parasite genotype, there may be very little genetic variation in general resistance (resistance against all parasites genotypes simultaneously). This will act as an evolutionary constraint, and prevent, or slow, the evolution of increased host resistance.

Using a host that is known to be resistant to specific parasite genotypes, we tested whether these specific interactions significantly constrain the evolution of increased resistance, and found that they do not.

We found large amounts of heritable variation in the rate that different *D. melanogaster* lines transmit five different isolates of the sigma virus. This genetic variation in transmission was partitioned roughly equally across the genome, with the X chromosome having about half as much variation as the second and third chromosomes—as expected given that the X contains half as many genes as the other two chromosomes. The correlation between chromosome size and the amount of genetic variation suggests that multiple genes spread evenly across the genome control resistance. These findings, along with the results from two previous studies (Bangham et al. 2007, 2008), indicate that resistance to the sigma virus is a trait with high levels of genetic variation. In males, this variation translates into high heritability of between ~40% and ~50% for the different viruses.

We know that there is selection for increased resistance in natural populations, as sigma virus infections are costly to the fly, causing lower female fecundity and reduced over-wintering survival (Seecof 1966; Fleuriet 1981b, a; Yampolsky et al. 1999). Given that resistance is highly heritability and under selection, we expect resistance to the sigma virus to increase in the population. Whether this occurs will depend on the constraints on the evolution of resistance. One possible type of constraint is that trade-offs exist between resistance against different pathogen genotypes, preventing the evolution of resistance to all the viral genotypes in a population.

We found evidence of specific resistance, where host genes do not affect all the viruses in the same way. The clearest example, which has been described before, involves the fly gene *ref(2)P* which confers resistance to some viral isolates but not others (Fleuriet 1988; Contamine et al. 1989; Dru et al. 1993; Wayne et al. 1996; Bangham et al. 2007). Additionally, the genetic correlations between resistance to different viruses were less than one, indicating that specific genetic interactions exists elsewhere in the genome. However, the majority of the variation on the second and third chromosomes that affected transmission rates was general to all five viruses—contrary to the idea that trade-offs exist between resistance against different viruses. The effects of variation on the X chromosome on transmission rates tend to be specific to single viruses, but this may be an artifact

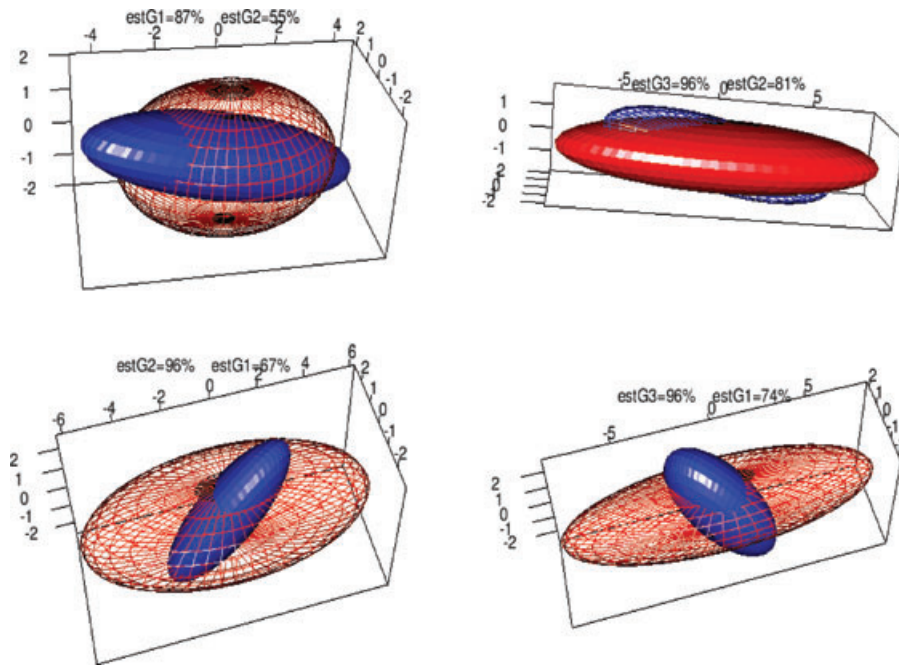


Figure 5. The plots show the distribution of line effects for three of the five original dimensions in a three-dimensional subspace. The subspace is defined by the first three eigenvectors of the first matrix (identified from the suffix of the first estG) which is plotted in red. The distribution of line effects for a second chromosome (identified from the suffix of the second estG) is plotted in blue. The % refers to the amount of variation that lies in this subspace compared to the total.

Table 5. Predicted change in the transmission rate in response to selection for resistance to a single virus or to a mixture of five viruses. The numbers in bold show the direct response to the virus used for selection.

Viruses	Viruses selected on in turn					Viruses selected on simultaneously
	U.S.A.	U.K.	Spain	France	Greece	
U.S.A.	-0.014	-0.013	-0.015	-0.013	-0.002	-0.013
U.K.	-0.008	-0.019	-0.017	-0.013	-0.001	-0.013
Spain	-0.008	-0.015	-0.025	-0.012	-0.001	-0.012
France	-0.008	-0.013	-0.015	-0.019	-0.001	-0.013
Greece	-0.012	-0.018	-0.021	-0.018	-0.003	-0.003

For each virus, we defined a selection gradient (β) that results in an equivalent strength of selection for reduced transmission (i.e., that which would occur if the viruses had the same prevalence and virulence in the population). This was done by calculating selection gradients that produce an equal drop (in percentage) in the probability that an infected male transmits the virus to his offspring. This assumes that infected males and females rarely mate.

of small genetic variances for the X chromosome leading to poor estimates of the variances and covariances.

We conclude that most of the genetic variation in transmission rates is general, even though a small amount of genetic variation on each chromosome is involved in specific interactions with particular viruses. Although we selected viral isolates to maximize their phylogenetic diversity, our results are likely to hold within wild populations where there is a similar amount of genetic variation between viruses in their transmission rates as we observed (Wilfert and Jiggins 2010). Furthermore, using the multivariate breeder’s equation, we were able to show that fly

populations have the potential to evolve general resistance to all five of these viral isolates. It thus seems unlikely that trade-offs in resistance against these viral isolates are a constraint preventing the evolution of resistance in *D. melanogaster* populations.

In light of these results, we ask: Why are flies not more resistant to the sigma virus? Well perhaps the role of genetic correlations and specificities should not be ruled out entirely. We found that although selection for general resistance (to all five viruses) is possible, the rate at which selection can increase resistance will tend to be slowed by specific interactions with particular viruses. This becomes important when you consider parasites,

which evolve rapidly and offer a constantly shifting target for selection (Woolhouse 2002). This shifting target, combined with the slow rate of evolution toward that moving target, will reduce the average resistance of hosts to their parasites. Another possibility is that trade-offs exist with other components of fitness; growth rate, fecundity, competitive ability, to name a few, could all contribute to constraining the evolution of resistance against the sigma virus.

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