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Genetic variation affecting host–parasite interactions: major-effect quantitative trait loci affect the transmission of sigma virus in *Drosophila melanogaster*

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Abstract

In natural populations, genetic variation affects resistance to disease. Whether that genetic variation comprises lots of small-effect polymorphisms or a small number of large-effect polymorphisms has implications for adaptation, selection and how genetic variation is maintained in populations. Furthermore, how much genetic variation there is, and the genes that underlie this variation, affects models of co-evolution between parasites and their hosts. We are studying the genetic variation that affects the resistance of *Drosophila melanogaster* to its natural pathogen — the vertically transmitted sigma virus. We have carried out three separate quantitative trait locus mapping analyses to map gene variants on the second chromosome that cause variation in the rate at which males transmit the infection to their offspring. All three crosses identified a locus in a similar chromosomal location that causes a large drop in the rate at which the virus is transmitted. We also found evidence for an additional smaller-effect quantitative trait locus elsewhere on the chromosome. Our data, together with previous experiments on the sigma virus and parasitoid wasps, indicate that the resistance of *D. melanogaster* to co-evolved pathogens is controlled by a limited number of major-effect polymorphisms.

Keywords: *Drosophila melanogaster*, host–parasite co-evolution, QTL analysis, polymorphism, sigma virus

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Introduction

Parasites exert strong selection pressures on their hosts, and hosts exert strong selection pressures on parasites. Key to understanding responses to these selection pressures is the genetic variation that affects host–parasite interactions. Genetic variation affects disease resistance in a wide range of organisms (Henter 1995a, b; Holub 2001; Lazzaro *et al*. 2004; Hirschhorn & Daly 2005; Riehle *et al*. 2006; Dubuffet *et al*. 2007). The amount and type of genetic variation affecting disease resistance has important consequences for the evolution of parasite virulence and infectivity, and the evolution of the host immune systems. Furthermore, whether that genetic variation takes the shape of lots of small-effect polymorphisms or a small number of large-effect polymorphisms affects responses to selection and drift, and how genetic variation is maintained in populations (reviewed Barton & Keightley 2002).

The *Drosophila melanogaster*–sigma virus system is an important model of host–parasite co-evolution. The sigma virus is a common natural pathogen of *D. melanogaster* that is transmitted only vertically, from parent to offspring. Sigma is transmitted at a high rate through females (who usually pass it to 100% of offspring), and at a lower rate through males (who pass it to between 0% and 90% of offspring). Variation in paternal transmission is crucial to whether the virus can invade a population and the prevalence that it reaches (Fleuriet 1991). This is because sigma virus infection is harmful to flies, reducing both egg viability and survival overwinter (Fleuriet 1981). Therefore, if it was transmitted solely through females, it would be lost from the population, and a sufficient rate of paternal

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transmission is needed to overcome the costs imposed on the host.

In a previous study, we found that there is considerable genetic variation on chromosome two that alters the transmission of the sigma virus through males, but does not affect maternal transmission or replication of the sigma virus following injection into its host (Bangham *et al*. 2008). Several *Drosophila* genes that confer resistance to the sigma virus have been mapped, but none can explain this variation. Variation in one gene — *ref(2)P* — affects the replication and transmission of the sigma virus (Dru *et al*. 1993; Wayne *et al*. 1996; Bangham *et al*. 2007). *ref(2)P* encodes a protein that can physically interact with the sigma viral proteins (Wyers *et al*. 1993), although its mode of antiviral action is unknown. In our previous study, we found that a polymorphism in *ref(2)P* that affects replication and transmission of sigma explained little of the variation in paternal transmission. Other loci affecting host responses to sigma have been mapped only approximately. Little more is known about these loci, or whether these are naturally occurring polymorphisms (reviewed by Brun & Plus 1998). One of these loci does affect paternal transmission, but this locus is on chromosome three and not chromosome two. Several other loci (located on chromosomes two and three) affect replication of the sigma virus after it has been injected into flies but are not thought to affect transmission through males (Gay 1978).

As a first step to finding out how many (and which) genes are responsible for variation in paternal transmission of this pathogen, we have carried out three separate quantitative trait locus (QTL) mapping analyses on chromosome two. All three analyses identified a major-effect locus in a similar location, and we also found evidence for an additional smaller-effect QTL elsewhere on the chromosome.

Materials and methods

Drosophila melanogaster *stocks, viral isolates and general methods*

In a previous experiment, we found that variation on the second chromosome of *Drosophila melanogaster* (about 40% of the genome) had a significant effect of on sigma virus transmission (Bangham *et al*. 2008). This was measured on 83 chromosome-substitution lines (created by Lazzaro *et al*. 2004), each of which has a different homozygous second chromosome that had been sampled from a population in Pennsylvania (USA) which had been substituted into a common isogenic genetic background (see Lazzaro *et al*. 2004 for details). These 83 lines had a range of paternal transmissions between 0% and 88% (see Bangham *et al*. 2008). The three lines chosen were near the extremes of this continuous distribution (i.e. they were not outliers).

Because the sigma virus is only transmitted vertically, we used a fly stock that was infected with the sigma virus isolate Ap30, and used this to cross the virus into our fly lines (described fully in Bangham *et al*. 2008 and Carpenter *et al*. 2007). The infected *SM5/Pm*; *spa*^{pol} fly stock (henceforth called *SM5/Pm*) had the same genetic background as the second chromosome-substitution lines. To assay for infection by the sigma virus, adults are exposed to pure carbon dioxide for 15–17 min at 12 °C. By 2 h post-exposure, uninfected flies are awake from this anaesthesia, but flies infected with the sigma virus are dead or paralysed.

To map the QTLs responsible for variation in the rate of transmission through males, we created three independent sets of recombinant lines infected with the sigma virus (see Appendix S1, Supplementary material for details of how these lines were created). We selected three chromosome-substitution lines with high paternal transmission and three lines with low paternal transmission, none of which carried the known *ref(2)P* resistance mutation. The three sets of recombinant lines went through one generation of recombination in the F_1 females, and recombinant isogenic stocks were constructed using the balancer stock. These were genotyped at markers 10–15 cM apart (see Table S1, Supplementary material).

To measure the rate of transmission from infected recombinant males to offspring, 5-day-old male recombinants were collected and were crossed in pairs to two 3–4 day-old uninfected females (stock P18). Two days after the cross was set up, the parents were removed and the parental males were assayed for sigma infection, and any vials of offspring for which one or other male parent was not infected were discarded. Fifteen to sixteen days after this final cross was set up, offspring were assayed for infection. In total, in cross 1, we assayed 43 772 flies (in 1135 vials, 76 different lines, a median of 15 replicates for each line); in cross 2, we assayed 51 653 flies (in 989 vials, 64 different lines, a median of 16 replicates per line); and in cross 3, we assayed 72 923 flies (in 1339 vials, 55 different lines, a median of 25 replicates per line).

Statistical analysis of genetic variation

The statistical analysis to identify QTLs was carried out using the R (version 2.2.1) software and language, and QTLs were mapped using Haley–Knott regression (Haley & Knott 1992). Multiple regression was performed using a general linear mixed-effects model implemented using R's lme function (Laird & Ware 1982; Pinheiro & Bates 2000). First, to obtain the minimal adequate model, we started with the maximal model containing the recombinant line and all levels of experimental replication nested within line as random effects. At this stage, we did not include genotype as a factor, so there were no fixed effects. By comparing this model to progressively simpler models, we

sequentially removed nonsignificant random effects. Next, for each recombinant line, we calculated the probabilities of the two alternative genotypes at every centimorgan position along the chromosome, conditional on the observed flanking marker genotype. Next, we tested for an association between viral transmission and genotype at each centimorgan location using multiple regression. Viral infection rates were measured as proportions (proportion of offspring infected with the virus), so these were arcsine-square-root transformed to remove the dependency of the variance of the observation on the observation itself. These were regressed onto the conditional probability of an individual being homozygous for the high transmission line allele. The strength of association between that location and the transmission of the virus was described as an *F*-statistic, which was converted into a chi-squared statistic by multiplying *F* by the numerator degrees of freedom.

Because we are performing multiple correlated tests when performing a scan of the chromosome, we determined an experiment-wide significance threshold for the association between marker genotype and phenotype by permutation (Churchill & Doerge 1994). The phenotype data were permuted over the different recombinant lines, the resulting data set was analysed as described above, and the maximum chi-squared statistic across all the centimorgan locations was recorded. This was carried out 1000 times to generate a null distribution of the chi-squared statistic for each cross. The confidence intervals on the QTL location were determined by bootstrapping, where the analysis was repeated on 1000 different data sets generated by resampling recombinant lines with replacement. At each round of bootstrapping, the location of the maximum chi-squared statistic was recorded and empirical 99% and 95% confidence intervals obtained from the resulting distribution (Visscher *et al*. 1996). Because we analysed arcsine-square-root transformed data, the effect of the QTL on viral transmission was obtained by back-transforming [using $(\sin X)^2$] the estimated transmission rate (X) of each of the two genotypes obtained from the regression into a proportion, and then calculating the difference between these values. The approximate confidence limits on the effect of the QTLs on viral transmission were estimated using the 'intervals' function of lme (Pinheiro & Bates 2000), and were back transformed in the same way.

We wanted to test whether the three crosses had yielded significantly different QTL locations. Taking pairs of crosses or all three crosses together, we compared the likelihood of the observed data under a model in which each cross had the same QTL location to the likelihood under a model allowing each cross to have different QTL locations. To determine the maximum likelihood under a model in which pairs of crosses (or all three crosses combined) had the same QTL location, we fitted a model to all three data sets separately and recorded the likelihood at each centimorgan location. We then determined the maximum likelihood for the model in which the three crosses could have different located QTL by summing the maximum likelihood over the chromosome obtained for each cross. Then, for each pair of crosses (or all three crosses), we added together the likelihoods at each position and identified the location with the maximum likelihood. For each comparison, we compared the maximum likelihood for the model that had QTLs at different locations to the model with QTL at the same location. The resulting log-likelihood ratio test statistic is equivalent to the chi-squared statistic, and this was compared to the chi-squared distribution with one degree of freedom (or two degrees of freedom for the comparison of one-vs.-three comparison).

We wanted to test whether one QTL or two QTLs best describe the variation in the three data sets. So for each data set, we fitted a model with two non-interacting QTLs to all possible pairwise combinations of QTL locations (in centimorgans), and recorded the pair of locations that had the highest likelihood (Haley & Knott 1992). We compared the likelihood of this model (with two QTLs) to that of the model with a single QTL, using a log-likelihood ratio test. The log-likelihood ratio test statistic is equivalent to the chi-squared statistic. We tested the significance of this by comparing the chi-squared values to the values obtained from the permutations of the single QTL model on the data set of each respective cross.

Results

Single-QTL models

We started by regressing the genotype of the flies against the viral transmission rate at every centimorgan location along the chromosome. In all three crosses, there is a strong association between genotype and transmission, as indicated by a large peak in the chi-squared statistic, in a similar region of the chromosome (Fig. 1). Furthermore, at this location the difference between the viral transmission rate estimated for the two genotypes is similar for the three crosses. In cross 1, the maximum chi-squared statistic is at 38 cM ($\chi^2 = 110.76$, d.f. = 1, $P < 0.0001$) and is associated with a change in transmission of 54%. In cross 2, the maximum chi-squared statistic is at 36 cM ($\chi^2 = 60.74$, d.f. = 1, $P < 0.0001$) and transmission changed by 45%. In cross 3, the maximum *F*-statistic is at 47 cM ($\chi^2 = 70.39$, d.f. = 1, $P < 0.0001$) and transmission changed by 57% (Fig. 2; Table 1). Table 1 gives confidence intervals on these effect sizes.

Assuming that these peaks in the chi-squared statistic were caused by a single QTL, we calculated confidence limits on the QTL location for each cross by bootstrapping. These three confidence limits overlapped (Fig. 2; Table 1)

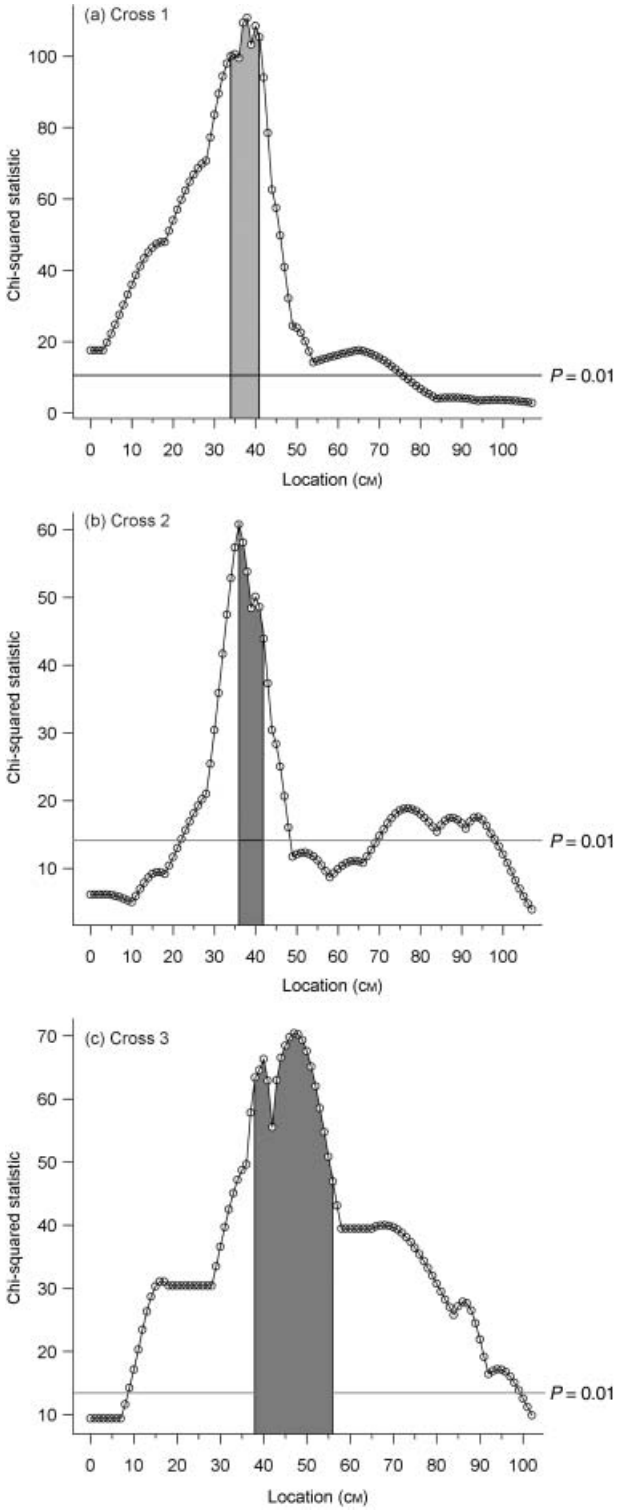

Fig. 1 Showing the locations of the QTLs using single-QTL models. The chi-squared statistic obtained by regressing genotype against the rate of paternal transmission of the sigma virus. The horizontal line is the 1% significance threshold from permutation, and the shaded region is the 95% confidence interval on the QTL location obtained by bootstrapping.

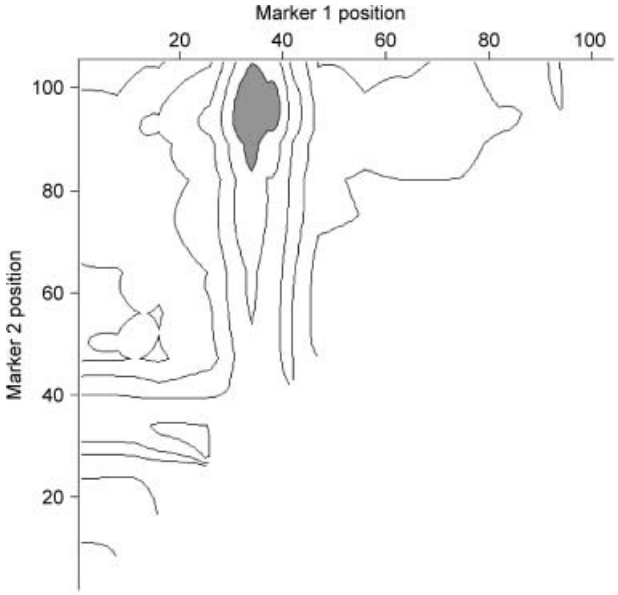

Fig. 2 Showing the locations of the two QTLs from cross 2. Drop in the LOD score of all pairwise combinations of QTL locations for cross 2 (the position of the maximum LOD of 13.67 is 34 cM and 94 cM). The contours show the decline in LOD score (in units of 1) relative to the maximum-likelihood model. The region indicating a LOD drop of < 1 is shaded grey.

suggesting that the same QTL might be responsible for variation in the paternal transmission of the sigma virus in the three crosses. Therefore, to test whether there is evidence for QTL in different locations in the three crosses, we compared a model in which the three crosses had different QTL locations to a model in which the QTL was in the same location. This was repeated for each combination of pairs of crosses (Table 2). The QTL locations for crosses 1 and 2 were not significantly different from one another, but the location of the cross 3 QTL was significantly (or nearly significantly) different to the locations for crosses 1 and 2 (Table 2). Having found no significant difference between the QTLs located by crosses 1 and 2, we combined the data sets from crosses 1 and 2 and carried out a second QTL analysis, which yielded a QTL at 38 cM with an effect size of 54% (Table 2). Whether the data were from cross 1 or cross 2 did not have a significant effect on the effect size of the QTL effect (interaction of genotype and cross: $\chi^2 = 1.89$, $P = 0.1682$).

Two-QTL models

To test whether more than one QTL influenced transmission of the virus, we compared models that allowed two QTLs to the models that allowed only a single QTL described above. For cross 2, a model with two QTLs fitted the data significantly better than a model with a single QTL (Table 3), and the locations of these QTL — at 34 cM and

Table 1 QTL identified under the single QTL model

Cross	QTL location (cM)	95% confidence intervals on location	Effect on paternal transmission*	95% confidence intervals of effect
1	38	34–41	0.54	0.43–0.61
2	36	36–42	0.45	0.33–0.53
3	47	38–56	0.57	0.35–0.72
1 and 2	38	36–42	0.54	0.43–0.61

*The difference in the proportion of infected offspring between the high line and the low line homozygotes.

Table 2 Test of whether the QTLs are in different locations in the three crosses

Cross	Model	Location (cM)	χ^{2*}	Degrees of freedom	*P*†
1 and 2	Same location	38	1.93	1	0.16
	Different locations	39, 37			
1 and 3	Same location	41	3.35	1	0.07
	Different locations	39, 48			
2 and 3	Same location	39	7.25	1	< 0.01
	Different locations	37, 48			
1, 2 and 3	Same location	39	7.25	2	< 0.01
	Different locations	39, 37, 48			

Analyses assume a single QTL in each cross *twice the difference in the log likelihoods of the same location and different location models, †from chi-squared (χ^2) distribution.

Table 3 Comparison of models with one QTL and two QTLs

Cross	Number of QTLs in model	QTL locations (cM)	Effect on paternal transmission	χ^{2*}	*P* value
1	1	38	0.54	3.73	0.33
	2	31, 39	0.25, 0.33		
2	1	36	0.45	20.29	< 0.0001
	2	34, 94	0.39, 0.23		
3	1	47	0.57	5.86	0.11
	2	35, 87	0.45, 0.15		

*Twice the difference of the log likelihoods of models with one and two QTLs, *P* value from chi-squared (χ^2) distribution with one degree of freedom.

94 cM altering viral transmission by 39% and 23%, respectively, — were well separated. This is reflected in Fig. 1, which shows a second peak in the χ^2 statistic around 94 cM for cross 2. For crosses 1 and 3, the two-QTL models were not significantly better than the one-QTL models (Table 3).

For cross 2, we used two ways to assess the confidence interval on the location of the two QTLs. First, we plotted the drop in the logarithm of the odds (LOD) score relative to the maximum-likelihood model for all possible combinations of QTL locations (Fig. 2). Assuming that the two loci are independent, the LOD drop can give approximate 95% confidence intervals. An LOD drop of 1 (shaded in Fig. 2) covers a small region around the best locations (for cross 2, approximately 30–40 cM for one QTL and 82–105 for the other). However, confidence intervals obtained in this way have been shown to be too short and the more conservative criterion of a LOD drop of two better approximates a 95% confidence interval (van Ooijen 1992). For cross 2, this larger confidence interval retains a narrow interval for the larger QTL but indicates a poor ability to locate the second QTL with the interval spanning more than half of the chromosome. As an alternative approach to examine whether the presence of the smaller QTL altered the confidence interval of the location of the larger QTL in cross 2, we repeated the bootstrapping described above with the genotype at the smaller QTL location included as a fixed effect in the model. This had little effect on the location of the main QTL, which had 95% confidence intervals of between 36 cM and 42 cM.

Discussion

We have found evidence for a major-effect polymorphism affecting paternal transmission of the sigma virus in *Drosophila melanogaster* derived from a natural population. Three separate QTL analyses identified a major-effect locus in a similar location (affecting paternal transmission by about 50% in the single QTL model, or 40% in the two-QTL model). QTL studies normally only analyse the recombinants from a single cross, which makes it impossible to judge whether the variation uncovered is rare or whether it makes an important contribution to the variation within a population. The similarity in the size and position of QTLs identified in these three analyses indicates that they may all contain the same major-effect polymorphism. There was a significant conflict between the location of the QTL in cross 3 and the other two crosses, but this could be caused by nearby linked QTLs as well as a genuine difference in QTL location. Indeed, one of the crosses also shows evidence for a second, smaller-effect locus. The lines used for making the crosses were chosen because of their extreme phenotypes; there may be other loci that have moderate or small effects that were not sampled in these parents.

The region of the major-effect QTLs identified encompasses many genes (approximately 190 genes within the 95% confidence intervals for the single QTL identified from the combined data sets of crosses 1 and 2). High-resolution mapping has often broken major-effect QTLs into numerous smaller-effect loci (Mackay & Lyman 2005). Whether our major-effect QTL represents a polymorphism (or polymorphisms) at a single locus, or several linked loci will become clearer when the polymorphism is mapped to a narrower region or when candidate genes are tested. As the rate of recombination per gene is lower near to centromeres, small-effect loci spread across the genome might manifest as large-effect QTLs near to centromeres (Noor *et al*. 2001). However, the recombination rate around our QTLs is relatively high (Marais *et al*. 2003), so this is unlikely to be an explanation for the QTLs identified in our experiments.

A great deal of attention has been paid to natural polymorphisms in the chromosome-two gene *ref(2)P* that affect the replication and transmission of sigma (Gay 1978; Dru *et al*. 1993; Wayne *et al*. 1996; Bangham *et al*. 2007). The lines used in the three analyses in the present study were deliberately chosen because they did not possess the well-known *ref(2)P* resistance mutation that affects sigma replication and maternal transmission (Dru *et al*. 1993; Wayne *et al*. 1996; Bangham *et al*. 2007), but it is possible that other polymorphisms within the gene might affect paternal transmission in this study. However, *ref(2)P* is at position 54 cM on chromosome two — not within the 95% confidence intervals of the QTLs identified in crosses 1 and 2, and only just within the 95% confidence intervals of the QTL located in cross 3. Therefore polymorphisms in *ref(2)P* are unlikely to underlie the QTLs identified in our experiments.

The architecture of genetic variation can shed light on the type of selection maintaining this variation. What might the existence of major-effect polymorphisms tell us about the co-evolution between *D. melanogaster* and sigma virus? Genetic variation in the shape of many small-effect polymorphisms could be maintained through mutation-selection balance, but this is unlikely to maintain the large-effect polymorphisms in this study. One possibility is that this variation is maintained by negative frequency-dependent selection, as predicted by many theoretical models of co-evolution (e.g. Stahl *et al*. 1999). This would require trade-offs between resistance and other traits (such as resistance to other viral genotypes, or some other component of fitness). A second possibility is that a 'resistance' allele is spreading under directional selection, and that the polymorphisms we have picked up in this study are transient. This has been proposed as an explanation for the resistant/susceptible *ref(2)P* polymorphism in natural populations of *D. melanogaster* (Wayne *et al*. 1996; Bangham *et al*. 2007).

It is possible that the major-effect QTLs affecting paternal transmission of sigma virus represent one half of a 'gene-for-gene' interaction between sigma and *D. melanogaster* — that major-effect alleles carried by the host might have corresponding major-effect alleles carried by sigma that overcome resistance. There is evidence that *ref(2)P* is involved in a gene-for-gene interaction with sigma. Two distinct genotypes of the sigma virus have been found in natural populations: 'infective' viruses that can infect flies carrying the resistant *ref(2)P* allele and 'avirulent' viruses that cannot (Fleuriet 1980), and the spread of this infective viral genotype might have been a response to selection imposed by the resistant *ref(2)P* allele. It is possible that paternal transmission of sigma too could be affected by gene-for-gene interactions — Fleuriet (1991) found that paternal transmission was affected by interactions between viral strains and host strains in crosses between laboratory populations and African populations. The major-effect polymorphisms identified in the present study are consistent with this.

There are several examples of major-effect resistance polymorphisms as outcomes of host–parasite co-evolution. There are at least two such polymorphisms that affect antiviral resistance in flies [our QTL and *ref*(2)*P*]. There are also two major-effect polymorphisms that make *D. melanogaster* resistant to different species of parasitoid wasps, the only other co-evolved parasites of *Drosophila* that have been studied in detail (Poirie *et al*. 2000). Major-effect polymorphisms have also been described in natural populations of other organisms, including mosquitoes and many plants (Wilfert & Schmid-Hempel 2008). It is possible that selection is sometimes more likely to maintain major-effect polymorphisms than minor-effect polymorphisms, as theory suggests that natural selection can favour a

mixture of highly resistant and susceptible individuals in populations (Boots & Haraguchi 1999). However, these models do not consider the genetics of resistance traits, so it is unclear whether it predicts that resistance should be controlled by a few major-effect polymorphisms. Furthermore, additional data are needed to test whether the genetic architecture of resistance to pathogens is different from that of other traits.

In conclusion, there is an emerging picture that resistance to transmission and replication of the sigma virus is affected by natural variation in major-effect loci. Identifying those genes will give us some clues as to the ways in which animals respond to viruses, and which components of this response are subject to selection. It is still not clear why susceptible variants of genes affecting transmission are maintained in populations, despite the advantage to resistance, but the answer to this question will give insights into the process of co-evolution between parasites and their hosts.

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References

Bangham J, Obbard DJ, Kim KW, Jiggins FM (2007) The age and evolution of an antiviral resistance mutation in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 2027–2034.

Bangham J, Kim K-W, Webster CL, Jiggins FM (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.

Barton N, Keightley P (2002) Understanding quantitative genetic variation. *Nature Reviews Genetics*, **3**, 11–21.

Boots M, Haraguchi Y (1999) The evolution of costly resistance in host-parasite systems. *American Naturalist*, **153**, 359–370.

Brun P, Plus N (1998) The viruses of *Drosophila*. In: *The Genetics and Biology of Drosophila* (eds Ashburner M, Wright TRF), pp. 625–702. Academic Press, London.

Carpenter JA, Obbard DJ, Maside X, Jiggins FM (2007) The recent spread of a vertically transmitted virus through populations of *Drosophila melanogaster*. *Molecular Ecology*, **16**, 3947–3954.

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics*, **138**, 963–971.

Clancy DJ, Kennington WJ (2001) A simple method to achieve consistent larval density in bottle cultures. *Drosophila Information Service*, **84**, 168–169.

Dru P, Bras F, Dezelee S, Gay P, Petitjean AM, Pierre-Deneubourg 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.

Dubuffet A, Dupas S, Frey F, Drezen JM, Poirie M, Carton Y (2007) Genetic interactions between the parasitoid wasp *Leptopilina boulardi* and its *Drosophila* hosts. *Heredity*, **98**, 21–27.

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. *Archives of Virology*, **69**, 261–272.

Fleuriet A (1991) Polymorphism of the hereditary rhabdovirus sigma in wild populations of its host, *Drosophila melanogaster*. *Genetics, Selection, Evolution*, **23**, 25–37.

Gay P (1978) *Drosophila* genes which intervene in multiplication of sigma virus. *Molecular and General Genetics*, **159**, 269–283.

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, **69**, 315–324.

Henter HJ (1995a) The potential for coevolution in a host-parasitoid system. 1. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution*, **49**, 427.

Henter HJ (1995b) The potential for coevolution in a host-parasitoid system. 2. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution*, **49**, 439.

Hirschhorn JN, Daly MJ (2005) Genome–wide association studies for common diseases and complex traits. *Nature Reviews Genetics*, **6**, 95–108.

Holub EB (2001) The arms race is ancient history in *Arabidopsis*, the wildflower. *Nature Reviews Genetics*, **2**, 516–527.

Laird NM, Ware JH (1982) Random-effects models for longitudinal data. *Biometrics*, **38**, 963–974.

Lazzaro BP, Sceurman BK, Clark AG (2004) Genetic basis of natural variation in *D. melanogaster* antibacterial immunity. *Science*, **303**, 1873–1876.

Mackay TFC, Lyman RF (2005) *Drosophila* bristles and the nature of quantitative genetic variation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **360**, 1513–1527.

Marais G, Mouchiroud D, Duret L (2003) Neutral effect of recombination on base composition in *Drosophila*. *General Research*, **81**, 79–87.

Noor MA, Cunningham AL, Larkin JC (2001) Consequences of recombination rate variation on quantitative trait locus mapping studies: simulations based on the *Drosophila melanogaster* genome. *Genetics*, **159**, 581–588.

Pinheiro JC, Bates DM (2000) *Mixed Effects Models in S and S-Plus*. Springer Verlag, New York.

Poirie M, Frey F, Hita M, Huguet E, Lemeunier F, Periquet G, Carton Y (2000) *Drosophila* resistance genes to parasitoids: chromosomal location and linkage analysis. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1417–1421.

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

Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J (1999) Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. *Nature*, **400**, 667–671.

van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. *Theoretical and Applied Genetics*, **84**, 803–811.

Visscher PM, Thompson R, Haley CS (1996) Confidence intervals for QTL locations using bootstrapping. *Genetics*, **143**, 1013–1020.

Wayne ML, Contamine D, Kreitman M (1996) Molecular population genetics of ref (2) P, a locus which confers viral resistance in *Drosophila*. *Molecular Biology and Evolution*, **13**, 191–199.

Wilfert L, Schmid-Hempel P (2008) The genetic architecture of susceptibility to parasites. *BMC Evolutionary Biology*, **8**, 187.

Wyers F, Dru P, Simonet B, Contamine D (1993) Immunological cross–reactions and interactions between the *Drosophila melanogaster* ref (2) P protein and sigma rhabdovirus proteins. *Journal of Virology*, **67**, 3208–3216.

Supplementary material

The following supplementary material is available for this article:

Appendix S1 Methods for creating recombinant lines

Table S1 Molecular markers used in the QTL analysis

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2008.03873.x
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