Natural genetic variation in *Drosophila melanogaster* reveals genes associated with *Coxiella burnetii* infection

Rosa M. Guzman,1,‡ Zachary P. Howard,1,‡ Zying Liu,1 Ryan D. Oliveira,2 Alisha T. Massa,2 Anders Omsland,3 Stephen N. White,2,4,5 and Alan G. Goodman 1,‡,*

1School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, USA
2Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, USA
3Paul G. Allen School for Global Animal Health, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, USA
4USDA-ARS Animal Disease Research, Pullman, WA 99164, USA
5Center for Reproductive Biology, Washington State University, Pullman, WA 99164, USA
1Equal author contribution.

Abstract

The gram-negative bacterium *Coxiella burnetii* is the causative agent of Query (Q) fever in humans and coxiellosis in livestock. Host genetics are associated with *C. burnetii* pathogenesis both in humans and animals; however, it remains unknown if specific genes are associated with severity of infection. We employed the *Drosophila* Genetics Reference Panel to perform a genome-wide association study to identify host genetic variants that affect host survival to *C. burnetii* infection. The genome-wide association study identified 64 unique variants ($P < 10^{-5}$) associated with 25 candidate genes. We examined the role each candidate gene contributes to host survival during *C. burnetii* infection using flies carrying a null mutation or RNAi knockdown of each candidate. We validated 15 of the 25 candidate genes using at least one method. This is the first report establishing involvement of many of these genes or their homologs with *C. burnetii* susceptibility in any system. Among the validated genes, *FER* and *tara* play roles in the JAK/STAT, JNK, and decapentaplegic/TGF-β signaling pathways which are components of known innate immune responses to *C. burnetii* infection. *CG42673* and *DIP-e* play roles in bacterial infection and synaptic signaling but have no previous association with *C. burnetii* pathogenesis. Furthermore, since the mammalian ortholog of *CG13404* (*PLGRKT*) is an important regulator of macrophage function, *CG13404* could play a role in host susceptibility to *C. burnetii* through hemocyte regulation. These insights provide a foundation for further investigation regarding the genetics of *C. burnetii* susceptibility across a wide variety of hosts.

Keywords: genome-wide association study; bacteria; pathogenesis; immunity; host response

Introduction

*Coxiella burnetii* is the causative agent of Query (Q) fever, a zoonotic disease that poses a serious threat to both human and animal health (Maurin and Raoult 1999). Because of its morbidity, low infectious dose, and the environmental stability of *C. burnetii*, the US NIH and CDC classify it as a Category B priority pathogen (Madariaga et al. 2003). Humans primarily become infected from sheep, goats, and cattle through inhalation of contaminated aerosols (Marrie et al. 1996; McQuiston et al. 2002; Schimmer et al. 2008). Therefore, reducing bacterial load in the livestock is critical to preventing Q fever outbreaks. *C. burnetii* is endemic worldwide and sporadic outbreaks have recently been reported in the United States (Karakousis et al. 2006; Anderson et al. 2013; Kersh et al. 2013; Sondgeroth et al. 2013; Dahigren et al. 2015). A recent large outbreak of Q Fever on a goat farm in the Netherlands cost 307 million Euros in public health management efforts and agricultural interventions (Schimmer et al. 2008; Roest et al. 2011a, 2011b; van Asseldonk et al. 2013). To date, no commercial Q fever vaccine is available for humans or animals in the United States, and antibiotic therapy is the only option for treating human infection. Culling infected or at-risk animals is often employed to control outbreaks (Roest et al. 2011b, 2012, 2013). Additionally, the lack of animal models with genetic malleability and the strict requirements in BSL3 animal facilities to work with Select Agent phase 1 virulent strains of *C. burnetii* make it difficult to study host–pathogen interactions in vivo.

Host genetics influence the development of *C. burnetii* infection in both humans and other animals (Chigo et al. 2002; Leone et al. 2004; Raoult et al. 2005; Meghari et al. 2008; Delaby et al. 2012; De Lange et al. 2014). Experimental studies in human and mouse cells correlate defective monocyte/macrophage activation and migration with ineffective granuloma formation, and overexpression of interleukin (IL)-10 is present in patients with chronic Q fever (Meghari et al. 2008; Delaby et al. 2012; Bewley 2013; Mehrjaj et al. 2013; Ka et al. 2014). Two recent studies genotyped human populations and revealed that genetic variation in innate immune genes, such as those encoding pattern recognition receptors and IFNG, are associated with susceptibility to Q fever.
(Wielders et al. 2015; Ammerdorffer et al. 2016). Despite this importance, specific genetic variants associated with susceptibility to C. burnetii infection remain largely unknown. In addition, it is unknown how host genetic factors affect bacterial load and shedding in susceptible reservoir hosts, namely livestock.

Previous studies profiled mammalian host responses to C. burnetii infection. These studies show that the bacteria downregulate the host innate immune response during acute infection and determine that the resolution of Q fever is associated with the re-establishment of type I interferon (IFN) signaling (Chigo et al. 2002; Fauqaret et al. 2014; Corvel et al. 2014). Directed studies in humans reveal that single-nucleotide polymorphisms (SNPs) in innate immune receptors and signaling genes such as TLR1, STAT1, IFNγ, and MYD88 are associated with acute or chronic Q fever (Schoeffelen et al. 2015; Wielders et al. 2015). Since these studies used a targeted approach to examine SNPs in only a set of candidate genes, we aimed to undertake a global, genome-wide analysis to identify gene variants associated with C. burnetii infection using Drosophila melanogaster as the host model.

We recently demonstrated that adult D. melanogaster is susceptible to infection with the BSL2 NMII clone 4 strain of C. burnetii and that this strain replicates in flies (Bastos et al. 2017). Importantly, this previous work established D. melanogaster as a suitable model for studying host-pathogen interactions during C. burnetii infection despite the bacteria being a mammalian pathogen and not a natural pathogen of insects. Additionally, mechanisms of immunity differ between mammals and insects, most notably the lack of an adaptive immune response in insects. Furthermore, while many components of innate immunity are mammalian orthologs or highly conserved functions that allow for extrapolation to mammalian systems.

To identify host genetic variants associated with sex-specific susceptibility or tolerance to C. burnetii infection, the human orthologs of the genes we identified may also play important roles in human immune cell regulation, highlighting the conserved nature of gene function between insect and mammalian models of C. burnetii infection.

Materials and methods
Drosophila melanogaster and C. burnetii stocks
Fly stocks were obtained from the Bloomington Drosophila Stock Center, the Vienna Drosophila Resource Center, Exelixis at Harvard Medical School, and the Kyoto Stock Center. Fly stocks were maintained at room temperature in standard meal agar fly food at 25°C and 65% humidity. All fly strains are listed in Supplementary Table S1. CoxIEL burnetii Nine Mile phase II (NMII) clone 4 RSA439 was propagated in Acidified Citrate Cysteine Medium 2 as previously described (Omsland et al. 2009). CoxIEL burnetii stocks were quantified using real-time PCR to measure bacterial genome equivalent (GE), as previously described (Coleman et al. 2004).

Fly infections and hazard ratio phenotype determination
Each DGRP line was separated into groups of 40 male and 40 female adult flies (2–7 days old) (Supplementary Tables S2 and S3) and injected with phosphate-buffered saline (PBS) or 10⁵ GE of C. burnetii diluted in PBS to establish infection. We infected flies at a multiplicity of infection of 10⁵ GE C. burnetii/fly based on the previous study establishing D. melanogaster as a model for C. burnetii infection (Bastos et al. 2017). For infections, flies were anesthetized with CO₂ and injected with 23 nL of bacteria or PBS using a pulled glass capillary and an automatic nanoliter injector (Drummond Scientific, Broomall, PA, USA), as previously described (Hiroya et al. 2018). Individual flies were injected at the ventrolateral surface of the fly thorax and placed into new vials. Male and female flies were housed in separate vials. After injection, survival was monitored daily for 30 days with the flies maintained at 25°C and 68% humidity. We used Prism v8.0 (GraphPad Software, Inc.) to determine hazard ratios and P-values [log-rank (Mantel-Cox) test] for survival curves for males and females from each DGRP line. All survival analyses take the full 30-day trial into account, and raw data for the DGRP lines are found in Supplementary File S1. Lines having less than 3% mortality in the mock-infected group were not included in downstream analyses (Chow et al. 2013).

Genome-wide association using hazard ratios and candidate gene analyses
To determine phenotype-to-genotype association, hazard ratios were log_{10} transformed and submitted to the dgrp2 webtool (http://dgrp2.gnets.ncsu.edu/), which adjusts the phenotype for the effects of Wolbachia infection and major inversions (Mackay et al. 2012; Huang et al. 2014). Three separate analyses were run using male, female, and combined hazard ratios for the DGRP lines (Supplementary Tables S2-S4). R was used to create Quantile-
Validation of candidate genes

To empirically determine the effect of knockout or knockdown of the candidate gene on severity to infection, 40 adult flies from each null mutant and RNAi knockdown for each candidate gene were injected with PBS or C. burnetii, as stated previously. RNAi knockdown was performed using straight-winged progeny from crosses between the CyO-balanced Act5C-GAL4 driver line and the corresponding dsRNA-containing RNAi lines (Supplementary Table S1). Sibling progeny flies carrying the CyO balancer were used as control flies. Genetic background strains for each null mutant strain were used as control flies. We conducted all survival experiments for each candidate gene twice independently and we used Prism v8.0 (GraphPad Software, Inc.) to determine hazard ratio and P-value [log-rank (Mantel-Cox) test] for each independent survival experiment to ensure that the two experiments were not significantly different from each other (data not shown). We then combined the results from both experiments to determine single hazard ratios and generate survival graphs (Ahlers et al. 2019; Dudzic et al. 2019). Raw survival data for validation experiments are found in Supplementary File S2. Significance levels of combined survival experiments for each genotype were binned into one of three categories: no significance (P > 0.01), low significance (0.01 > P > 0.0001), or high significance (P < 0.0001) (Supplementary Tables S6 and S7). We considered a gene to validate if the significance level changed between control and null mutant or RNAi knockdown genotypes for the sexes corresponding to GWA analysis type. For validated gene candidates in the average category, significance levels changed for both sexes, and validated gene candidates in the difference category changed for one sex but not the other.

Splicing, branch point variation, and codon usage analysis

The Ensembl project (http://uswest.ensembl.org/index.html) and the Human Splicing Finder (http://www.umd.be/HSF/) were used to determine splicing and branch point variation from curated sequences to determine codon usage fraction based on frequency of amino acids per thousand.

Data availability

Strains and stocks are available upon request. Genomic sequence for the DGRP is available at http://dgrp.gnets.ncsu.edu/. Supplementary material and all raw survival data (Supplementary Files S1 and S2) are available at FigShare: https://doi.org/10.25386/genetics.13490244. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

Results

Susceptibility to C. burnetii infection is dependent on host genetic background

Previously, we determined that flies deficient in the IMD signaling pathway genes, PGRP-LC and Relish, exhibit increased susceptibility to C. burnetii infection. We also determined that the gene eiger contributed to decreased tolerance to C. burnetii infection in flies, as eiger mutant flies were less susceptible to C. burnetii infection (Bastos et al. 2017). Therefore, we hypothesized that susceptibility to C. burnetii infection in Drosophila is associated with host genetics, and that the broad base genetic variation in the DGRP could identify other candidate genes that effect susceptibility to C. burnetii infection via a GWAS. To determine the susceptibility of each DGRP line to infection, adult males and females of each line were mock-infected or infected with C. burnetii. We then monitored survival and calculated hazard ratios that were used as input for the GWA analysis (Figure 1). In total, we calculated 193 and 195 hazard ratios for males and females, respectively. The survival curves reveal an approximately log-normal distribution of hazard ratios ranging from ∼0.719 to 1.643 for male flies (0.191–44.01, non-log-transformed) and ∼0.714 to 1.200 for female flies (0.1932–15.85, non-log-transformed) (Supplementary Tables S2 and S3, Figure S1A), which indicates that genetic polymorphisms in the DGRP lines affect susceptibility to C. burnetii infection. Interestingly, male flies are more susceptible than female flies overall to C. burnetii infection, with a mean hazard ratio of 1.90 for male flies and 1.56 for female flies (P = 0.0015) (Supplementary Figure S1A). Notably, we observe three distinct survival phenotypes for both male and female flies among all DGRP lines. These survival phenotypes are defined by the hazard ratio, which compares the mortality rate of C. burnetii-infected flies to mock-infected flies. Hazard ratio analysis has been used to examine flies’ mortality rate to West Nile virus compared to mock-infection and to Pseudomonas entomophila based on route of infection (Martins et al. 2013; Ahlers et al. 2019). In general, susceptible DGRP lines display increased mortality to C. burnetii infection compared to mock-infection and positive log₁₀ hazard ratios. Tolerant DGRP lines show no change in survival between C. burnetii and mock-infection and have log₁₀ hazard ratios close to zero. We also observe that certain DGRP lines exhibit decreased mortality compared to the mock-infected group, as noted...
by the negative log_{10} hazard ratio in Supplementary Tables S2 and S3. Negative hazard ratios following microbial infection are not uncommon. This means that the genetic background of the particular DGRP line results in increased survival compared to the mock/injury control (Martins et al. 2013; Ahlers et al. 2019). A study on dietary restriction also shows negative hazard ratios (Mccracken et al. 2020).

GWA analyses of DGRP hazard ratios reveal candidate gene variants

The DGRP facilitates rapid GWA analyses using a quantitative phenotype via submission of a data set to the online webtool (Mackay et al. 2012). To determine polymorphisms in the DGRP population that affect susceptibility to C. burnetii, we submitted hazard ratios for analysis. We log_{10}-transformed the hazard ratios prior to submission for GWA analysis (Supplementary Figure S1A) to yield an approximately normal distribution (Shapiro–Wilk test, \( P > 0.1 \)) due to GWA analyses relying on parametric tests (Chow et al. 2013). We determined that hazard ratios are significantly positively correlated between male and female flies (\( P = 4.99 \times 10^{-7} \)), but with an \( r^2 \) value of 0.121, which indicates a weak correlation and potential sex-dependent genotypes (Supplementary Figure S1B). Thus, we submitted hazard ratios as separate files for male and female analyses, and a single, combined file in order to identify polymorphisms that may be sex-dependent and to increase power for polymorphisms that are sex-independent. We termed the sex-independent analysis the average analysis, which results in top candidate variants that affect both sexes while the sex-dependent analysis which we termed difference analysis, results in top candidates that affect one sex but not the other. In total, we submitted 193, 195, and 191 hazard ratios for males, females, and average and difference, respectively (Supplementary Tables S2–S4).

We tested a total of 1,893,791 polymorphisms in the male analysis, 1,897,049 polymorphisms in the female analysis, and 1,889,141 polymorphisms in the combined analysis. These analyses were not sufficiently powered to detect polymorphisms at a Bonferroni-corrected \( P \)-value of \( 2.64 \times 10^{-8} \). Therefore, we employed a genome-wide suggestive \( P \)-value threshold of \( 10^{-5} \) which has been used for studies employing the DGRP (He et al. 2014; Chow et al. 2016; Kelsey and Clark 2017; Schmidt et al. 2017; Lavoy et al. 2018; Mackay and Huang 2018; Palu et al. 2020; Talsness et al. 2020). Using this \( P \)-value, we obtained a total of 69 associated polymorphisms from the GWA analyses, which included five duplicate variants (Figure 2 and Supplementary Table S5). Q–Q plots revealed no significant inflation due to dataset distribution, lambda values ranged from 0.993 (females) to 1.002 (difference), and \( P \)-values derived from these analyses appear to be reduced overall on the lines from the Q–Q plots and lambda values below 1 (Supplementary Figure S2, A–D). Another interpretation of the Q–Q plots and GWAS significance values is that there is random association of our genome-wide suggestive variants. However, previous work has shown that weak signals in DGRP studies produce meaningful results. For example, candidate gene association and phenotypic correlation can be preserved among GWAS from different labs and using different populations of inbred fly lines (Everman et al. 2019; Pitchers et al. 2019). Furthermore, weak DGRP signals have been used to identify genes that genuinely regulate the phenotypic output of the DGRP studies (Chow et al. 2016; Palu and Chow 2018; Ahlers et al. 2019; Palu et al. 2020). Nevertheless, we calculated FDR corrections that would result in an equivalent \( P \)-value cutoff of \( P < 10^{-5} \) for each of our GWA analyses (Benjamini and Hochberg 1995; Pinheiro et al. 2020). For the female GWAS, the FDR correction is 0.88; for the male, it is 0.94; for the average, it is 0.99; for the difference, it is 0.78. As described in Everman et al. (2019), an FDR equivalent for a \( P < 10^{-5} \) cutoff ranged from 0.49 to 0.82 for previously published studies (Mackay et al. 2012; Everman and Morgan 2018). One reason our FDR corrections are higher is because our study uses additional DGRP lines than those cited above, which results in a greater number of tests (N) and additional variants with MAFs > 0.05. Nevertheless, the very lenient \( P \)-value cutoff we used gives us the opportunity to test more candidate genes in our validation experiments to rule out false positives that may have been selected using our lenient cutoff. While the DGRP may be underpowered, the ease with which one can perform empirical validation using Drosophila genetics makes the model as a whole very useful. Importantly, our goal is not to claim the associations reported here as definitive markers for host susceptibility to C. burnetii infection but to broadly identify candidate genes for future mechanistic studies in the context of C. burnetii or other pathogenic infections.

Of the 64 unique polymorphisms identified from the GWA, 14 variants are intergenic (21.9%), three of which are within 200 base pairs upstream of nearest gene; 39 are within introns (60.9%); eight are within exons (12.5%); one is within the 5’ UTR (1.6%); and two are in antisense-coding RNA within exon/introns (3.1%) (Supplementary Table S5). Of the eight SNPs within exons, six are silent and two are missense mutations. The 64 unique variants correspond to 31 unique genes that we narrowed to 25 candidate genes for validation experiments. Candidate genes were chosen based on stocks available and the location and type of gene disruption used in the available stocks. Due to these limitations, we did not perform validation experiments for CG42455, side, Mtb8, Or92a, Cpr100A, or CG32694. We also report the relative male: female ratio gene expression data of each candidate gene using information available on Flybase (FB2019_02) (Table 1). We used the DGRP genome assembly (BDGP R5/dm3) to gather putative functions and regulatory annotations for each gene using Flybase and modENCODE and found that 12 are in
TFBS (48%); nine are within regions predicted to be transcriptionally silent (36%); one is within a long noncoding RNA (4%); and three are in enhancers only (12%) (Table 1). Lastly, we report the human ortholog with the highest weighted score from the DRSC Integrative Ortholog Prediction Tool (DIOPT) on Flybase.

Validation of candidate genes

We next tested the 25 candidate genes from the different GWA analyses (Table 1) by infecting and monitoring survival during C. burnetii infection for 30 days in flies carrying a null mutation in the candidate gene or knocked down for the candidate gene by RNAi. We defined validation of candidate genes when the null mutant or RNAi knockdown line that has a different threshold of survival $P$-value significance than its genetic control, as described in the Materials and methods section and Supplementary Tables S6 and S7. Of the 25 candidate genes, 6 validated in null mutants only (24%), five in RNAi knockdown only (20%), 4 in both null mutants and RNAi (16%), and 10 did not validate with either method (40%) (Figure 3A). Survival of $w^{1118}$ males and females (Figure 3B) during C. burnetii infection was used as the genetic control for several null mutants, including RhoGEF64C$^{MB04730}$ (Figure 3C), tara$^1$ (Figure 3D), and CG13404$^{07277}$ (Figure 3E). We selected these candidate genes to represent how we determined validation based on $P$-value and survival trend for validating genes from different categories, i.e. null-only, RNAi-only, or both. $w^{1118}$ females (Figure 3B) are not susceptible to C. burnetii infection ($P = 0.0333$) but $w^{1118}$ males (Figure 3B') are highly susceptible ($P < 0.0001$) which corroborates our previous work (Bastos et al., 2017). We selected the candidate gene RhoGEF64C$^{MB04730}$ from male-only GWA (Figure 3, C–C') and we observe that survival in null mutants (Figure 3C) is overall tolerant ($P = 0.0014$) compared to $w^{1118}$ males (Figure 3B'). In contrast, there is no significant change in survival between control and RNAi-knockdown flies (Figure 3C') (control, $P = 0.0374$; RNAi, $P = 0.0130$). Thus, RhoGEF64C$^{MB04730}$ males validated only in null mutants. The candidate gene tara was selected from the female-only GWA and we observe that in null mutants (Figure 3D) and RNAi knockdown flies (Figure 3D'), the absence of the gene results in decreased survival compared to control genotypes. Specifically, tara$^1$ females are susceptible to infection ($P < 0.0001$).
Table 1 Candidate genes associated with top variants from GWA analyses

| Candidate gene | Top variant (BDGP R5/dm3) | Type                  | P-value   | Analysis | Male/female expression | Regulatory annotations | Putative gene function | Human ortholog |
|----------------|----------------------------|-----------------------|-----------|----------|------------------------|------------------------|------------------------|------------------|
| CG34351        | 2L_4702261_SNAPSHOT        | Intronic              | 7.5 x 10^-6 | Female   | 2.3                    | Poorly annotated       | Regulation of G-proteins | RGS7BP          |
| DIP-z          | 2L_6394872_SNAPSHOT        | Exonic, synonymous    | 2.9 x 10^-6 | Male     | 3.8                    | Euohromatin transcriptionally silent (or intergenic) | Interaction with Dprs | OPCML            |
| rk             | 2L_13999491_SNAPSHOT       | Intronic              | 8.4 x 10^-6 | Difference | -                      | Transcriptionally silent | GPCR; burisicon receptor, melanization | LGR5             |
| shn            | 3R_7099616_SNAPSHOT        | Exonic, synonymous    | 1.9 x 10^-7 | Female   | 1.4                    | TFBS hot spot          | Zinc finger C2H2 transcription factor | HIVEP2           |
| GNBP-like3     | 2R_16414194_SNAPSHOT       | Exonic, synonymous    | 6.1 x 10^-6 | Male     | 1.5                    | Euohromatin transcriptionally silent (or intergenic) | Beta 1,3-glucan recognition/binding | CRYBG1           |
| CG42741        | 2R_18004195_SNAPSHOT       | Intronic              | 5.3 x 10^-6 | Difference | 10                     | Zinc finger C2H2 transcription factor | Rho guanyl-nucleotide exchange factor | ARHGEF3          |
| trh            | 3L_376337_SNAPSHOT         | Intronic              | 4.4 x 10^-7 | Difference | 2.6                    | TFBS                   | bHLH-PAS transcription factor | NPA1             |
| CG32264        | 3L_3750617_SNAPSHOT        | Intronic              | 7.5 x 10^-6 | Average  | 2.6                    | Transcriptionally silent | Actin binding          | PHACTR2          |
| RhoGEF64C      | 3L_4738164_SNAPSHOT        | Intronic              | 7.4 x 10^-6 | Male     | 7                     | Euohromatin transcriptionally silent (or intergenic) | Rho guanyl-nucleotide exchange factor | ARHGEF3          |
| Pura           | 3L_7623383_SNAPSHOT        | Intronic              | 2.2 x 10^-7 | Average  | 1.5                    | IncRNA                 | Rho guanyl-nucleotide exchange factor | PLEKHG4          |
| daily          | 3L_8851042_SNAPSHOT        | Intronic              | 6.7 x 10^-6 | Average  | 1.9                    | Putative enhancer but not hot spot | Co-receptor for growth factors/morphogens | GPC5             |
| CG42673        | 3L_9540740_SNAPSHOT        | Intronic              | 3.5 x 10^-6 | Difference | 4.5                    | TFBS                   | Nitric-oxide synthase binding | NOS1AP           |
| dpr6           | 3L_10044744_SNAPSHOT       | Intronic              | 3.8 x 10^-6 | Difference | 0.035                  | Transcriptionally silent | Interaction with Dips | CADM1            |
| AstC-R2        | 3L_18481371_SNAPSHOT       | Exonic, synonymous    | 1.4 x 10^-6 | Difference | 4.2                    | Between two TFBS       | Allstatatin receptor | SSTR2            |
| ich             | 3R_4787301_SNAPSHOT        | Intronic              | 9.3 x 10^-7 | Average  | -                      | TFBS hot spot          | Zinc finger C2H2 transcription factor | PRDM15           |
| FER            | 3R_5218712 JNS             | Intronic              | 2.7 x 10^-6 | Female   | 3.8                    | Active enhancer        | Protein tyrosine kinase activity | FER              |
| tara           | 3R_12079260_SNAPSHOT       | Intronic              | 7.7 x 10^-6 | Female   | 1.1                    | Active enhancer, TFBS hot spot | Transcriptional co-regulator | SERTAD1          |
| CG31221        | 3R_15278653_SNAPSHOT       | Intronic              | 6.4 x 10^-6 | Male     | 2.7                    | Near TFBS              | LDL receptor           | LRP1B            |
| loco           | 3R_18456211_SNAPSHOT       | Antisense RNA         | 2.3 x 10^-6 | Average  | 9.6                    | Antisense RNA, enhancer, TFBS | Regulation of G-proteins | GRS12            |
| CG1544         | 3R_27026419_SNAPSHOT       | Intronic              | 9.4 x 10^-6 | Difference | 2.1                    | TFBS                   | Oxoglutarate dehydrogenase Actin binding | DHTKD1           |
| CG34417        | X_6434578_SNAPSHOT         | Intergenic, 226bp up-stream | 9.9 x 10^-6 | Average  | —                      | TFBS hot spot, putative enhancer/promoter | Sagging activity | SMTN             |
| CG12075        | X_8751630_SNAPSHOT         | Intronic              | 2.7 x 10^-6 | Difference | 1                      | Silent chromatin state TFBS hot spot | Lipid signaling | NCOR1            |
| Smer           | X_12610056_SNAPSHOT        | Intronic              | 8.9 x 10^-6 | Average  | 0.25                   | Chromatin binding, transcriptional regulation | Calcium regulation, IP3 signaling | NET1             |
| iP3K2          | X_13210675_SNAPSHOT        | Intronic              | 7.0 x 10^-6 | Average  | 2.4                    | Putative enhancer site | Plasminogen receptor | PLGKRT           |
| CG13404        | X_14160126_SNAPSHOT        | Exonic, synonymous    | 6.1 x 10^-6 | Female   | 4.3                    | Active enhancer, TFBS hot spot | - | - |

* Human orthologs from DIOPT. Ortholog with highest weighted score reported.

Compared to w¹¹¹⁸ females (Figure 3B) and tara RNAi females (Figure 3D) are also susceptible (P = 0.0025) compared to control (P = 0.0123). Thus, tara validated for females in both null mutants and RNAi knockdown flies. We selected the candidate gene CG13404 from female-only GWA and we observe that null mutants (Figure 3E) are not susceptible to infection (P = 0.2737) like w¹¹¹⁸ females (Figure 3B). In contrast, CG13404 RNAi females (Figure 3E) are susceptible to infection (P < 0.0001) while control genotype females are not (P = 0.3914). Thus, CG13404 validated only in RNAi knockdown flies. Finally, to determine the relative effects on survival in males and females for all candidate genes, we calculated the relative hazard ratio of the null mutant (Supplementary Figure S3A) or RNAi knockdown (Supplementary Figure S3B) flies as compared to their respective genetic controls. Relative hazard ratios greater than one mean that the loss of that gene reduces survival to C. burnetii infection while relative hazard ratios less than one mean that the loss of that gene improves survival to C. burnetii infection.

**ENCODE analysis of validated genes**

Splicing and branching of precursor mRNA and abundance of tRNA codons are known to affect gene expression (Kršljićová et al. 2004; Wang and Burge 2008; Sauna and Kimchi-Sarfaty 2011; Will and Luhrmann 2011; Singh and Cooper 2012;...
Therefore, we used data available from the ENCODE project to determine regulatory annotations for the variants in genes that validated in host survival experiments. Table 2 summarizes the splicing and branch point analysis in terms of percent variation from wild type and codon usage as a fraction of frequency of amino acid (SNP) per thousand over frequency of amino acid (wild type) per thousand. Several SNPs varied at the predicted mRNA splicing sites, branch points, or codon usage compared to wild-type sequences such as the variants affecting the validated genes CG34351, DIP-ε, Pura, tara, FER, and IP3K2. The insertion (3R_S218712) within FER results in a -92.69% difference from wild-type splicing and has no variation in branch point splicing from wild type. This change in splicing for the FER indel indicates a change in the coding sequence, resulting in a possible frameshift or premature stop codon, which could lead to a truncated protein with altered function. This example highlights the importance of analyzing splicing and branch point variations in validating genes, as they can have significant impacts on gene expression and protein function.
the site is broken but a 3 base pair insertion offsets the destruction. Similarly, the SNP (3R_12079260) within tara differs 64.82% from wild-type splicing which also indicates a new splice site creation with no destruction. The frequency of the wild-type DIP-e codon is 3.72-times higher than that of the DIP-e SNP (2L_6394872), which suggests that decreased abundance of tRNA codon availability for the transcript variant may affect its translation and thus its function during C. burnetii infection. Changes in codon usage fraction for shn and CG13404 may affect these gene variants albeit to a lesser extent.

Discussion

In this study, we describe the application of an unbiased GWAS using the DGRP to reveal variants in genes associated with C. burnetii infection. We show that 15 genes conferred a significant difference in host survival during C. burnetii infection in null mutants, RNAi knockdown, or both gene disruption methods. We also compiled regulatory annotations of the variants in validating genes and show that certain gene variants affect splicing and codon usage, which may in turn downregulate gene expression. These data support the use of previously generated null mutant or RNAi knockdown gene disruption fly stocks to validate our candidate genes. While C. burnetii is not a natural D. melanogaster pathogen, there is evidence that it is an endosymbiont of tick arthropods and may have co-evolved in these animals (Duron et al. 2015). Previous studies show that few genome-wide significant variants are identified when using a non-natural versus natural D. melanogaster pathogen (Magwire et al. 2012), or when the pathogen has low prevalence in D. melanogaster (Chapman et al. 2020). Similarly, our GWA analyses did not identify any variants that met the expected Bonferroni-corrected P-value significance level. Instead, this study provides a platform to examine potential host factors that regulate C. burnetii infection given the limited genetic tools available for the bacteria. It is currently not possible to perform a genetic screen in mammals using the BSL2 strain of C. burnetii due to the inability of the strain to infect wild-type animals. Furthermore, a genetic screen using a Select Agent strain would encounter its own logistical roadblocks. Using our innovative approach, we uncover genes and processes that are relevant to bacterial infections in general. Additionally, directed studies of these genes could be performed using a Select Agent strain of C. burnetii. For these reasons, we do not take the validating genes in this study to be absolute but rather a first step toward understanding their cross-species role in the host response to infection.

Previous studies have shown that C. burnetii infection differentially affects male and female mice (Leone et al. 2004; Textoris et al. 2010), and our results corroborate these studies. First, male and female hazard ratios differ significantly among the DGRP lines in the initial screen (Supplementary Figure S1A). We interpret the difference in hazard ratios between sexes as a conserved phenotype with mammalian organisms. Secondly, expression of the candidate genes in adult flies differs between sexes (Table 1). For example, the expression of RhofGEP64C and DIP-e are 7.0 and 3.8-times more expressed in males than females, which is a potential reason why they validated in the male-only category, but not a definite cause. Validating genes from the difference category have higher differential sex expression, such as CG42741 and CG42673, which are 10 and 4.5-times more expressed in males than females, respectively. In contrast, validating genes in average category have closer relative expression, such as PurA and IF3K2, which are only 1.5 and 2.4-times more expressed in males than females, respectively. These results suggest that gene expression may drive sex-specific differences in host survival to C. burnetii infection. Lastly, genes we identified and validated as sex-specific, such as CG13404 and FER, have known functions in immunity, as discussed below.

Host survival in CG13404 RNAi-knockdown female flies indicated they were significantly more susceptible to infection compared to control genotype. The human ortholog of CG13404 is the plasminogen receptor gene PLGRKT, which is important for macrophage polarization and efferocytosis, two key components of inflammation regulation (Vago et al. 2019). The absence of Plg-RKT causes defective plasminogen binding and inflammatory macrophage migration in both male and female mice pups, but only female PLGRKT−/− pups die 2 days after birth (Miles et al. 2017). Our results corroborate this study because CG13404 was a top candidate from the female-only GWA. We hypothesize the role of FGRKT in macrophage regulation connects CG13404 to immunity. In D. melanogaster immunity, hemocytes are the professional phagocytic cells. They are present in flies during both larval and adult stages, and they recognize, engulf, and destroy dying host cells and pathogens (Hoffmann 2003; Yano et al. 2008; Regan et al. 2013). Hemocytes are critical for innate immune signaling by mediating the secretion of antimicrobial peptides (AMPs) in response to pathogens through the Toll, JAK/STAT, and Immune deficiency (Imd) pathways (Hoffmann 2003; Lemaître and Hoffmann 2007). Recent studies in our lab show that hemocytes support C. burnetii replication and induce Imd-specific AMPs (Bastos et al. 2017; Hiroyasu et al. 2018). However, our screen did not identify any genes in the classical Imd or Toll pathway. Nevertheless, the involvement of CG13404, the fly ortholog of Plg-RKT, during C. burnetii infection may exemplify conserved, sex-specific differences in mammalian macrophage and fly hemocyte regulation.

FER expression leads to activation of the DPP-mediated pathway, which has recently been shown to improve survival of Klebsiella pneumoniae through STAT3 when overexpressed (Murray 2006; Dolgachev et al. 2018; Li et al. 2019). Coxiella burnetii induces expression of STAT3 and IL-10 during murine infection (Murray 2006; Textoris et al. 2010; Millar et al. 2015). Textoris et al. show that male mice have increased gene expression of STAT3 and IL-10 during infection which may account for the higher susceptibility of Q fever observed in men. Our study corroborates this study because FER was a top candidate in the female-only GWAS. We hypothesize that the absence of FER in females disrupts the immune response required to control infection and leads to significantly decreased host survival.

In addition to FER, the other three genes that validated in both gene disruption methods reveal new connections between immunity and C. burnetii infection. Tara encodes a transcriptional co-regulator that interacts with chromatin remodeling complexes, cell cycle proteins, the JNK signaling pathway, and plays a role in ataxin-1-induced degeneration (Fernandez-Funez et al. 2000; Calgaro et al. 2002; Branco et al. 2008; Afonso et al. 2015). The human ortholog of tara is SERTAD1, which is also a transcriptional co-regulator (Biswas et al. 2010; Savitz et al. 2013). Interestingly, induction of SERTAD1 is expressed independently of IFN during Nipah virus infection (Glennon et al. 2015). IFN induction is tissue-dependent during C. burnetii infection (Hedges et al. 2016); therefore, it is plausible that tara is targeted by the bacteria during infection. DIP-e encodes a protein belonging to the immunoglobulin superfamily of defective proboscis extension response (Dpr) and Dpr-interacting proteins (DIP), which form a complex network of cell surface receptors in synaptic specificity. The human ortholog of DIP-e is OPCL1, an immunoglobulin protein best
characterized as a tumor suppressor (Cui et al. 2008; Birtley et al. 2019), and there is currently no reported role for either gene during bacterial infections. CG42673 remains uncharacterized, but another DGRP GWAS reports that loss of function of CG42673 in blood cells significantly impairs the cellular immune response to Staphylococcus aureus (Nazario-Toole 2016). Interestingly, this study also shows that dpr10 significantly affects S. aureus phagosome maturation while our own top candidate, dpr6 validated by RNAi knockdown. It is possible that CG42673 functions as an enhancer like its human ortholog NOS1AP (Grossmann et al. 2015; Hein et al. 2015) and regulates C. burnetii infection.

While some gene candidates validated as expected, other candidates validated as per our statistical tests, but in opposite directions. For example, we observed differences between the DGRP predictive effect of top candidates and validating genes. The GWA output predicts that the RhoGEF64 gene (CG13404, Supplementary Table S5) has increased host susceptibility to infection (effect = 0.1709). However, survival of RhoGEF64C MR04730 null mutant males was significantly improved compared to control genotype (Figure 3C) during C. burnetii infection. The opposite survival trend in the null mutant flies is likely if the SNP is a gain-of-function mutation, which is difficult to test but worth pursuing in further studies. Similarly, the SNP (X_14160126) in CG13404 (effect = 0.1213) predicts decreased host susceptibility to infection but RNAi knockdown females were significantly more susceptible compared to the control genotype (Figure 3E). One explanation for these opposing results is the possibility of gene product threshold effects, and overall susceptible or tolerant phenotypes during infection must be tested at the host level with subsequent functional experiments. Furthermore, the effect of the gene variants cannot be inferred from GWA alone, and knockout or knockdown of the associated gene may yield a different phenotype than that which was predicted for the variant in question. The use of null mutants or gene knockdown by RNAi is common practice to validate DGRP candidate gene variants (Howick and Lazzaro 2017; Palu et al. 2019, 2020), but the most precise way to test the effect that a gene variant has is to use gene-editing technology to knock-in the specific gene variant (Yoo et al. 2020). While we did not test the effect of each individual allele variant on host survival during C. burnetii infection, we conclude that the presence or absence of the genes in which the variants lie affects the outcome of infection.

In conclusion, this study builds on our previously developed framework utilizing D. melanogaster as an animal model to dissect the innate immune responses to C. burnetii infection (Bastos et al. 2017; Hiroyasu et al. 2018). We observe that C. burnetii infection significantly depends on host genetic background of the fly. In contrast, genetic studies in relevant natural hosts such as ticks, livestock, and humans are severely limited. Thus, we propose that the validating genes in this study can be used to test new hypotheses regarding host responses, taking into consideration the genes’ function in flies, their regulatory annotations, and their orthologs’ function in humans or other animals. These studies may reveal novel mechanisms of transmission among different host species or help identify at-risk human and livestock populations through genotyping efforts.

Acknowledgments

We thank Marcos A. Perez and Laura R.H. Ahlers for critical review of this manuscript. We are grateful to the editors and anonymous reviewers for their constructive feedback during the review process. We thank Michael D. Knight, Sarah A. Borgnes, Olivia M. Hayden, Marina Martin, and Emily L. Kindelberger for assistance in injecting fruit flies. We thank Codie Durfee for technical assistance. We are thankful to the Drosophila Genomics Resource Center (P40OD010949), the Bloomington Drosophila Stock Center (P40OD018537), the Vienna Drosophila Stock Center, Exelixis and TRIp at Harvard Medical School (R01GM084947) for providing reagents and fly stocks.

Funding

This investigation was supported by funds from Washington State University and the National Institutes of Health Public Health Service grant R21AI128103 (to A.G.G.). R.D.O. was supported by NIH Training Grant T32AI007025. This investigation’s contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Conflicts of interest

None declared.

Literature cited

Afonso DJS, Liu D, Machado DR, Pan H, Jepson JEC, et al. 2015. TARANIS functions with cyclin A and Cdk1 in a novel arousal center to control sleep in Drosophila. Curr Biol. 25:1717–1726. http://doi.org/10.1016/j.cub.2015.05.037.

Aguilera M, Salinas R, Rosales E, Carminati S, Colombo MJ, et al. 2009. Actin dynamics and Rho GTPases regulate the size and formation of parasitophorous vacuoles containing Coxiella burnetii. Infect Immun. 77:4609–4620. http://doi.org/10.1128/IAI.00301-09.

Ahlers LRH, Trammell CE, Carrell GF, Mackinson S, Torrevillas BK, et al. 2019. Insulin potentiates JAK/STAT signaling to broadly inhibit flavivirus replication in insect vectors. Cell Rep. 29:1946–1960.e5. http://doi.org/10.1016/j.celrep.2019.10.029.

Ammerdorffer A, Stappers MH, Oosting M, Schoffelen T, Hagenaars JC, et al. 2016. Genetic variation in TLR10 is not associated with chronic Q fever, despite the inhibitory effect of TLR10 on Coxiella burnetii-induced cytokines in vitro. Cytokine. 77:196–202. http://doi.org/10.1016/j.cyto.2015.09.005.

Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, et al. 2013. Diagnosis and management of Q fever—United States, 2013: recommendations from CDC and the Q Fever Working Group. MMWR Recomm Rep. 62:1–30.

Bastos RG, Howard ZP, Hiroyasu A, Goodman AG. 2017. Host and bacterial factors control susceptibility of Drosophila melanogaster to Coxiella burnetii infection. Infect Immun. 85.e00218-17. doi:10.1128/IAI.00218-17.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B (Methodol). 57:289–300. http://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Benoit M, Barbarat B, Bernard A, Olive D, Mege J-L. 2008a. TARANIS functions with cyclin A and Cdk1 in a novel arousal center to control sleep in Drosophila. Curr Biol. 25:1717–1726. http://doi.org/10.1016/j.cub.2015.05.037.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B (Methodol). 57:289–300. http://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Benoit M, Ghigo E, Capo C, Raoult D, Mege J-L. 2008b. The uptake of apoptotic cells drives Coxiella burnetii replication and macrophage polarization: a model for Q fever endocarditis. PLoS Pathog. 4: e1000066. http://doi.org/10.1371/journal.ppat.1000066.
Blewery KR. 2013. Animal models of Q fever (Coxiella burnetii). Comp Med. 63:469–476.

Birtle JR, Alomary M, Zanini E, Antony J, Maben Z, et al. 2019. Inactivating mutations and X-ray crystal structure of the tumor suppressor OPCML reveal cancer-associated functions. Nat Commun. 10:3134. http://doi.org/10.1038/s41467-019-10966-8.

Biswas SC, Zhang Y, Iyirhiaro G, Willett RT, Rodriguez Gonzalez Y, et al. 2010. Sertad1 plays an essential role in developmental and pathological neuron death. J Neurosci. 30:3973–3982. http://doi.org/10.1523/JNEUROSCI.6421-09.2010.

Bou Sleiman MS, Osman D, Massouras A, Hoffmann AA, Lemaitre B, et al. 2015. Genetic, molecular and physiological basis of variation in Drosophila gut immunocompetence. Nat Commun. 6:7829. http://doi.org/10.1038/ncomms8829.

Branco J, Al-Ramahi I, Ukani L, Perez AM, Fernandez-Funez P, Chapman JR, Dowell MA, Chan R, Unckless RL. 2020. The genetic basis of natural variation in Drosophila melanogaster immune defense against Enterococcus faecalis. Genes. 11:234. http://doi.org/10.3390/genes11020234.

Everman ER, McNeil CL, Hackett JL, Bain CL, Macdonald SJ. 2019. Dissection of complex, fitness-related traits in multiple Drosophila mapping populations offers insight into the genetic control of stress resistance. Genetics. 211:1449–1467. http://doi.org/10.1034/1534-9419.20140101.

Everman ER, Morgan TJ. 2018. Antagonistic pleiotropy and mutation accumulation contribute to age-related decline in stress response. Evolution. 72:303–317. http://doi.org/10.1111/evo.13408.

Fadista J, Manning AK, Florez JC, Group L. 2016. The (in)famous GWAS P-value threshold revisited and updated for low-frequency variants. Eur J Hum Genet. 24:1202–1205. http://doi.org/10.1038/ejhg.2015.269.

Gelbart WM. 1989. The decapentaplegic gene: a TGF-beta homologue controlling pattern formation in Drosophila. Development. 107:65–74.

Gigo E, Capo C, Tung CH, Raoul D, Garvel JP, et al. 2002. Coxieilla burnetii survival in THP-1 monocytes involves the impairment of phagosome maturation: IFN-gamma mediates its restoration and bacterial killing. J Immunol. 169:4488–4495.

Gibson J, Russ TC, Clarke T-K, Howard DM, Hillary RF, et al. 2019. A meta-analysis of genome-wide association studies of epigenetic age acceleration. PLoS Genet. 15.e1008104. http://doi.org/10.1038/s41996-020-0628-5.

Glennon NB, Jabado O, Lo MK, Shaw ML. 2015. Transcriptome profiling of the virus-induced innate immune response in Pseudomonas aeruginosa and its attenuation by nipah virus interferon antagonist functions. J Virol. 89:7550–7566. http://doi.org/10.1128/JVI.00302-15.

Gorvel JP, Textoris J, Banchereau R, Ben Amara A, et al. 2000. Identification of genes that modify ataxin-1-induced neurodegeneration. Nature. 408:101–106. http://doi.org/10.1038/35040584.

Graveley BR, Brooks AN, Carlson JW, Duff MO, Landolin JM, del Nido PJ, et al. 2015. Phospho-tyrosine dependent protein–protein interaction network of ticks led to the emergence of the Q fever pathogen, Coxieilla burnetii. PLoS Pathog. 11:e1004892. http://doi.org/10.1371/journal.ppat.1004892.

Gudjonsson J, Knoop JA, Ingason A, Sigfusson A, et al. 2004. Identification of genes that modify FER gene enhances innate immune response and improves survival in a murine model of pneumonia. Gene Ther. 25:359–375. http://doi.org/10.1038/s41434-018-0022-y.

Henson RL, Hulihan SM, Aps J, Ross A, et al. 2010. Phospho-tyrosine-dependent protein–protein interaction network. Mol Syst Biol. 6:326. http://doi.org/10.1038/msb.2010.97.

He BZ, Ludwig MZ, Dickerson DA, Barse L, Arun B, et al. 2014. Effect of genetic variation in a Drosophila model of diabetes-associated serine proteases in Drosophila. Cell Rep. 27:1050–1061.e3. http://doi.org/10.1016/j.celrep.2019.03.101.

Duron O, Noel V, McCoy KD, Bonazzi M, Sidi-Boumedine K, et al. 2015. The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, Coxieilla burnetii. PLoS Pathog. 11:e1004892. http://doi.org/10.1371/journal.ppat.1004892.
misfolded human proinsulin. Genetics. 196:557–567. http://doi.org/10.1534/genetics.113.157800.

Hedges JF, Robison A, Kimmel E, Christensen K, Lucas E, et al. 2016. Type I interferon counters or promotes Coxiella burnetii replication dependent on tissue. Infect Immun. 84:1815–1825. http://doi.org/10.1128/IAI.01540-15.

Hein MY, Hubner NC, Poser I, Cox J, Nagaraj N, et al. 2015. A human interactome in three quantitative dimensions organized by stoichiometries and abundances. Cell. 163:712–723. http://doi.org/10.1016/j.cell.2015.09.053.

Hiroyasu A, DeWitt DC, Goodman AG. 2018. Extraction of hemocytes from Drosophila melanogaster larvae for microbial infection and analysis. J Vis Exp. 135:57077.http://doi.org/10.3791/57077.

Hoffmann JA. 2003. The immune response of Drosophila. Nature. 426:33–38. http://doi.org/10.1038/nature02021.

Howick VM, Lazzaro BP. 2017. The genetic architecture of defence as resistance to and tolerance of bacterial infection in Drosophila melanogaster. Mol Ecol. 26:1533–1546. http://doi.org/10.1111/mec.14017.

Hu Y, Flockhart I, Vinayagam A, Bergwitz C, Berger B, et al. 2011. An integrative approach to ortholog prediction for disease-focused and other functional studies. BMC Bioinformatics. 12:357. http://doi.org/10.1186/1471-2105-12-357.

Hua X, Li B, Song L, Hu C, Li X, et al. 2018. Stimulator of interferon genes (STING) provides insect antiviral immunity by promoting Dredd caspase–mediated NF-κB activation. Journal of Biological Chemistry. 293:11878–11890.

Huang W, Massouras A, Inoue Y, Peiffer J, Ramia M, et al. 2014. Natural variation in genome architecture among 205 Drosophila melanogaster genetic reference panel lines. Genome Res. 24:1193–1208.http://doi.org/10.1101/gr.171546.113.

Jeacock L, Faria J, Horn D. 2018. Codon usage bias controls mRNA and protein abundance in trypanosomatids. eLife. 7:e32496. http://doi.org/10.7554/eLife.32496.

Ka MB, Gondois-Rey F, Capo C, Testoris J, Million M, et al. 2014. Imbalance of circulating monocyte subsets and PD-1 dysregulation in Q fever endocarditis: the role of IL-10 in PD-1 modulation. PLoS One. 9:e107533.http://doi.org/10.1371/journal.pone.0107533.

Karakousis PC, Trucksis M, Dumler JS. 2006. Chronic Q fever in the environments in the United States. J Clin Microbiol. 44:2283–2287. http://doi.org/10.1128/jcm.02365-05.

Kelsey KJP, Clark AG. 2017. Variation in position effect variegation within a natural population. Genetics. 207:1157–1166. http://doi.org/10.1534/genetics.117.300306.

Kersh GJ, Fitzpatrick KA, Self JS, Priestley RA, Kelly AJ, et al. 2013. Presence and persistence of CoxIEL burnetii in the environments of goat farms associated with a Q fever outbreak. Appl Environ Microbiol. 79:1697–1703. http://doi.org/10.1128/aem.03472-12.

Komar AA. 2016. The Yin and Yang of codon usage. Hum Mol Genet. 25:R77–R85. http://doi.org/10.1038/hmg/ddw207.

Královčová J, Houngninou-Molango S, Krámer A, Veřejnová I. 2004. Branch site haplotypes that control alternative splicing. Hum Mol Genet. 13:3189–3202. http://doi.org/10.1093/hmg/ddh334.

Lavoy S, Chittoor-Vinod VG, Chow CY, Martin I. 2018. Genetic modifiers of neurodegeneration in a Drosophila model of Parkinson’s disease. Genetics. 209:1345–1356. http://doi.org/10.1534/genetics.118.301119.

Lemaître B, Hoffmann J. 2007. The host defense of Drosophila melanogaster. Annu Rev Immunol. 25:697–743. http://doi.org/10.1146/annurev.immunol.25.022106.141615.

Leone M, Honstetter M, Lepidi H, Capo C, Bayard F, et al. 2004. Effect of sex on Coxiella burnetii infection: protective role of 17β-estriol. J Infect Dis. 189:339–345. http://doi.org/10.1086/380798.

Li P, Ma Z, Yu Y, Hu X, Zhou Y, et al. 2019. FER promotes cell migration via regulating JNK activity. Cell Prolif. 52:http://doi.org/10.1111/cpr.12656.

Mackay TF, Richards S, Stone EA, Barbadilla A, Ayroles JF, et al. 2012. The Drosophila melanogaster genetic reference panel. Nature. 482:173–178. http://doi.org/10.1038/nature10811.

Mackay TFC, Huang W. 2018. Charting the genotype-phenotype map: lessons from the Drosophila melanogaster genetic reference panel: charting the genotype-phenotype map. WIREs Dev Biol. 7:e289.http://doi.org/10.1002/wdev.289.

Madariaga MG, Rezai K, Trenholme GM, Weinstein RA. 2003. Q fever: a biological weapon in your backyard. Lancet Infect Dis. 3:709–721.

Magwire MM, Fabian DK, Schweyen H, Cao C, Longdon B, et al. 2012. Genome-wide association studies reveal a simple genetic basis of resistance to naturally coevolving viruses in Drosophila melanogaster. PLoS Genet. 8:e1003057.http://doi.org/10.1371/journal.pgen.1003057.

Marrie TJ, Stein A, Janigan D, Raoul D. 1996. Route of infection determines the clinical manifestations of acute Q fever. J Infect Dis. 173:484–487.

Martin M, Hiroyasu A, Guzman RM, Roberts SA, Goodman AG. 2018. Analysis of Drosophila STING reveals an evolutionarily conserved antimicrobial function. Cell Rep. 23:3537–3550.e6. http://doi.org/10.1016/j.celrep.2018.05.029.

Martins NE, Faria VG, Teixeira I, Magalhães S, Sucena E. 2013. Host adaptation is contingent upon the infection route taken by pathogens. PLoS Pathog. 9:e1003601.http://doi.org/10.1371/journal.ppat.1003601.

Maurin M, Raoul D. 1999. Q fever. Clin Microbiol Rev. 12:518–553.

McCracken AW, Buckle E, Simons MJ. 2020. The relationship between longevity and diet is genotype dependent and sensitive to desiccation in Drosophila melanogaster. J Exp Biol. 223:je230185. doi:10.1242/jeb.230185.

McQuiston JH, Childs JE, Thompson HA. 2002. Q fever. J Am Vet Med Assoc. 221:796–799.

Megharia S, Bechah Y, Capo C, Lepidi H, Raoul D, et al. 2008. Persistent Coxiella burnetii infection in mice overexpressing IL-10: an efficient model for chronic Q fever pathogenesis. PLoS Pathog. 4:e23.http://doi.org/10.1371/journal.ppat.0040023.

Mehraj V, Testoris J, Ben Amara A, Ghigo E, Raoul D, et al. 2013. Monocyte responses in the context of Q fever: from a static polarized model to a kinetic model of activation. J Infect Dis. 208:942–951. http://doi.org/10.1093/infdis/jit266.

Miles LA, Baik N, Lighvani S, Khaldoyanidi S, Varki NM, et al. 2017. Deficiency of plasminogen receptor, Plg-R KT, causes defects in plasminogen binding and inflammatory macrophage recruitment in vivo. J Thromb Haemost. 15:155–162.http://doi.org/10.1111/jth.13532.

Millar JA, Valdés R, Kacharia FR, Landfear SM, Cambronne ED, et al. 2015. CoxIEL burnetii and Leishmania mexicana residing within similar parasitophorous vacuoles elicit disparate host responses. Front Microbiol. 6.http://doi.org/10.3389/fmicb.2015.00794.

Murray MJ. 2006. The Fes/Fer non-receptor tyrosine kinase cooperates with Src42A to regulate dorsal closure in Drosophila Development. 133:3063–3073.http://doi.org/10.1242/dev.02467.

Nazario-Toole AE. 2016. Genome wide association studies of phagocytosis and the cellular immune response in Drosophila melanogaster. Digital Repository at the University of Maryland.http://doi.org/10.13016/M26V1H.
Omsland A, Cockrell DC, Howe D, Fischer ER, Virtseneva K, et al. 2009. Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. *Proc Natl Acad Sci U S A.* 106:4430–4434. http://doi.org/10.1073/pnas.0812074106.

Palu RAS, Chow CY. 2018. Boldsplot/ELOV6 is a conserved modifier of disease and the ER stress response. *PLoS Genet.* 14:e1007557. http://doi.org/10.1371/journal.pgen.1007557.

Palu RAS, Dalton HM, Chow CY. 2020. Decoupling of apoptosis from activation of the ER stress response by the Drosophila Metallophilase superdeather. *Genetics.* 214:913–925. http://doi.org/10.1534/genetics.119.303004.

Palu RAS, Ong E, Stevens K, Chung S, Owings KG, et al. 2019. Natural genetic variation screen in Drosophila identifies wnt signaling, mitochondrial metabolism, and redox homeostasis genes as modifiers of Apoptosis. *G3 (Bethesda).* 9:4995–4005. http://doi.org/10.1534/g3.119.400722.

Pennings JLA, Kremers MNT, Hodemaekers HM, Hagenaaars JCP, Koning HJ, et al. 2015. Disregulation of serum gamma interferon levels in vascular chronic *Q* Fever patients provides insights into disease pathogenesis. *Clin Vaccine Immunol.* 22:664–671. http://doi.org/10.1128/CVI.0078-15.

Pinheiro W, Nye J, Márquez EJ, Kowalski A, Dworkin I, et al. 2019. A multivariate genome-wide association study of wing shape in *Drosophila melanogaster*. *Genetics.* 211:1429–1447. http://doi.org/10.1534/genetics.118.301342.

Raoult D, Marrie T, Mege J. 2005. Natural history and pathophysiology of Q fever. *Lancet Infect Dis.* 5:219–226. http://doi.org/10.1016/s0950-2688(10)00252-9.

Regan JC, Brandão AS, Leitão AB, Mantas Dias ÁR, Sucena É, et al. 2013. Steroid hormone signaling is essential to regulate innate immune cells and fungal bacterial infection in *Drosophila*. *PLoS Pathog.* 9:e1003720. http://doi.org/10.1371/journal.ppat.1003720.

Roest HI, Bossers A, van Zijderveld FG, Rebel JM. 2013. Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis and control of the zoonotic disease Q fever. *Vet Q.* 33:148–160. http://doi.org/10.1534/g3.113.000722.

Roett HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, et al. 2011a. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. *Emerg Infect Dis.* 17:668–675. http://doi.org/10.3201/eid1704.101562.

Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, et al. 2011b. The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiol Infect.* 139:1–12. http://doi.org/10.1016/j.elder.1313.

Ross W, van Gelderen B, Dinkla A, Frangoulidou D, van Zijderveld F, et al. 2012. Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. *PLoS One.* 7:e48949. http://doi.org/10.1371/journal.pone.0048949.

Salinas RP, Ortiz Flores RM, Distel JS, Aguilerama CO, Colombo MJ, et al. 2015. *Coxiella burnetii* phagocytosis is regulated by GTPases of the Rho family and the RhoA effectors mDia1 and ROCK. *PLoS One.* 10:e0145211–e0145211. http://doi.org/10.1371/journal.pone.0145211.

Sauna ZE, Kimchi-Sarfaty C. 2011. Understanding the contribution of synonymous mutations to human disease. *Nat Rev Genet.* 12:683–691. http://doi.org/10.1038/nrg3051.

Savitz J, Frank MB, Victor T, Behak M, Marino JH, et al. 2013. Inflammation and neurological disease-related genes are differentially expressed in depressed patients with mood disorders and correlate with morphometric and functional imaging abnormalities. *Brain Behav Immun.* 31:161–171. http://doi.org/10.1016/j.bbi.2012.10.007.

Schimmer B, Morooy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, et al. 2008. Large Ongoing Q Fever Outbreak in the South of The Netherlands, 2008. Euro surveillance: bulletin European sur les maladies transmissibles = European communicable disease bulletin 13.

Schmidt JM, Battaly P, Gledhill-Smith RS, Good RT, Lumb C, et al. 2017. Insights into DDT resistance from the Drosophila melanogaster genetic reference panel. *Genetics.* 207:1178–1193. http://doi.org/10.1534/genetics.117.300310.

Schoffelen T, Ammeredorffer A, Hagenaaars JC, Bleeker-Rovers CP, Wegdam-Blans MC, et al. 2015. Genetic variation in pattern recognition receptors and adaptor proteins associated with development of chronic Q fever. *J Infect Dis.* 212:818–829. http://doi.org/10.1093/infdis/jiv113.

Sheehan G, Garvey A, Croke M, Kavanagh K. 2018. Intrahepatic humoral immune defences in mammals and insects: The same, with differences? *Virulence.* 9:1625–1639. http://doi.org/10.1080/21505594.2018.1526531.

Singh RK, Cooper TA. 2012. Pre-mRNA splicing in disease and therapeutics. *Trends Mol Med.* 18:472–482. http://doi.org/10.1016/j.molmed.2012.06.006.

Sondgeroth KS, Davis MA, Schlee SL, Allen AJ, Ewermann JF, et al. 2013. Seroprevalence of *Coxiella burnetii* in Washington State domestic goat herds. *Vector Borne Zoonotic Dis.* 13:779–783. http://doi.org/10.1089/vbz.2013.1331.

Strange BE, Stahl EA, Raj T. 2011. Progress and promise of genome-wide association studies for human complex trait genetics. *Genetics.* 187:367–383. http://doi.org/10.1534/genetics.110.120907.

Tafesh-Edwards G, Eleftherianos I. 2020. Drosophila immunity against natural and nonnatural viral pathogens. *Virology.* 540:165–171. http://doi.org/10.1016/j.viro1.2019.12.001.

Talness DM, Owings KG, Coelho E, Mercenne G, Pleins JM, et al. 2020. A Drosophila screen identifies NKKC1 as a modifier of NGLY1 deficiency. *eLife.* 9:e57831. http://doi.org/10.7554/eLife.57831.

Tewtris J, Ban LH, Capo C, Raout D, Leone M, et al. 2010. Sex-related differences in gene expression following *Coxiella burnetii* infection in mice: potential role of circadian rhythm. *PLoS One.* 5:e12190. http://doi.org/10.1371/journal.pone.0012190.

Vigo JP, Sugimoto MA, Lima KM, Negreiros-Lima GL, Baik N, et al. 2019. Plasminogen and the plasminogen receptor, Plg-RKT, regulates macrophage phagocytic and functional changes. *Front Immunol.* 10:1458. http://doi.org/10.3389/fimmu.2019.01458.

van Asseldonk MA, Prins J, Bargevoet RH. 2013. Economic assessment of Q fever in the Netherlands. *Prev Vet Med.* 112:27–34. http://doi.org/10.1016/j.prevetmed.2013.06.002.

Wang JB, Lu H-L, St Leger RJ. 2017. The genetic basis for variation in resistance to infection in the *Drosophila melanogaster genetic reference panel*. *PLoS Pathog.* 13:e1006260. http://doi.org/10.1371/journal.ppat.1006260.

Wang Z, Burge CB. 2008. Splicing regulation: From a parts list of regulatory elements to an integrated splicing code. *RNA.* 14:802–813. http://doi.org/10.1261/rna.876308.

Weber MM, Faris R, van Schaik EJ, McLachlan JT, Wright Wu, et al. 2016. The type IV secretion system effector protein CirA Stimulates the GTPase activity of RhoA and is required for virulence in a mouse model of *Coxiella burnetii* infection. *Infect Immun.* 84:2524–2533. http://doi.org/10.1128/IAI.01554-15.

Wielders CC, Hackert VH, Schimmer B, Hodemaekers HM, de Klerk A, et al. 2015. Single nucleotide polymorphisms in immune
response genes in acute Q fever cases with differences in self-reported symptoms. Eur J Clin Microbiol Infect Dis. 34: 943–950. http://doi.org/10.1007/s10096-014-2310-9.

Will CL, Luhrmann R. 2011. Spliceosome structure and function. Cold Spring Harbor Perspect Biol. 3:a003707. http://doi.org/10.1101/cshperspect.a003707.

Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, et al. 2008. Autophagic control of listeria through intracellular innate immune recognition in Drosophila. Nat Immunol. 9:908–916. http://doi.org/10.1038/ni.1634.

Yoo S, Nair S, Kim H, Kim Y, Lee C, et al. 2020. Knock-in mutations of scarecrow, a Drosophila homolog of mammalian Nkx2.1, reveal a novel function required for development of the optic lobe in Drosophila melanogaster. Dev Biol. 461:145–159. http://doi.org/10.1016/j.ydbio.2020.02.008.

Zhang Y-B, Hu J, Zhang J, Zhou X, Li X, et al. 2016. Genome-wide association study identifies multiple susceptibility loci for craniofacial microsomia. Nat Commun. 7:10605. http://doi.org/10.1038/ncomms10605.

Zhou Z, Dang Y, Zhou M, Li L, Yu C, et al. 2016. Codon usage is an important determinant of gene expression levels largely through its effects on transcription. Proc Natl Acad Sci U S A. 113: E6117–E6125. http://doi.org/10.1073/pnas.1606724113.

Communicating editor: J. Gleason