Dissecting genetic and sex-specific host heterogeneity in pathogen transmission

potential

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Abstract

Heterogeneity in disease transmission is widespread and, when not accounted for, can produce unpredictable outbreaks of infectious disease. Despite this, precisely how different sources of variation in host traits drive heterogeneity in disease transmission is poorly understood. Here we dissected the sources of variation in pathogen transmission using *Drosophila melanogaster* and *Drosophila C Virus* as a host-pathogen model system. We found that infected lifespan, viral growth, virus shedding, and viral load at death were all significantly influenced by fly genetic background, sex and female mating status. To understand how variation in each of these traits may generate heterogeneity in disease transmission, we estimated individual transmission potential by integrating data on virus shedding and lifespan alongside previously collected data on social aggregation. We found that ~15% of between-individual heterogeneity in disease transmission was explained by a significant interaction between genetic and sex-specific variation. We also characterised the amount of variation in viral load, virus shedding, and lifespan following infection that could be explained by genetic background and sex. Amongst the determinants of individual variation in disease transmission these sources of host variation play roles of varying importance, with genetic background generally playing the largest role. Our results highlight the importance of characterising sources of variation in multiple host traits when studying disease transmission at the individual-level.

Keywords: Superspreaders; viral transmission; *Drosophila C Virus*; *Drosophila melanogaster*; individual variation; heterogeneity; V_i ; R_0 ; genetic variation; sexual dimorphism

Introduction

Individual host heterogeneity in disease spread is commonly observed across a wide range of infectious diseases (Woolhouse et al., 1997; Lloyd-Smith et al., 2005; Paull et al., 2011). Such heterogeneity is so common that it has been generalised into the ‘20-80 rule’ because of the frequent observation that 20% of hosts contribute to roughly 80% of transmission (Shaw & Dobson, 1995; Wilson et al., 2002; Woolhouse et al., 1997). More extreme forms of heterogeneity can result in very rare ‘superspreading’ individuals capable of causing large outbreaks of infectious disease in human and animal populations (Brooks-Pollock, Roberts, & Keeling, 2014; Lloyd-Smith et al., 2005). A superspreader of particular infamy is Mary Mallon who became known as ‘Typhoid Mary’ by infecting over 50 people with *Salmonella typhi* while working as a cook in New York during the early 20th century (Marineli, Tsoucalas, Karamanou, & Androutsos, 2013). More recently, the 2003 outbreaks of SARS in Singapore and Hong Kong were greatly accelerated by a few superspreading individuals who caused over 70% of all SARS transmission (Li et al., 2004).

Outbreaks of infectious disease are often difficult to predict, especially when the effect of superspreaders are not accounted for by traditional assessments of outbreak risk. A widely used metric for the rate of pathogen spread is the basic reproductive number, R_0 , which estimates the average number of expected secondary infections caused by a single infected individual in a completely susceptible population. By focussing on the population average, R_0 conceals outliers with a potentially higher propensity to spread disease (Lloyd-Smith et al., 2005; Paull et al., 2012; VanderWaal & Ezenwa, 2016). A clearer understanding of what drives heterogeneity in disease transmission requires a framework capable of accounting for such between-individual variation, which could enable more efficient control strategies that specifically target and treat high-risk individuals (Lloyd-Smith et al., 2005). The importance of predicting high-risk individuals before outbreaks occur has pushed understanding the causes of heterogeneity in disease transmission to the

forefront of epidemiology and disease ecology research (Gervasi, Civitello, Kilvitis, & Martin, 2015; Paull et al., 2012; Stein, 2011; VanderWaal & Ezenwa, 2016).

Despite being commonplace, the underlying causes of heterogeneity in pathogen transmission remain elusive. Individual variation in host contact networks may be an important factor: it was Typhoid Mary's position as a cook which exposed her to so many susceptible individuals. However, what enabled Typhoid Mary to stay in this role was her status as an asymptomatic carrier of the infection, which led to her release from quarantine on several occasions (Marineli et al., 2013). Similarly, the absence of symptoms in a number of SARS superspreaders delayed their admission to hospital and allowed them to continue spreading the virus (Centers for Disease Control and Prevention (CDC), 2003). These examples help underline that achieving a detailed understanding of the sources of heterogeneity in pathogen transmission is challenging because it results from complex interactions between multiple host behavioural, physiological, and immune traits. By dissecting the underlying genetic and sex-specific sources of variation in these traits we can assess how they influence three key components of pathogen transmission: contact rate between infected and susceptible individuals, the likelihood that contact will result in infection, and the duration of infection (VanderWaal & Ezenwa, 2016).

Infected-susceptible host contact rate is predominantly determined by host behaviours affecting locomotion and aggregation. Contact rates are also affected by population density (Keeling & Rohani, 2007), social group size (Patterson & Ruckstuhl, 2013), and behavioural syndromes (Keiser, Pinter-Wollman, et al., 2016). Social networks often exhibit extreme heterogeneity in the wild (Godfrey, 2013; Rushmore et al., 2013) and factors such as host genotype, sex condition, age and personality have been demonstrated to affect social aggregation in lab systems (de Bono & Bargmann, 1998; Keiser, Howell, et al., 2016; Saltz, 2011; Siva-Jothy & Vale, 2019). Once individuals acquire an infection, their ability to clear and shed pathogens

is chiefly determined by physiological and immune mechanisms. Variation in these mechanisms chiefly influence the likelihood of pathogen transmission and the duration of infection (Grassly & Fraser, 2008; VanderWaal & Ezenwa, 2016). Many genetic and environmental sources of variation in physiological immunity have been described (Bou Sleiman et al., 2015; Lazzaro Brian P & Little Tom J, 2009; Ponton et al., 2013) including coinfection (Budischak et al., 2015; Lass Sandra et al., 2013), nutrition (Cornet, Bichet, Larcombe, Faivre, & Sorci, 2014; Vale, Choisy, & Little, 2013), and stress (Beldomenico & Begon, 2010; Capitanio et al., 2008). It is relevant to note that most studies have addressed the effects of behavioural, physiological and immune traits on transmission in isolation of one another. However, there is increasing evidence that transmission heterogeneity is often explained by coupled heterogeneities in these traits and how they may covary (Bolzoni, Real, & Leo, 2007; Farrington, Whitaker, Unkel, & Pebody, 2013; White, Forester, & Craft, 2018). To fully understand the sources of heterogeneity in pathogen transmission, it is therefore essential to measure multiple behavioural, physiological, and immune traits in hosts.

In the present work we aimed to test how common sources of variation between individuals (genetic background, sex and mating status) contribute to individual heterogeneity in pathogen transmission potential. The fruit fly, *Drosophila melanogaster*, is a powerful and genetically tractable model of infection, immunity and behaviour (Apidianakis & Rahme, 2009; Sokolowski, 2001). This makes it an ideal model system to investigate heterogeneity in pathogen transmission in the highly controlled conditions of a laboratory. We infected males and females from a range of naturally derived genotypes with *Drosophila C Virus* (DCV), a horizontally transmitted fly pathogen that causes behavioural, physiological and metabolic pathologies (Arnold, Johnson, & White, 2013; Chtarbanova et al., 2014; Gupta, Stewart, Rund, Monteith, & Vale, 2017; Vale & Jardine, 2015). We then quantified host traits and infection outcomes that directly impact pathogen transmission: (1) the infected lifespan, (2) the internal viral load, (3) how much virus was shed, and (4) the viral load

121 at death (VLAD). Finally, we integrated these measurements alongside previously
 122 described data on variation in social aggregation (Siva-Jothy & Vale, 2019) into a
 123 composite metric of individual transmission potential, V (Lloyd-Smith et al., 2005;
 124 VanderWaal & Ezenwa, 2016). Estimations of individual transmission potential, V ,
 125 allowed us to assess how genetic and sex-specific variation affects between-
 126 individual heterogeneity in pathogen transmission.

Materials & Methods

Flies & Rearing Conditions

Flies used in experiments were 3-5 days old and came from ten lines of the *Drosophila* Genetic Resource Panel (DGRP). These genetic backgrounds are five of the most resistant and susceptible to systemic *Drosophila* C Virus infection (Magwire et al., 2012). Virgin females were isolated from males within 7 hours of eclosion. Mated females and males were produced by rearing one female with one male for 24 hours. Mating was confirmed using oviposition within the following 24 hours and these egg's subsequent development. Flies were reared in plastic vials on a standard diet of Lewis medium at $18 \pm 1^\circ\text{C}$ with a 12 hour light:dark cycle. Stocks were tipped into new vials approximately every 14 days. One month before the experiments, flies were maintained at low density (~ 10 flies per vial) for two generations at $25 \pm 1^\circ\text{C}$ with a 12 hour light:dark cycle.

Virus Culture & Infection

The *Drosophila* C Virus (DCV) isolate used in this experiment was originally isolated in Charolles, France and grown in Schneider *Drosophila* Line 2 (DL2) as previously described (Vale and Jardine, 2015b), diluted ten-fold (10^8 infectious units per ml) in TRIS-HCl solution (pH=7.3), aliquoted and frozen at -70°C until required. To infect with DCV, flies were pricked in the pleural suture with a 0.15mm diameter pin, bent at 90° $\sim 0.5\text{mm}$ from the tip, dipped in DCV.

Measuring Lifespan and Viral Load at Death

Lifespan and viral load at death were measured in the same fly. Following DCV infection, flies were isolated and reared in standard vials. Flies were then monitored every day until all individuals died, whereupon they were removed from vials, fixed in 50 μl of TRI-reagent and frozen at -70°C , to await DCV titre at death quantification. For twenty-seven of thirty treatment groups, the lifespan following infection and viral load

at death was measured for n=17-20, three treatment groups consisted of n=7-15 flies (Table S1).

Viral Growth and Shedding Measurement Setup

Due to destructive sampling, we measured the viral load and shedding of single flies at a single time point, either 1-, 2- or 3-days post-infection (DPI). Following DCV infection, single flies were placed into 1.5ml Eppendorf tubes with ~50µl of Lewis medium in the bottom of the tube. To measure viral shedding, flies were transferred to tubes for 24 hours, immediately following 1 or 2 days after systemic infection. After living in these tubes for a further 24 hours, flies were removed and homogenised in 50µl of TRI-reagent, tubes were also washed out with 50µl of TRI-reagent by vortexing. These samples were then frozen at -70°C, to await DCV quantification by qPCR. For each combination of sex and genetic background over the three days viral load and virus shedding was measured, n=7-15 flies were measured (Table S2-S4).

DCV RNA Extraction

RNA was extracted from viral load at death and viral shedding samples by Phenol-Chloroform extraction. Samples were thawed on ice for 30 minutes before being incubated at room temperature for 5 minutes to allow dissociation of nucleo-protein complex. Samples were then centrifuged at 12,000×g for 10 minutes at 4°C after which large debris was removed. For phase separation, samples were shaken vigorously for 15 seconds, 10µl of chloroform added, incubated at room temperature for a further 3 minutes before being centrifuged at 12,000×g for 15 minutes at 4°C. Following phase separation, the upper aqueous layer was removed from each sample and added to 25µl of isopropanol, tubes were then inverted twice, before being centrifuged at 12,000×g for 10 minutes at 4°C. Precipitated RNA was then washed by removing the supernatant, and re-dissolving the RNA pellet in 50µl of 75% ethanol before being centrifuged at 7,500×g for 5 minutes at 4°C. RNA suspension was achieved by removing 40µl of the ethanol supernatant, allowing the rest to dry

by evaporation and dissolving the remaining RNA pellet in 20µl of RNase-free water. We extracted RNA from flies after 1, 2 or 3 days of infection using a semi-automatic MagMAX Express Particle Processor using the MagMAX-96 total RNA isolation kit manufacturer's protocol (Life Technologies, 2011) with the elution step extended to 18 minutes. RNA samples were stored at -70°C to await reverse transcription.

Reverse transcription & qPCR Protocol

Extracted RNA was reverse-transcribed with M-MLV reverse transcriptase and random hexamer primers, before being diluted 1:1 with nuclease free water. cDNA samples were stored at -20°C to await qPCR analysis. DCV titre was quantified by qPCR using Fast SYBR Green Master Mix in an Applied Biosystems StepOnePlus system. Samples were exposed to a PCR cycle of 95°C for 2 minutes followed by 40 cycles of: 95°C for 10 seconds followed by 60°C for 30 seconds. Forward and reverse primers used included 5'-AT rich flaps to improve fluorescence (DCV_Forward: 5' AATAAATCATAAGCCACTGTGATTGATACAACAGAC 3'; DCV Reverse: 5' AATAAATCATAAGAAGCACGATACTTCTTCCAAACC 3'). Across all plates, two technical replicates were carried out per sample. DCV titre was calculated by absolute quantification, using a standard curve created from a 10-fold serial dilution (1-10⁻¹²) of DCV cDNA. Our detection threshold was calculated for each plate using the point at which two samples on our standard curve gave the same Ct value. The point of redundancy in a standard curve was taken to be equivalent to 0 viral particles. Due to our detection protocol measuring viral copies of RNA, we cannot comment on the viability of any detected virus. We transformed our measurements of viral RNA transformed in order for them to represent the amount of virus growing inside a whole fly rather than the amount in the qPCR well sample. To account for dilution between RNA extraction and qPCR we transformed DCV RNA samples by a factor of 3125, to represent the amount of DCV growing in, or shed by, flies. The mean qPCR efficiency was 116% with a standard error of ±2.9%

Calculating Between-Individual Variation in Transmission Potential, V

We used measures of virus shedding, lifespan following infection, and social aggregation to predict individual transmission potential. We integrated these measures based on a simple framework that describes transmission potential as a function of contact rate between susceptible and infected individuals, the likelihood that such contact will result in infection, and the duration of the infectious period (VanderWaal & Ezenwa, 2016). Using previously analysed data on social aggregation (Siva-Jothy & Vale, 2019), we used nearest neighbour distance as a measure of contact rate. Flies that aggregated more closely to conspecifics, have a higher contact rate, and are therefore more likely to spread DCV. We also assume that transmission likelihood increases with virus shedding. We therefore take the amount of virus shed by flies as a proximate measure of the likelihood that contact will result in infection. Using these traits, individual transmission potential, V , was calculated as:

$$V = \frac{(\text{Virus Shedding Titre}) \times (\text{Lifespan})}{(\text{Aggregation Distance})}$$

Aggregation distance, lifespan following infection and virus shedding were all measured in separate experiments. Therefore, to calculate V as a measure of individual transmission potential, we simulated theoretical individuals by bootstrapping trait values sampled from each of these three datasets. We simulated 60 individuals for each combination of sex and genetic background, assuming no specific covariance structure between traits, that is, all possible trait combinations were considered.

Statistical Analysis

Across all experiments, generalised linear models (GLMs) were used to analyse continuous response variables and logistic regressions were used to analyse

proportions. An effect of sex or mating was analysed in separate models comparing males or virgin females to the same dataset of mated females, respectively.

To analyse lifespan, two GLMs were constructed containing a three-way interaction genetic background, VLAD, and sex or mating (Table S6). The two GLMs for VLAD, contained either a two-way interaction between genetic background and sex or a two-way interaction between genetic background and mating (Table S6).

Due to zero-inflation, we used two models to sequentially analyse both viral load and virus shedding data. Viral load and virus shedding are broken down into qualitative (the proportion of non-zero values) and quantitative variation (differences between non-zero values). First, we conducted logistic regressions on all of the values in these datasets and analysed the proportion of values that were greater than zero. Logistic regressions analysing sex-differences in viral load included DPI (a 3-level factor: 1, 2 or 3 days) and an interaction between genetic background and sex (Table S6). For analysing the effect of mating in females on viral load, logistic regressions included DPI and an interaction between genetic background and mating (Table S6). Logistic regressions of virus shedding used a similar model that also included quantitative viral load as a predictor (Table S6). After these logistic regressions, zeroes were removed from all datasets to analyse the subset of positive-values. The GLMs used to analyse these subsets included the same predictors as their corresponding logistic regressions, for viral load: an interaction between genetic background and sex or mating, alongside DPI, with the inclusion of quantitative viral load for virus shedding (Table S6).

Due to zero-inflation V was also analysed with a logistic regression followed by a GLM. A logistic regression was used to analyse the proportion of V values that were greater than zero with a two-way interaction between sex and genetic background as predictors (Table S6). Zero-values of V were then removed from the dataset, and

269 a GLM was used to analyse differences in the size of V , with an interaction between
270 sex and genetic background included as a predictor (Table S6).

271

272 We calculated the amount of deviance and variance explained by predictors in
273 logistic regressions and GLMs, respectively, by dividing the total deviance or
274 variance explained by the model. Where appropriate, we corrected for multiple
275 testing using Bonferroni correction. All statistical analyses and graphics produced in
276 R 3.3.0 using the *ggplot2* (Wickham, 2016), *lme4* (Bates, Mächler, Bolker, & Walker,
277 2015) and *multcomp* (Hothorn, Bretz, & Westfall, 2008) packages.

Results

Lifespan Following Infection

Infected lifespan varied significantly between males and females and the extent of this variation differed between host genetic backgrounds (Figure 1a; Table 1). Genetic background explained the most variance of any predictor across models assessing mating (7%) and sex (10.9%). Interactions with sex and mating also explained a further 2.7% and 1.5%, respectively (Figure 5; Table 1). We found no evidence that mating affected the lifespan of females following DCV infection (Figure 1a; Table 1). Viral load at death (VLAD) was not affected by genetic background, sex or female mating status (Figure 1b; Table 2), and flies that died sooner following infection had greater VLAD than those that died later (Figure 1c; Table 1).

Figure 1. Mean $\pm$ SE (a) lifespan in days following infection and (b) the viral load at death in males (red), mated females (blue), and virgin females (pale blue) of ten genetic backgrounds. The x-axis shows the line number from the DGRP panel and is in ascending order according to male flies. (c) the relationship between lifespan following infection and the viral load of flies at death. Each point is an individual male (red), mated female (blue), or virgin female (pale blue) fly. The nature of this relationship within each genetic background is represented by a line of best fit with outlier backgrounds labelled.

Viral Load

A substantial number of flies did not have detectable loads of DCV. These zero-values reflect qualitative variation and are likely caused by viral titres below the detection threshold of our qPCR and therefore reflect individuals with very low DCV loads, or no virus at all. We found extensive genetic variation in qualitative DCV load (Figure 2a; Table 3) which was affected by sex (Figure 2a; Table 3) and female mating status (Figure 2a; Table 3). Relatively little deviance was explained by sex (0.002%), mating (0.13%), or genetic background in models testing sex (1.18%) and mating (2.83%) effects. The predictors that explained the most deviance were the interactions between genetic background and sex (5.58%) or mating (4.92%) (Figure 5; Table 3). The size of non-zero DCV loads reflects quantitative variation and was affected by similar interactions between mating and sex with genetic background (Figure 2b; Table 4). While <1% of variance was explained by sex and mating, much more was explained by genetic background (7.94% and 11%) alongside its interactions with sex (19.2%) and mating (4.38%) (Figure 5; Table 4).

The number of detectable DCV loads decreased following 1-day post-infection (pairwise comparison, $p < 0.0001$) and remained lower than day 1 at day 3 (pairwise comparison, $p = 0.0016$). There were significant changes in quantitative DCV load variation over the first three days of infection. Viral load peaked 2-days post-infection (pairwise comparison, $p = 0.0012$), before decreasing to the same level as 1-day post-infection at 3-days post-infection (pairwise comparison, $p = 0.068$).

Figure 2. Mean±SE (a) proportion of flies with non-zero loads of DCV and the (b) viral titre of flies with non-zero DCV loads, over the first 3 days of infection. Across both panels, numbers in each pane denote the genetic background from the DGRP, while the colour of bars, points and lines represent sex and mating status. Males are shown in red, mated females in blue, and virgin females in pale blue. Bars of the same colour in each in pane in panel (a) represent (from left to right) days 1, 2 and 3 of infection.

335

336 Virus Shedding

337 Similar to measures of viral load, we did not detect DCV in the shedding of a number
 338 of flies. Here, we interpret zeroes to be reflective of individuals that shed very low
 339 titres of DCV, or no virus at all. Qualitative variation in DCV shedding was significantly
 340 affected by genetic background, with sex modulating the extent of this difference
 341 (Figure 3a; Table 5). Sex however, explained <1% of the deviance, while genetic
 342 background and its interaction with sex explained 2.2% and 3.07% (Figure 5). Mating
 343 did not affect qualitative DCV shedding (Figure 3a; Table 5) and explained <1% of
 344 the deviance (Figure 5; Table 5). In flies where DCV was detected in shedding,
 345 quantitative DCV shedding was affected by genetic background and the extent of
 346 this variation was determined by female mating status, but not sex (Figure 3b; Table
 347 6). The amount of variance explained by sex and in our models was <1%, in
 348 comparison with genetic background (9.48% and 5.82%) and its interactions with sex
 349 (8.87%) or mating (6.53%) (Figure 5; Table 6). Qualitative and quantitative DCV
 350 shedding peaked at day 2 (Figures 3a; Tables 5 & 6, pairwise comparisons,
 351 $p < 0.0001$). Across all treatment groups, there was no significant relationship between
 352 viral load and shedding (Figure S1; Table 6).

353

Figure 3. Mean±SE (a) proportion of flies shedding non-zero titres of DCV and the (b) titre of the non-zero virus shedding subset over the first 3 days of infection. Panels denote genetic background, while the colour of bars, points and lines represent sex and mating status. Males are shown in red, mated females in blue, and virgin females in pale blue. Bars of the same colour in each in pane in panel (a) represent (from left to right) days 1, 2 and 3 of infection.

Variation in Transmission Potential, V

We incorporated the lifespan and virus shedding data described above alongside previously gathered data on genetic and sex-specific variation in social aggregation

365 to calculate individual disease transmission potential, V (Lloyd-Smith et al., 2005;
366 VanderWaal & Ezenwa, 2016). As a result of many flies not shedding DCV (Figure
367 3a), the distribution of transmission potential, V , was zero-inflated (Figure 4a). Zero
368 values of V represent individuals with no transmission risk (Figure 4a), as flies that
369 shed no virus had no transmission potential, irrespective of their aggregation and
370 lifespan. The distribution of V was also characterised by a right-extreme tail,
371 comprised of individuals with high-risk transmission potentials relative to the
372 population average (Figure 4a). Qualitative variation in V (the proportion of flies where
373 $V > 0$) differed between males and females with the extent of this difference also
374 determined by genetic background (Figure 4b; Table 7). Sex (0.28%), genetic
375 background (2.3%) and the interaction between the two (2.83%) explained relatively
376 little deviance in our models (Figure 5; Table 7). In quantitative variation in V , sex
377 explained <1%, while genetic background and its interaction with sex explained
378 4.13% and 11.4% of variance respectively (Figure 5; Table 8).

Figure 4. Bootstrap simulation results of transmission potential (V) ($n=60$): (a) the population-level distribution of V relative to the mean of the population. The red dashed line demarcates the average transmission potential of the population (similar to R_0), a traditional metric used to describe a population's outbreak risk. The mean $\pm$ SE of (b) the proportion of flies with a non-zero transmission potential and (c) the transmission potential of flies with a non-zero transmission potential. In figure panels (b) and (c) sex is denoted by colour with males in red and females in blue. The x-axis of panels (b) and (c) is in ascending order of the male genetic backgrounds.

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390

391 **Figure 5.** Summary of the percentage of variance or deviance explained by a subset
 392 of predictors in analyses of disease transmission potential and outcomes of infection.

Discussion

We identified genetic and sex-specific variation in three key outcomes of DCV infection: lifespan following infection, virus shedding, and virus load. When combined with social aggregation data, this variation resulted in genetic and sex-specific variation in individual transmission potential, V . While all of these outcomes of infection influence transmission potential, due to many individuals not shedding any virus, virus shedding exerted more influence over V than variation in lifespan following infection and social aggregation. Due to this central role, below we discuss potential explanations for the effect of mating, as well as genetic and sex-specific variation on virus shedding, and link these to genetic and sex-specific variation in V .

The effect of host genetic background in generating heterogeneity in transmission

The genetic variation in virus shedding affected both qualitative and quantitative variation in DCV shedding. As the distributions of neither social aggregation nor lifespan following infection were zero-inflated, variation in virus shedding appears to be a key determinant of qualitative and quantitative variation in V . Differences between genetic backgrounds in qualitative shedding was a key determinant of variation in V , as there is no risk of pathogen transmission in the absence of shedding. Among individuals that shed DCV, between-individual heterogeneity in V was achieved through different routes. Some genetic backgrounds, such as males from RAL-818, showed a high proportion of individuals that are likely to spread DCV (Figure 4b), but only to relatively few individuals (Figure 4c). Conversely, other groups, such as females of the RAL-380 genetic backgrounds, showed one of the lowest proportions of individuals able to achieve transmission (Figure 4b), but the individuals that did achieve transmission include outliers with values of V that were orders of magnitude higher than the population average (Figure 4c).

Quantitative and qualitative variation in DCV shedding differed in how they were affected by host genetic background. Qualitative variation was affected by genetic

background as part of an interaction with host sex, while this interaction has no significant effect on quantitative DCV shedding (Tables 5 & 6). Similar differences are seen in the amount of deviance and variance genetic background explains in models of qualitative and quantitative variation in DCV shedding. Genetic background accounts for only 2.27% of deviance in qualitative DCV shedding whereas it accounts for 9.48% of the variance in quantitative DCV shedding (Figure 5). Genetic variation therefore appears to play an important role in determining shedding and affects qualitative and quantitative shedding in different ways. Similar effects of genetic backgrounds on parasite shedding have been reported in the Ramshorn snail species, *Biomphalaria glabrata*, during infection with *Schistosoma mansoni*. Genetic backgrounds differ in how many parasite eggs they shed and how quickly they start shedding after infection (Tavalire et al., 2016). The differences we see in the proportion of flies shedding DCV may be caused by a similar pattern of variation in individual's delaying virus shedding. Delaying the onset of shedding could be affected by a range of DCV infection symptoms. These include paralysis of muscles in the crop organ of the foregut, abdominal swelling, broad nutritional stress and reduced defecation rate (Chtarbanova et al., 2014).

Genetic background also appears to play a key role in transmission potential, we detected a significant effect on both qualitative and quantitative variation in *V*. The amount of deviance and variance explained by genetic background does not hugely differ (2.3% and 4.13%, respectively). However, when part of an interaction with sex, genetic background accounts for 11.4% of the variance in quantitative variation in DCV shedding, whereas this same interaction only accounts for 2.83% of the deviance in qualitative variation in shedding (Figure 5). Alongside other studies, this highlights the potential significance of genetic variation in pathogen shedding to generating transmission heterogeneity. For example, genetic variation in transmission was demonstrated using families of turbot fish (*Scophthalmus maximus*) which produced outbreaks that differed in how quickly individuals showed symptoms of

infection and died (Anacleto et al., 2019). Shedding may underlie this genetic variation in transmission as it was not directly measured and there were no significant differences in infection duration and contact rate (Anacleto et al., 2019). Common garden experiments have revealed shedding dynamics capable of influencing the population-level transmission dynamics of wild populations of the plant, *Plantago lanceolata*. In controlled laboratory settings, multi-strain coinfection was shown to increase the number of spores released of the fungal pathogen, *Podosphaera plantaginis*. Measures of natural populations have also demonstrated outbreak severity increases at higher levels of coinfection (Susi, Barrès, Vale, & Laine, 2015). The relationship between spore shedding and coinfection has also been shown to be affected by host genotype, with genotypes significantly differing in the number of spores released over a number of days post-infection (Susi, Vale, & Laine, 2015). Genetic variation in transmission potential has also been demonstrated in the freshwater ciliate, *Paramecium caudatum*, following *Holospira undulata* infection (Fellous et al., 2012). The genotype of the first individual to be infected was a key determinant of pathogen transmission as host genotype appears to affect the infectious potential of shed pathogens (Fellous et al., 2012). *H. undulata* infectiousness increases with host population density, as reduced variation in contact rate makes infectiousness the primary determinant of transmission (Magalon et al., 2010).

The effect of host sex in generating heterogeneity in transmission

We also observed clear qualitative and quantitative differences in V between males and females, which is suggestive of sex-specific variation in disease transmission. While the extent of any difference between males and females is also determined by genetic background, a greater proportion of males tend to transmit DCV than females across these backgrounds. In DCV shedding, a greater proportion of males from several genetic backgrounds (RAL-379, RAL-738 and RAL-818) shed DCV than females (Figure 3a).

Interestingly, we see significant sex-specific differences in qualitative, but not quantitative, variation in DCV shedding. Other work has also shown a number of sex differences in pathogen and parasite shedding (Sanchez, Devevey, & Bize, 2011; Sheridan, Poulin, Ward, & Zuk, 2000; Thompson, Gipson, & Hall, 2017). Often these biases link to differences in the selection pressures applied by sexual reproduction (Duneau & Ebert, 2012). Comparisons of mated and virgin female flies revealed mating effects which produced quantitative, but not qualitative, differences in shedding. While we did not measure V in virgin females, this mating effect may offer explanations for the sex differences seen in shedding and therefore V .

Sex-specific variation in qualitative differences in shedding exerts a significant influence over shedding (Figure 3a). It is important to note however, that in isolation, sex accounts for a miniscule 0.64% of the deviance in qualitative variation in shedding. Sex appears to play a more important role in conjunction with genetic background, the interaction between the two explaining 3.07% of deviance (Figure 5). While significant, sex-specific variation may play a relatively minor role in shedding. A variety of factors appear to underlie sex-differences in shedding across host-pathogen systems. For example, male-biased infection is common to many mammal hosts but generally absent from arthropod hosts (Sheridan et al., 2000). In the water flea, *Daphnia magna*, parasite spores are released into the environment upon death and females have been shown to release significantly more than males (Thompson et al., 2017). In the vole, *Microtus gryalis*, the faeces of females contains significantly more parasite eggs than that of males (Sanchez et al., 2011). Given that we see female-biased mortality to DCV infection (Figure 1a), it is perhaps surprising that shedding is not also female-biased. This could be due to

shedding being measured during the first three days of infection, whereas mortality occurred much later. We might therefore see sex-differences in shedding during the later stages of infection.

Both the qualitative and quantitative differences in V between males and females were determined alongside genetic background. While sex explained very little deviance and variance in qualitative and quantitative variation in V (Figure 6), its interaction with genetic background explained 2.83% and 11.4 %, respectively. Sex could therefore be an important source of variation in individual disease transmission. Sex differences in transmission or virus shedding, lifespan and social aggregation are commonly observed in a wide range of species (Duneau & Ebert, 2012; Ferrari, Cattadori, Nespereira, Rizzoli, & Hudson, 2004; Kaltz & Shykoff, 2001; Sanchez et al., 2011). Sex-specific variation has been relatively well-studied because sexes are easily distinguished in the wild, and examples of sexual dimorphism in physiological and behavioural traits are relatively common (Duneau and Ebert, 2012). Many mammalian hosts exhibit male-biased transmission (Ezenwa et al., 2016; Grear, Luong, & Hudson, 2012; Luong, Grear, & Hudson, 2009; Rhines, 2013), although there are exceptions of female-bias (Sanchez et al., 2011). In the white-footed mouse, *Peromyscus leucopus*, male-biased transmission is thought to be driven by sex differences in contact network connectivity, which has been linked to testosterone production (Foo, Nakagawa, Rhodes, & Simmons, 2017; Grear et al., 2012). Testosterone may be particularly relevant to transmission as its immunosuppressive (Foo et al., 2017) effects may also alter infectiousness and infection duration.

Female Mating Status in Shedding

Mated and virgin females did not qualitatively differ in DCV shedding; however, individuals did exhibit quantitative variation in shedding. While only 0.098% of the variance in quantitative shedding was explained by mating, the interaction between mating and genetic background explained 8.87% of the variance (Figure 5). This suggests that alongside host genetic background, mating might exert an important level of influence over shedding. One potential explanation for this mating effect are post-mating physiological changes in the intestine that can increase in defecation rates (Apger-McGlaughon and Wolfner, 2013). However, if this change is responsible for the significant effect of female mating, the virgin females from particular genetic backgrounds that shed more than mated females (Figure 3b) may be tolerant to these physiological changes. Relatively few have considered how mating affects aspects of disease transmission outside of contact rates (Altizer et al., 2003; Thrall et al., 2000). Particularly alongside other work in *Drosophila* that has demonstrated female-specific costs of infection (Kubiak and Tinsley, 2017; Short et al., 2012), this result highlights the importance of mating-induced physiological changes to transmission heterogeneity.

The difference between qualitative and quantitative variation in shedding relates to assumptions we make regarding how often DCV is shed. If DCV is always present in shedding, measures of zero reflect quantities of virus that are below the detection threshold of qPCR. While this could result in infectious individuals evading detection, as oral infection typically requires very high dosage (Gupta et al., 2017; Palmer et al., 2018), low-titre zero-values pose a smaller transmission risk. If DCV is not always shed, within-individual variation in when shedding occurs could be central to transmission

heterogeneity (Chen, Sanderson, & Lanzas, 2013). This is particularly relevant to our study as virus shedding was only measured at a single time point per fly, and we do not know how shedding, and therefore V , may change over time. Within-host, temporal variation in shedding is observed in a range of host-pathogen systems (Chen et al., 2013; Matthews et al., 2006; Mideo, Alizon, & Day, 2008). For example, avian hosts tend to shed more parasites during the late afternoon (Brawner III and Hill, 1999; Martinaud et al., 2009).

By combining measures of virus shedding, lifespan and social aggregation into a simple framework our work demonstrates that genetic and sex-specific variation can affect individual heterogeneity in disease transmission potential. We also show that genetic and sex-specific variation, as well as mating, can produce variation outcomes of infection. Alongside its interaction with sex, genetic background explains 5.41% of qualitative, and 15.54% of quantitative, individual variation in transmission potential. While our results do not implicate a particular genetic background, males generally present a greater transmission risk than females. In addition to highlighting high-risk individuals, our results are congruous with the observation that the majority of infected individuals produce very few, if any, secondary cases of infection. Non-infectious individuals are particularly relevant to predicting outbreaks of infectious disease as they obscure high-risk individuals in traditional, population-wide estimations of outbreak risk. Our findings demonstrate the benefit of using a model laboratory system as well established as *D. melanogaster* to study disease transmission. The number of available protocols and methodologies are central to considering multiple traits central to disease transmission and holistically studying their underlying determinants.

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584

References

- Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J. L., Antonovics, J., Cunningham, A. A., ... Pulliam, J. R. C. (2003). Social organization and parasite risk in mammals: Integrating theory and empirical studies. *Annual Review of Ecology Evolution and Systematics*, 34, 517–547. doi: 10.1146/annurev.ecolsys.34.030102.151725
- Anacleto, O., Cabaleiro, S., Villanueva, B., Saura, M., Houston, R. D., Woolliams, J. A., & Doeschl-Wilson, A. B. (2019). Genetic differences in host infectivity affect disease spread and survival in epidemics. *Scientific Reports*, 9(1), 4924. doi: 10.1038/s41598-019-40567-w
- Apger-McGlaughon, J., & Wolfner, M. F. (2013). Post-mating change in excretion by mated *Drosophila melanogaster* females is a long-term response that depends on sex peptide and sperm. *Journal of Insect Physiology*, 59(10), 1024–1030. doi: 10.1016/j.jinsphys.2013.07.001
- Apidianakis, Y., & Rahme, L. G. (2009). *Drosophila melanogaster* as a model host for studying *Pseudomonas aeruginosa* infection. *Nature Protocols*, 4(9), 1285–1294. doi: 10.1038/nprot.2009.124
- Arnold, P. A., Johnson, K. N., & White, C. R. (2013). Physiological and metabolic consequences of viral infection in *Drosophila melanogaster*. *The Journal of Experimental Biology*, 216(17), 3350–3357. doi: 10.1242/jeb.088138
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, 67(1). doi: 10.18637/jss.v067.i01
- Beldomenico, P. M., & Begon, M. (2010). Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology & Evolution*, 25(1), 21–27. doi: 10.1016/j.tree.2009.06.015

609 Bolzoni, L., Real, L., & Leo, G. D. (2007). Transmission Heterogeneity and Control
610 Strategies for Infectious Disease Emergence. *PLOS ONE*, 2(8), e747. doi:
611 10.1371/journal.pone.0000747

612 Bou Sleiman, M. S., Osman, D., Massouras, A., Hoffmann, A. A., Lemaitre, B., &
613 Deplancke, B. (2015). Genetic, molecular and physiological basis of variation in
614 *Drosophila* gut immunocompetence. *Nature Communications*, 6. doi:
615 10.1038/ncomms8829

616 Brawner III, W. R., & Hill, G. E. (1999). Temporal variation in shedding of coccidial
617 oocysts: implications for sexual-selection studies. *Canadian Journal of Zoology*,
618 77(2), 347–350. doi: 10.1139/z98-207

619 Brooks-Pollock, E., Roberts, G. O., & Keeling, M. J. (2014). A dynamic model of bovine
620 tuberculosis spread and control in Great Britain. *Nature*, 511(7508), 228–231. doi:
621 10.1038/nature13529

622 Budischak, S. A., Sakamoto, K., Megow, L. C., Cummings, K. R., Urban, J. F., & Ezenwa,
623 V. O. (2015). Resource limitation alters the consequences of co-infection for both
624 hosts and parasites. *International Journal for Parasitology*, 45(7), 455–463. doi:
625 10.1016/j.ijpara.2015.02.005

626 Capitanio, J. P., Abel, K., Mendoza, S. P., Blozis, S. A., McChesney, M. B., Cole, S. W., &
627 Mason, W. A. (2008). Personality and serotonin transporter genotype interact with
628 social context to affect immunity and viral set-point in simian immunodeficiency
629 virus disease. *Brain, Behavior, and Immunity*, 22(5), 676–689. doi:
630 10.1016/j.bbi.2007.05.006

631 Centers for Disease Control and Prevention (CDC). (2003). Severe acute respiratory
632 syndrome--Singapore, 2003. *MMWR. Morbidity and Mortality Weekly Report*, 52(18),
633 405–411.

- Chen, S., Sanderson, M., & Lanzas, C. (2013). Investigating effects of between- and within-host variability on Escherichia coli O157 shedding pattern and transmission. *Preventive Veterinary Medicine*, 109(1), 47–57. doi: 10.1016/j.prevetmed.2012.09.012
- Chtarbanova, S., Lamiable, O., Lee, K.-Z., Galiana, D., Troxler, L., Meignin, C., ... Imler, J.-L. (2014). Drosophila C virus systemic infection leads to intestinal obstruction. *Journal of Virology*, 88(24), 14057–14069. doi: 10.1128/JVI.02320-14
- Cornet, S., Bichet, C., Larcombe, S., Faivre, B., & Sorci, G. (2014). Impact of host nutritional status on infection dynamics and parasite virulence in a bird-malaria system. *The Journal of Animal Ecology*, 83(1), 256–265. doi: 10.1111/1365-2656.12113
- de Bono, M., & Bargmann, C. I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell*, 94(5), 679–689. doi: 10.1016/S0092-8674(00)81609-8
- Duneau, D., & Ebert, D. (2012). Host Sexual Dimorphism and Parasite Adaptation. *PLOS Biology*, 10(2), e1001271. doi: 10.1371/journal.pbio.1001271
- Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., & White, L. (2016). Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proc. R. Soc. B*, 283(1828), 20153078–20153078. doi: 10.1098/rspb.2015.3078
- Farrington, C. P., Whitaker, H. J., Unkel, S., & Pebody, R. (2013). Correlated Infections: Quantifying Individual Heterogeneity in the Spread of Infectious Diseases. *American Journal of Epidemiology*, 177(5), 474–486. doi: 10.1093/aje/kws260

657 Fellous, S., Duncan, A. B., Quillery, E., Vale, P. F., & Kaltz, O. (2012). Genetic influence on
658 disease spread following arrival of infected carriers. *Ecology Letters*, 15(3), 186–192.
659 doi: 10.1111/j.1461-0248.2011.01723.x

660 Ferrari, N., Cattadori, I. M., Nespereira, J., Rizzoli, A., & Hudson, P. J. (2004). The role of
661 host sex in parasite dynamics: field experiments on the yellow-necked mouse
662 *Apodemus flavicollis*. *Ecology Letters*, 7(2), 88–94. doi: 10.1046/j.1461-
663 0248.2003.00552.x

664 Foo, Y. Z., Nakagawa, S., Rhodes, G., & Simmons, L. W. (2017). The effects of sex
665 hormones on immune function: a meta-analysis. *Biological Reviews*, 92(1), 551–571.
666 doi: 10.1111/brv.12243

667 Gervasi, S. S., Civitello, D. J., Kilvitis, H. J., & Martin, L. B. (2015). The context of host
668 competence: A role for plasticity in host-parasite dynamics. *Trends in Parasitology*,
669 31(9), 419–425. doi: 10.1016/j.pt.2015.05.002

670 Godfrey, S. S. (2013). Networks and the ecology of parasite transmission: A framework for
671 wildlife parasitology. *International Journal for Parasitology: Parasites and Wildlife*,
672 2, 235–245. doi: 10.1016/j.ijppaw.2013.09.001

673 Grassly, N. C., & Fraser, C. (2008). Mathematical models of infectious disease transmission.
674 *Nature Reviews. Microbiology*, 6(3), 234–244. doi: 10.1038/nrmicro1845

675 Grear, D. A., Luong, L. T., & Hudson, P. J. (2012). Sex-biased transmission of a complex
676 life-cycle parasite: why males matter. *Oikos*, 121(9), 1446–1453. doi: 10.1111/j.1600-
677 0706.2012.20358.x

678 Gupta, V., Stewart, C., Rund, S. S. C., Monteith, K., & Vale, P. F. (2017). Costs and benefits
679 of sub-lethal *Drosophila* C virus infection. *Journal of Evolutionary Biology*. doi:
680 10.1111/jeb.13096

681 Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric
682 Models. *Biometrical Journal*, 50(3), 346–363.

683 Kaltz, O., & Shykoff, J. A. (2001). Male and female *Silene latifolia* plants differ in per-
684 contact risk of infection by a sexually transmitted disease. *Journal of Ecology*, 89(1),
685 99–109. doi: 10.1046/j.1365-2745.2001.00527.x

686 Keeling, M. J., & Rohani, P. (2007). *Chapter One Contact Tracing Operates by Refining the*
687 *Targeting of Other Control Measures. 1.4. What Are Mathematical Models?*

688 Keiser, C. N., Howell, K. A., Pinter-Wollman, N., Pruitt, J. N., Woolhouse, ME., Kortet, R.,
689 ... Edelstein-Keshet, L. (2016). Personality composition alters the transmission of
690 cuticular bacteria in social groups. *Biology Letters*, 12(7), 338–342. doi:
691 10.1098/rsbl.2016.0297

692 Keiser, C. N., Pinter-Wollman, N., Augustine, D. A., Ziemba, M. J., Hao, L., Lawrence, J.
693 G., & Pruitt, J. N. (2016). Individual differences in boldness influence patterns of
694 social interactions and the transmission of cuticular bacteria among group-mates.
695 *Proceedings of the Royal Society B: Biological Sciences*, 283(1829), 20160457–
696 20160457. doi: 10.1098/rspb.2016.0457

697 Kubiak, M., & Tinsley, M. C. (2017). Sex-Specific Routes To Immune Senescence In
698 *Drosophila melanogaster*. *Scientific Reports*, 7(1), 10417. doi: 10.1038/s41598-017-
699 11021-6

700 Lass Sandra, Hudson Peter J., Thakar Juilee, Saric Jasmina, Harvill Eric, Albert Réka, &
701 Perkins Sarah E. (2013). Generating super-shedders: co-infection increases bacterial
702 load and egg production of a gastrointestinal helminth. *Journal of The Royal Society*
703 *Interface*, 10(80), 20120588. doi: 10.1098/rsif.2012.0588

704 Lazzaro Brian P, & Little Tom J. (2009). Immunity in a variable world. *Philosophical*
705 *Transactions of the Royal Society B: Biological Sciences*, 364(1513), 15–26. doi:
706 10.1098/rstb.2008.0141

707 Li, Y., Yu, I. T. S., Xu, P., Lee, J. H. W., Wong, T. W., Ooi, P. L., & Sleight, A. C. (2004).
708 Predicting super spreading events during the 2003 severe acute respiratory syndrome
709 epidemics in Hong Kong and Singapore. *American Journal of Epidemiology*, 160(8),
710 719–728. doi: 10.1093/aje/kwh273

711 Life Technologies. (2011). *MagMAX-96 Total RNA Isolation Kit User Guide*. Carlsbad, CA
712 USA: Life Technologies Corporation.

713 Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., Getz, W. M., Schreiber, S. J., Kopp, P. E., ...
714 Kjelvik, M. K. (2005). Superspreading and the effect of individual variation on disease
715 emergence. *Nature*, 438(7066), 355–359. doi: 10.1038/nature04153

716 Luong, L. T., Grear, D. A., & Hudson, P. J. (2009). Male hosts are responsible for the
717 transmission of a trophically transmitted parasite, *Pterygodermatites peromysci*, to the
718 intermediate host in the absence of sex-biased infection. *International Journal for*
719 *Parasitology*, 39(11), 1263–1268. doi: 10.1016/j.ijpara.2009.03.007

720 Magalon, H., Nidelet, T., Martin, G., & Kaltz, O. (2010). Host growth conditions influence
721 experimental evolution of life history and virulence of a parasite with vertical and
722 horizontal transmission. *Evolution; International Journal of Organic Evolution*,
723 64(7), 2126–2138. doi: 10.1111/j.1558-5646.2010.00974.x

724 Magwire, M. M., Fabian, D. K., Schweyen, H., Cao, C., Longdon, B., Bayer, F., & Jiggins, F.
725 M. (2012). Genome-Wide Association Studies Reveal a Simple Genetic Basis of
726 Resistance to Naturally Coevolving Viruses in *Drosophila melanogaster*. *PLoS*
727 *Genetics*, 8(11). doi: 10.1371/journal.pgen.1003057

728 Marineli, F., Tsoucalas, G., Karamanou, M., & Androutsos, G. (2013). Mary Mallon (1869-
729 1938) and the history of typhoid fever. *Annals of Gastroenterology : Quarterly*
730 *Publication of the Hellenic Society of Gastroenterology*, 26(2), 132–134.

731 Martinaud, G., Billaudelle, M., & Moreau, J. (2009). Circadian variation in shedding of the
732 oocysts of *Isospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): An adaptive
733 trait against desiccation and ultraviolet radiation. *International Journal for*
734 *Parasitology*, 39(6), 735–739. doi: 10.1016/j.ijpara.2008.11.006

735 Matthews, L., Low, J. C., Gally, D. L., Pearce, M. C., Mellor, D. J., Heesterbeek, J. a P., ...
736 Woolhouse, M. E. J. (2006). Heterogeneous shedding of *Escherichia coli* O157 in
737 cattle and its implications for control. *Proceedings of the National Academy of*
738 *Sciences of the United States of America*, 103(3), 547–552. doi:
739 10.1073/pnas.0503776103

740 Mideo, N., Alizon, S., & Day, T. (2008). Linking within- and between-host dynamics in the
741 evolutionary epidemiology of infectious diseases. *Trends in Ecology & Evolution*,
742 23(9), 511–517. doi: 10.1016/j.tree.2008.05.009

743 Palmer, W. H., Medd, N. C., Beard, P. M., & Obbard, D. J. (2018). Isolation of a natural
744 DNA virus of *Drosophila melanogaster*, and characterisation of host resistance and
745 immune responses. *PLOS Pathogens*, 14(6), e1007050. doi:
746 10.1371/journal.ppat.1007050

747 Patterson, J. E. H., & Ruckstuhl, K. E. (2013). Parasite infection and host group size: a meta-
748 analytical review. *Parasitology*, 140(7), 803–813. doi: 10.1017/S0031182012002259

749 Paull, S. H., Song, S., McClure, K. M., Sackett, L. C., Kilpatrick, A. M., & Johnson, P. T.
750 (2011). From superspreaders to disease hotspots: linking transmission across hosts
751 and space. *Frontiers in Ecology and the Environment*, 10(2), 75–82. doi:
752 10.1890/110111

- Paull, S. H., Song, S., McClure, K. M., Sackett, L. C., Kilpatrick, A. M., & Johnson, P. T. J. (2012). From superspreaders to disease hotspots: linking transmission across hosts and space. *Frontiers in Ecology and the Environment*, 10(2), 75–82. doi: 10.1890/110111
- Ponton, F., Wilson, K., Holmes, A. J., Cotter, S. C., Raubenheimer, D., & Simpson, S. J. (2013). Integrating nutrition and immunology: A new frontier. *Journal of Insect Physiology*, 59(2), 130–137. doi: 10.1016/j.jinsphys.2012.10.011
- Rhines, A. S. (2013). The role of sex differences in the prevalence and transmission of tuberculosis. *Tuberculosis*, 93(1), 104–107. doi: 10.1016/j.tube.2012.10.012
- Rushmore, J., Caillaud, D., Matamba, L., Stumpf, R. M., Borgatti, S. P., & Altizer, S. (2013). Social network analysis of wild chimpanzees provides insights for predicting infectious disease risk. *Journal of Animal Ecology*, 82(5), 976–986. doi: 10.1111/1365-2656.12088
- Saltz, J. B. (2011). NATURAL GENETIC VARIATION IN SOCIAL ENVIRONMENT CHOICE: CONTEXT-DEPENDENT GENE-ENVIRONMENT CORRELATION IN DROSOPHILA MELANOGASTER. *Evolution*, 65(8), 2325–2334. doi: 10.1111/j.1558-5646.2011.01295.x
- Sanchez, A., Devevey, G., & Bize, P. (2011). Female-biased infection and transmission of the gastrointestinal nematode *Trichuris arvicolae* infecting the common vole, *Microtus arvalis*. *International Journal for Parasitology*, 41(13), 1397–1402. doi: 10.1016/j.ijpara.2011.09.004
- Shaw, D. J., & Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology*, 111(S1), S111–S133. doi: 10.1017/S0031182000075855

777 Sheridan, L. A. D., Poulin, R., Ward, D. F., & Zuk, M. (2000). Sex differences in parasitic
778 infections among arthropod hosts: is there a male bias? *Oikos*, 88(2), 327–334. doi:
779 10.1034/j.1600-0706.2000.880211.x

780 Short, S. M., Wolfner, M. F., & Lazzaro, B. P. (2012). Female *Drosophila melanogaster*
781 suffer reduced defense against infection due to seminal fluid components. *Journal of*
782 *Insect Physiology*, 58(9), 1192–1201. doi: 10.1016/j.jinsphys.2012.06.002

783 Siva-Jothy, J. A., & Vale, P. F. (2019). Viral infection causes sex-specific changes in fruit fly
784 social aggregation behaviour. *BioRxiv*, 630913. doi: 10.1101/630913

785 Sokolowski, M. B. (2001). *Drosophila*: genetics meets behaviour. *Nature Reviews. Genetics*,
786 2(11), 879–890. doi: 10.1038/35098592

787 Stein, R. A. (2011). Super-spreaders in infectious diseases. *International Journal of*
788 *Infectious Diseases : IJID : Official Publication of the International Society for*
789 *Infectious Diseases*, 15(8), e510-3. doi: 10.1016/j.ijid.2010.06.020

790 Susi, H., Barrès, B., Vale, P. F., & Laine, A.-L. (2015). Co-infection alters population
791 dynamics of infectious disease. *Nature Communications*, 6, 5975. doi:
792 10.1038/ncomms6975

793 Susi, H., Vale, P. F., & Laine, A.-L. (2015). Host Genotype and Coinfection Modify the
794 Relationship of within and between Host Transmission. *The American Naturalist*,
795 186(2), 000–000. doi: 10.1086/682069

796 Tavalire, H. F., Blouin, M. S., & Steinauer, M. L. (2016). Genotypic variation in host
797 response to infection affects parasite reproductive rate. *International Journal for*
798 *Parasitology*, 46(2), 123–131. doi: 10.1016/j.ijpara.2015.10.001

799 Thompson, O., Gipson, S. A. Y., & Hall, M. D. (2017). The impact of host sex on the
800 outcome of co-infection. *Scientific Reports*, 7(1), 910. doi: 10.1038/s41598-017-
801 00835-z

- Thrall, P. H., Antonovics, J., & Dobson, A. P. (2000). Sexually transmitted diseases in polygynous mating systems: prevalence and impact on reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, 267(1452), 1555–1563.
- Vale, P. F., Choisy, M., & Little, T. J. (2013). Host nutrition alters the variance in parasite transmission potential. *Biology Letters*, 9(2). doi: 10.1098/rsbl.2012.1145
- Vale, P. F., & Jardine, M. D. (2015). Sex-specific behavioural symptoms of viral gut infection and Wolbachia in *Drosophila melanogaster*. *Journal of Insect Physiology*, 82, 28–32. doi: 10.1016/j.jinsphys.2015.08.005
- VanderWaal, K. L., & Ezenwa, V. O. (2016). Heterogeneity in pathogen transmission: mechanisms and methodology. *Functional Ecology*, n/a-n/a. doi: 10.1111/1365-2435.12645
- White, L. A., Forester, J. D., & Craft, M. E. (2018). Covariation between the physiological and behavioral components of pathogen transmission: host heterogeneity determines epidemic outcomes. *Oikos*, 127(4), 538–552. doi: 10.1111/oik.04527
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Pogladyen, G., Read, A. F., & Skorpington, A. (2002). Chapter 2: Heterogeneities in macroparasite infections: patterns and processes. *The Ecology of Wildlife Diseases*. Oxford University Press, Oxford, 6–44.
- Woolhouse, M. E. J., Dye, C., Etard, J.-F., Smith, T., Charlwood, J. D., Garnett, G. P., ... Anderson, R. M. (1997). Heterogeneities in the transmission of infectious agents : *Proceedings of the National Academy of Sciences of the United States of America*, 94(January), 338–342.

827 Tables

Response Variable	Predictor	Df	F	%Variance Explained	p-value
Lifespan Following Infection	Sex	1	2.00	0.6	0.16
	Genetic Background	9	3.92	10.9	<0.0001
	VLAD	1	38.9	12.1	<0.0001
	Sex*Genetic Background	9	0.96	2.7	0.47
	Sex*VLAD	1	5.46	1.7	0.02
	Genetic Background*VLAD	9	0.63	1.8	0.77
	Sex*Genetic Background*VLAD	9	2.67	7.4	0.005
	Mating	1	2.74	0.9	0.099
	Genetic Background	8	2.43	7.0	0.01
	VLAD	1	32.3	10.2	<0.0001
	Mating*Genetic Background	8	0.54	1.5	0.84
	Mating*VLAD	1	3.78	1.2	0.053
	Genetic Background*VLAD	8	1.71	4.9	0.087
	Mating*Genetic Background*VLAD	8	1.46	4.2	0.16

828

829 **Table 1.** Model outputs for the generalized linear modelling tests performed on
830 lifespan following DCV infection. The VLAD acronym is used in place of 'viral load at
831 death'. Separate analyses were used to test the effect of sex and mating in females.

832

Response Variable	Predictor	Df	F	% Variance Explained	p-value
Viral Load at Death (VLAD)	Sex	1	0.17	0.05	0.68
	Genetic Background	9	0.96	2.53	0.47
	Sex*Genetic Background	9	0.92	2.43	0.50
	Mating	1	1.90	0.57	0.17
	Genetic Background	8	1.30	3.5	0.24
	Mating*Genetic Background	8	0.93	2.49	0.50

Table 2. Model outputs for the generalized linear modelling tests performed on the viral load at death of flies infected with DCV. Separate analyses were used to test the effect of sex and mating in females.

Response Variable	Predictor	Df	χ^2	% Deviance Explained	p -value
Qualitative DCV Load	Sex	1	0.019	0.002	0.89
	Genetic Background	9	9.58	1.18	0.39
	DPI	2	36.6	4.52	<0.0001
	Sex*Genetic Background	9	45.2	5.58	<0.0001
	Mating	1	1.01	0.13	0.31
	Genetic Background	8	22.4	2.83	0.008
	DPI	2	27.2	3.43	<0.0001
	Mating*Genetic Background	8	39.0	4.92	<0.0001

Table 3. Model outputs for the binomial logistic regression conducted on qualitative DCV loads (the proportion of non-zero DCV loads). The DPI acronym is used in place of 'days post-infection'. Separate analyses were used to test the effect of sex and mating in females.

843

Response Variable	Predictor	DF	F	% Variance Explained	p-value
Quantitative DCV Load	Sex	1	0.0062	0.003	0.94
	Genetic Background	9	2.24	7.94	0.02
	DPI	2	3.37	2.65	0.036
	Sex* Genetic Background	9	5.41	19.2	<0.0001
	Mating	1	0.68	0.26	0.41
	Genetic Background	8	3.18	11.0	0.0012
	DPI	2	4.66	3.60	0.01
	Mating* Genetic Background	8	1.42	4.38	0.19

844

845 **Table 4.** Model outputs for the GLM analysis conducted on quantitative DCV load (the
846 titres of non-zero DCV loads). The DPI acronym is used in place of 'days post-
847 infection'. Separate analyses were used to test the effect of sex and mating in
848 females.

Response Variable	Predictor	Df	χ^2	% Deviance Explained	p-value
Qualitative DCV Shedding	Sex	1	4.93	0.64	0.026
	Genetic Background	9	17.6	2.27	0.04
	Viral Load	1	0.03	0.004	0.85
	DPI	2	25.1	3.25	<0.0001
	Sex*Genetic Background	9	23.8	3.07	0.005
	Mating	1	1.33	0.18	0.25
	Genetic Background	8	19.0	2.53	0.025
	Viral Load	1	1.10	0.15	0.29
	DPI	2	7.66	1.02	0.022
	Mating*Genetic Background	8	8.12	1.08	0.42

849

850 **Table 5.** Model outputs for the GLM analysis conducted on qualitative DCV shedding
851 (the proportion of sheddings with non-zero readings of DCV). The DPI acronym is
852 used in place of 'days post-infection'. Separate analyses were used to test the effect
853 of sex and mating in females.

854

Response Variable	Predictor	Df	F	% Variance Explained	p-value
Quantitative DCV Shedding	Sex	1	0.67	0.28	0.42
	Genetic Background	9	2.52	9.48	0.009
	Viral Load	1	5.03	4.21	0.007
	DPI	2	0.23	0.095	0.63
	Sex*Genetic Background	9	1.73	6.53	0.082
	Mating	1	0.22	0.098	0.64
	Genetic Background	8	1.44	5.82	0.17
	Viral Load	1	11.2	10.1	<0.0001
	DPI	2	0.18	0.08	0.67
	Mating*Genetic Background	8	2.46	8.87	0.014

Table 6. Model outputs for the GLM analysis conducted on quantitative DCV shedding (the subset of shedding with non-zero readings of DCV). The DPI acronym is used in place of 'days post-infection'. Separate analyses were used to test the effect of sex and mating in females.

Response Variable	Predictor	Df	χ^2	% Deviance Explained	p-value
Qualitative V	Sex	1	4.58	0.28	0.032
	Line	9	38.2	2.30	<0.0001
	Sex*Line	9	47.0	2.83	<0.0001

861

862 **Table 7.** Model outputs for the logistic regression analysis conducted on qualitative
863 V (the proportion of non-zero V values).

864

Response Variable	Predictor	Df	F	% Variance Explained	p-value
Quantitative V	Sex	1	0.077	0.01	0.78
	Line	9	2.51	4.13	0.008
	Sex*Line	9	6.94	11.4	<0.0001

865

866 **Table 8.** Model outputs for the GLM analysis conducted on quantitative V (the
867 subset with non-zero V values).

Supplementary Figures and Tables

870

871 **Figure S1.** The relationship between the viral load of flies and the amount of virus
 872 they shed into their environment. The two distinct phenotypes, where individuals
 873 show a zero-value for shedding or load and a positive-value for the other trait, are
 874 marked by blue (supersponges) or red (supershedders).

875

	59	75	138	373	379	380	502	738	765	818
Male	20	20	18	19	17	18	17	19	18	19
Female	17	20	13	19	19	20	19	18	15	18
Virgin Female	20	18	7	18	20	19	19	20	16	20

Table S1 – The number of flies measured for lifespan and viral load at death for each combination of genetic background and sex/female mating status.

(a)		59	75	138	373	379	380	502	738	765	818
	Male	12	11	11	12	11	11	12	11	11	6
	Mated Female	13	12	11	12	12	12	11	12	12	12
	Virgin Female	12	14	NA	15	12	13	12	12	7	12

(b)		59	75	138	373	379	380	502	738	765	818
	Male	12	12	11	12	11	12	11	12	12	6
	Mated Female	14	12	11	11	12	11	11	11	12	13
	Virgin Female	12	14	NA	14	12	11	13	10	9	12

(c)		59	75	138	373	379	380	502	738	765	818
	Male	11	12	12	12	12	11	12	10	12	7
	Mated Female	11	11	10	13	12	11	13	11	12	12
	Virgin Female	11	13	NA	13	12	13	11	12	8	11

Table S2 – The number of viral load samples for each treatment group (a) 1 DPI, (b) 2 DPI and (c) 3 DPI.

(a)		59	75	138	373	379	380	502	738	765	818
Male	Male	5	9	2	5	4	6	8	6	5	3
	Mated Female	6	5	5	10	8	9	2	9	3	9
	Virgin Female	4	11	NA	11	7	9	4	3	6	4

(b)		59	75	138	373	379	380	502	738	765	818
Male	Male	1	5	3	1	1	3	7	6	1	4
	Mated Female	8	4	5	5	2	1	1	3	4	1
	Virgin Female	1	3	NA	9	7	7	6	1	5	4

(c)		59	75	138	373	379	380	502	738	765	818
Male	Male	1	5	5	5	7	7	1	4	7	6
	Mated Female	5	4	7	4	5	4	1	7	9	2
	Virgin Female	1	1	NA	6	6	6	5	6	3	3

Table S3 – The number of non-zero viral load samples for each treatment group (a) 1 DPI, (b) 2 DPI and (c) 3 DPI.

(a)		59	75	138	373	379	380	502	738	765	818
Male	Male	10	11	10	12	11	10	12	10	10	5
	Mated Female	13	12	11	11	12	12	11	12	12	12
	Virgin Female	11	14	NA	15	12	13	12	12	7	12

(b)		59	75	138	373	379	380	502	738	765	818
Male	Male	12	12	11	11	10	12	11	12	12	7
	Mated Female	14	12	11	11	12	11	11	9	12	13
	Virgin Female	11	13	NA	13	12	11	13	11	9	12

(c)		59	75	138	373	379	380	502	738	765	818
Male	Male	10	11	12	12	11	11	12	10	9	7
	Mated Female	11	9	10	13	9	9	13	8	12	11
	Virgin Female	10	12	NA	13	12	12	7	11	7	10

Table S4 – The number of virus shedding samples for each treatment group (a) 1 DPI, (b) 2 DPI and (c) 3 DPI.

(a)		59	75	138	373	379	380	502	738	765	818
	Male	0	7	4	6	3	7	4	6	2	4
	Mated Female	5	4	3	6	6	5	1	6	7	4
	Virgin Female	4	6	NA	8	6	4	6	3	5	5

(b)		59	75	138	373	379	380	502	738	765	818
	Male	10	7	6	5	9	9	5	11	7	7
	Mated Female	8	4	9	7	8	3	6	3	4	4
	Virgin Female	5	6	NA	7	10	5	8	8	6	6

(c)		59	75	138	373	379	380	502	738	765	818
	Male	4	4	8	3	9	2	7	2	3	6
	Mated Female	7	3	6	7	2	2	3	3	4	5
	Virgin Female	4	4	NA	7	7	4	4	5	2	4

Table S5 – The number of non-zero virus shedding samples for each treatment group (a) 1 DPI, (b) 2 DPI and (c) 3 DPI.

Response Variable	Analysis	Predictors
Lifespan	GLM	Sex * Genetic Background * VLAD Mating * Genetic Background * VLAD
VLAD	GLM	Sex * Genetic Background Mating * Genetic Background
Qualitative Load	Logistic Regression	Sex * Genetic Background + DPI Mating * Genetic Background + DPI
Quantitative Load	GLM	Sex * Genetic Background + DPI Mating * Genetic Background + DPI
Qualitative Shed	Logistic Regression	Sex * Genetic Background + Quant. Load + DPI Mating * Genetic Background + Quant. Load + DPI
Quantitative Shed	GLM	Sex * Genetic Background + Quant. Load + DPI Mating * Genetic Background + Quant. Load + DPI
Qualitative V	Logistic Regression	Sex * Genetic Background
Quantitative V	GLM	Sex * Genetic Background

896

897 **Table S6.** Summaries of the logistic regression and GLMs used to analyse the
898 response variables of our experiments. All interactions are fully-factorial and marked
899 using an asterisk (*).