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_l; 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

63 forefront of epidemiology and disease ecology research (Gervasi, Civitello, Kilvitis, &
64 Martin, 2015; Paull et al., 2012; Stein, 2011; VanderWaal & Ezenwa, 2016).
65
66 Despite being commonplace, the underlying causes of heterogeneity in pathogen
67 transmission remain elusive. Individual variation in host contact networks may be an
68 important factor: it was Typhoid Mary's position as a cook which exposed her to so
69 many susceptible individuals. However, what enabled Typhoid Mary to stay in this
70 role was her status as an asymptomatic carrier of the infection, which led to her
71 release from quarantine on several occasions (Marineli et al., 2013). Similarly, the
72 absence of symptoms in a number of SARS superspreaders delayed their admission
73 to hospital and allowed them to continue spreading the virus (Centers for Disease
74 Control and Prevention (CDC), 2003). These examples help underline that achieving
75 a detailed understanding of the sources of heterogeneity in pathogen transmission is
76 challenging because it results from complex interactions between multiple host
77 behavioural, physiological, and immune traits. By dissecting the underlying genetic
78 and sex-specific sources of variation in these traits we can assess how they influence
79 three key components of pathogen transmission: contact rate between infected and
80 susceptible individuals, the likelihood that contact will result in infection, and the
81 duration of infection (VanderWaal & Ezenwa, 2016).
82
83 Infected-susceptible host contact rate is predominantly determined by host
84 behaviours affecting locomotion and aggregation. Contact rates are also affected by
85 population density (Keeling & Rohani, 2007), social group size (Patterson &
86 Ruckstuhl, 2013), and behavioural syndromes (Keiser, Pinter-Wollman, et al., 2016).
87 Social networks often exhibit extreme heterogeneity in the wild (Godfrey, 2013;
88 Rushmore et al., 2013) and factors such as host genotype, sex condition, age and
89 personality have been demonstrated to affect social aggregation in lab systems (de
90 Bono & Bargmann, 1998; Keiser, Howell, et al., 2016; Saltz, 2011; Siva-Jothy & Vale,
91 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±1˚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±1˚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° ~0.5mm 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 50µl 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

155 at death was measured for n=17-20, three treatment groups consisted of n=7-15 flies
156 (Table S1).
157
158 ## Viral Growth and Shedding Measurement Setup
159 Due to destructive sampling, we measured the viral load and shedding of single flies
160 at a single time point, either 1-, 2- or 3-days post-infection (DPI). Following DCV
161 infection, single flies were placed into 1.5ml Eppendorf tubes with ~50µl of Lewis
162 medium in the bottom of the tube. To measure viral shedding, flies were transferred
163 to tubes for 24 hours, immediately following 1 or 2 days after systemic infection. After
164 living in these tubes for a further 24 hours, flies were removed and homogenised in
165 50µl of TRI-reagent, tubes were also washed out with 50µl of TRI-reagent by
166 vortexing. These samples were then frozen at -70°C, to await DCV quantification by
167 qPCR. For each combination of sex and genetic background over the three days vial
168 load and virus shedding was measured, n=7-15 flies were measured (Table S2-S4).
169
170 ## DCV RNA Extraction
171 RNA was extracted from viral load at death and viral shedding samples by Phenol-
172 Chloroform extraction. Samples were thawed on ice for 30 minutes before being
173 incubated at room temperature for 5 minutes to allow dissociation of nucleo-protein
174 complex. Samples were then centrifuged at 12,000×g for 10 minutes at 4°C after
175 which large debris was removed. For phase separation, samples were shaken
176 vigorously for 15 seconds, 10µl of chloroform added, incubated at room temperature
177 for a further 3 minutes before being centrifuged at 12,000×g for 15 minutes at 4°C.
178 Following phase separation, the upper aqueous layer was removed from each
179 sample and added to 25µl of isopropanol, tubes were then inverted twice, before
180 being centrifuged at 12,000×g for 10 minutes at 4°C. Precipitated RNA was then
181 washed by removing the supernatant, and re-dissolving the RNA pellet in 50µl of 75%
182 ethanol before being centrifuged at 7,500×g for 5 minutes at 4°C. RNA suspension
183 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 ($1\text{-}10^{-12}$) 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.

278 ## Results

279 ### Lifespan Following Infection

280 Infected lifespan varied significantly between males and females and the extent of
281 this variation differed between host genetic backgrounds (Figure 1a; Table 1).
282 Genetic background explained the most variance of any predictor across models
283 assessing mating (7%) and sex (10.9%). Interactions with sex and mating also
284 explained a further 2.7% and 1.5%, respectively (Figure 5; Table 1). We found no
285 evidence that mating affected the lifespan of females following DCV infection (Figure
286 1a; Table 1). Viral load at death (VLAD) was not affected by genetic background, sex
287 or female mating status (Figure 1b; Table 2), and flies that died sooner following
288 infection had greater VLAD than those that died later (Figure 1c; Table 1).

289

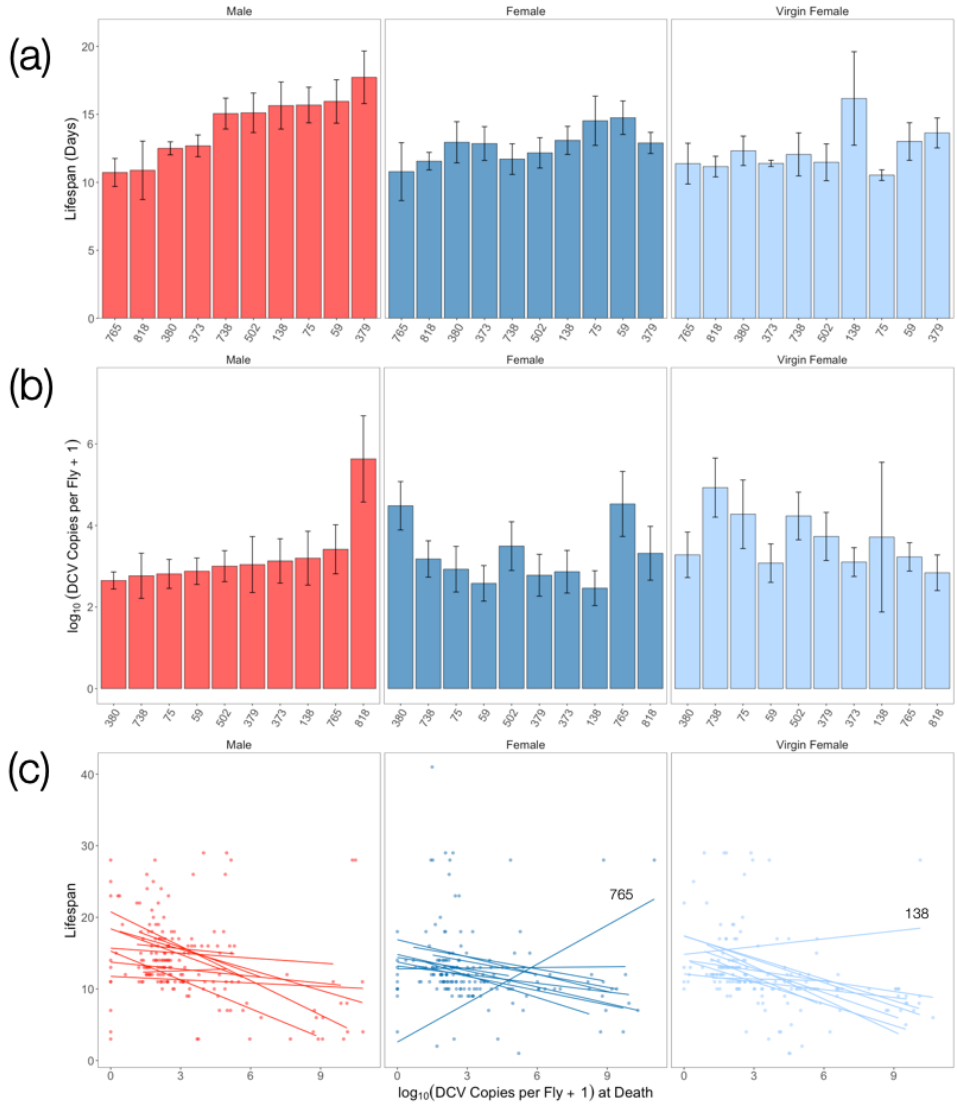


Figure 1. Mean±SE (a) lifespan in days following infection and (b) the viral load at death in males (red), mated females (blue), and virgin females (pale blue) of ten genetic backgrounds. The x-axis shows the line number form the DGRP panel and is in ascending order according to male flies. (c) the relationship 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, $p=0.068$).

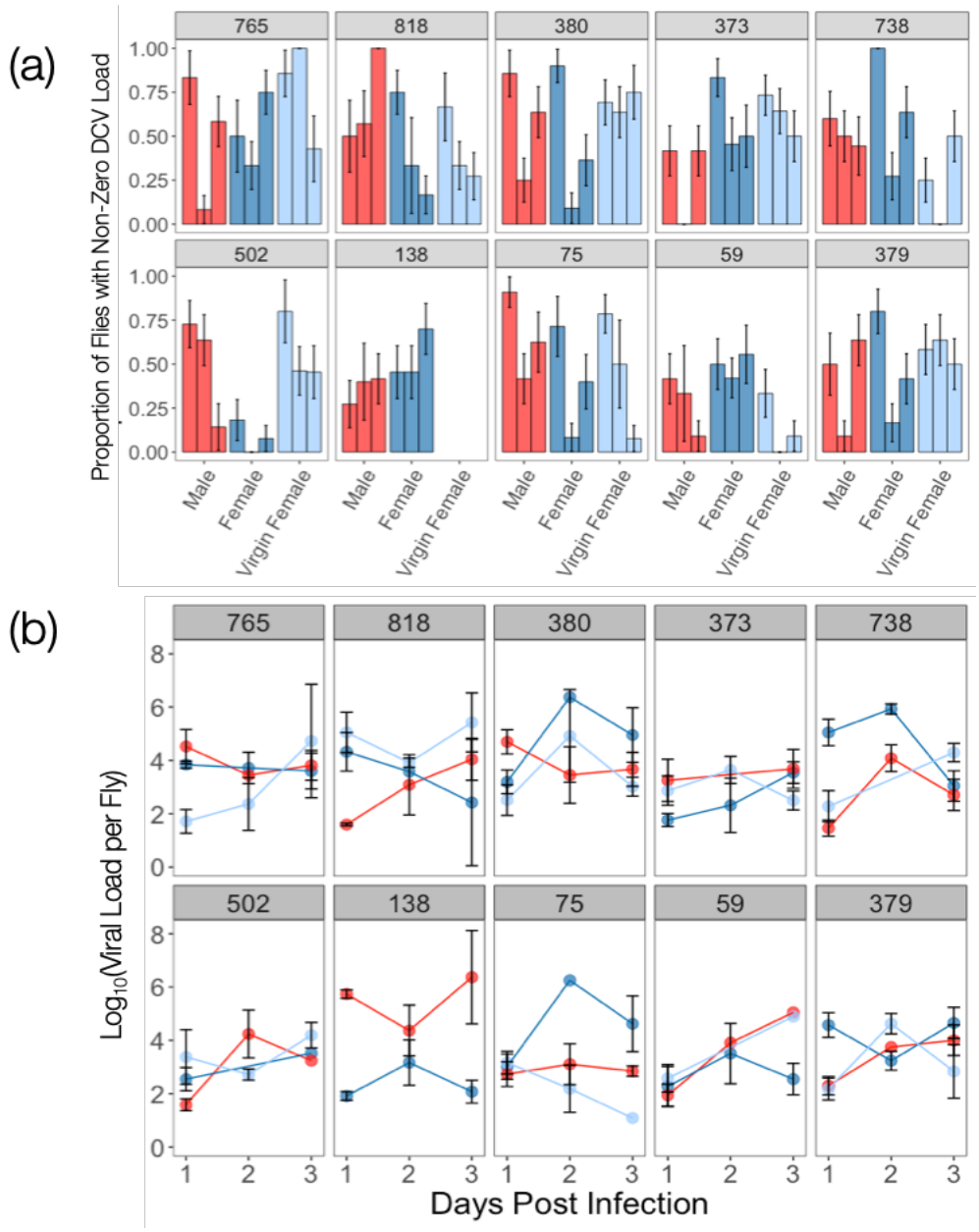


Figure 2. Mean±SE (a) proportion of flies with non-zero loads of DCV and the (b) viral titre of flies with non-zero DCV loads, over the first 3 days of infection. Across both panels, numbers in each pane denote the genetic background from the DGRP, while the colour of bars, points and lines represent 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

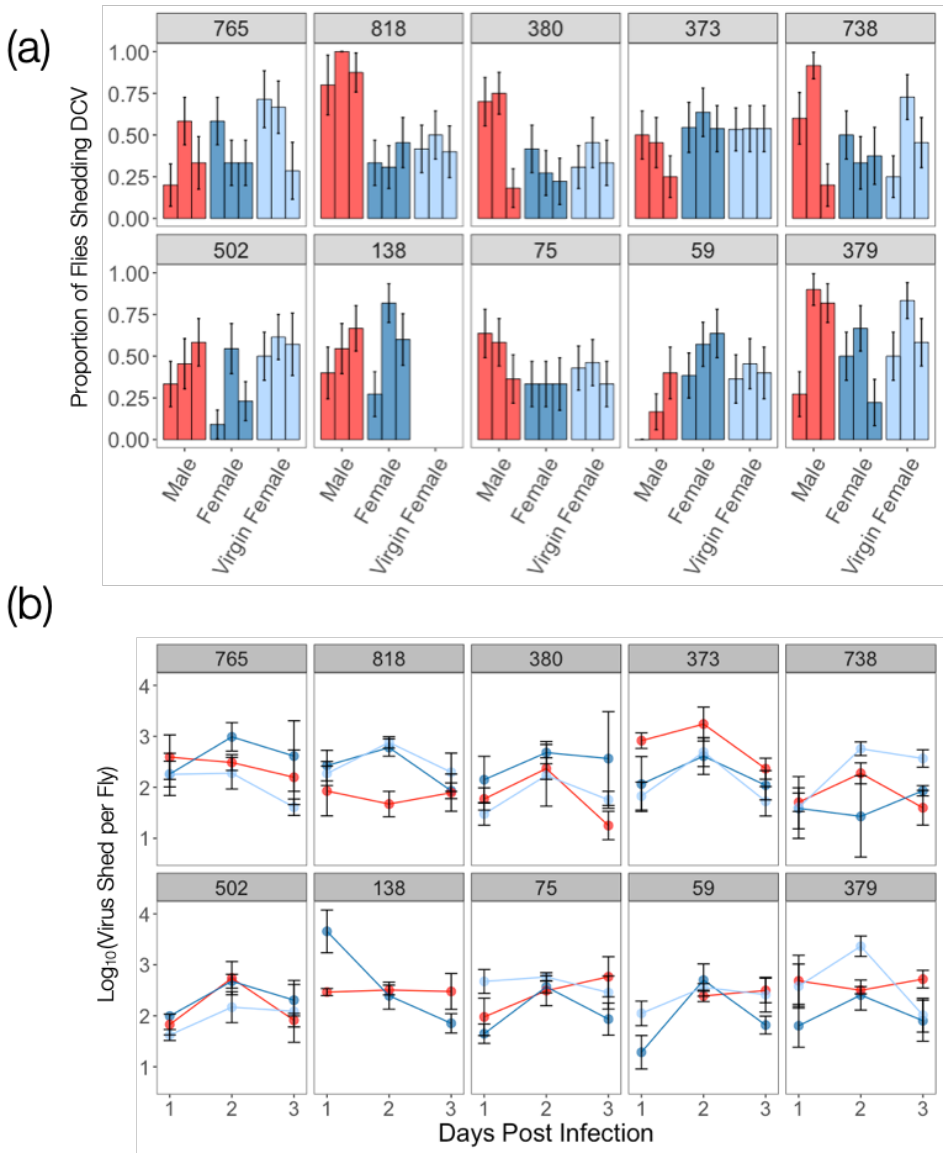


Figure 3. Mean±SE (a) proportion of flies shedding non-zero titres of DCV and the (b) titre of the non-zero virus shedding subset over the first 3 days of infection. Panels denote genetic background, while the colour of bars, points and lines represent sex and mating status. Males are shown in red, mated 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).

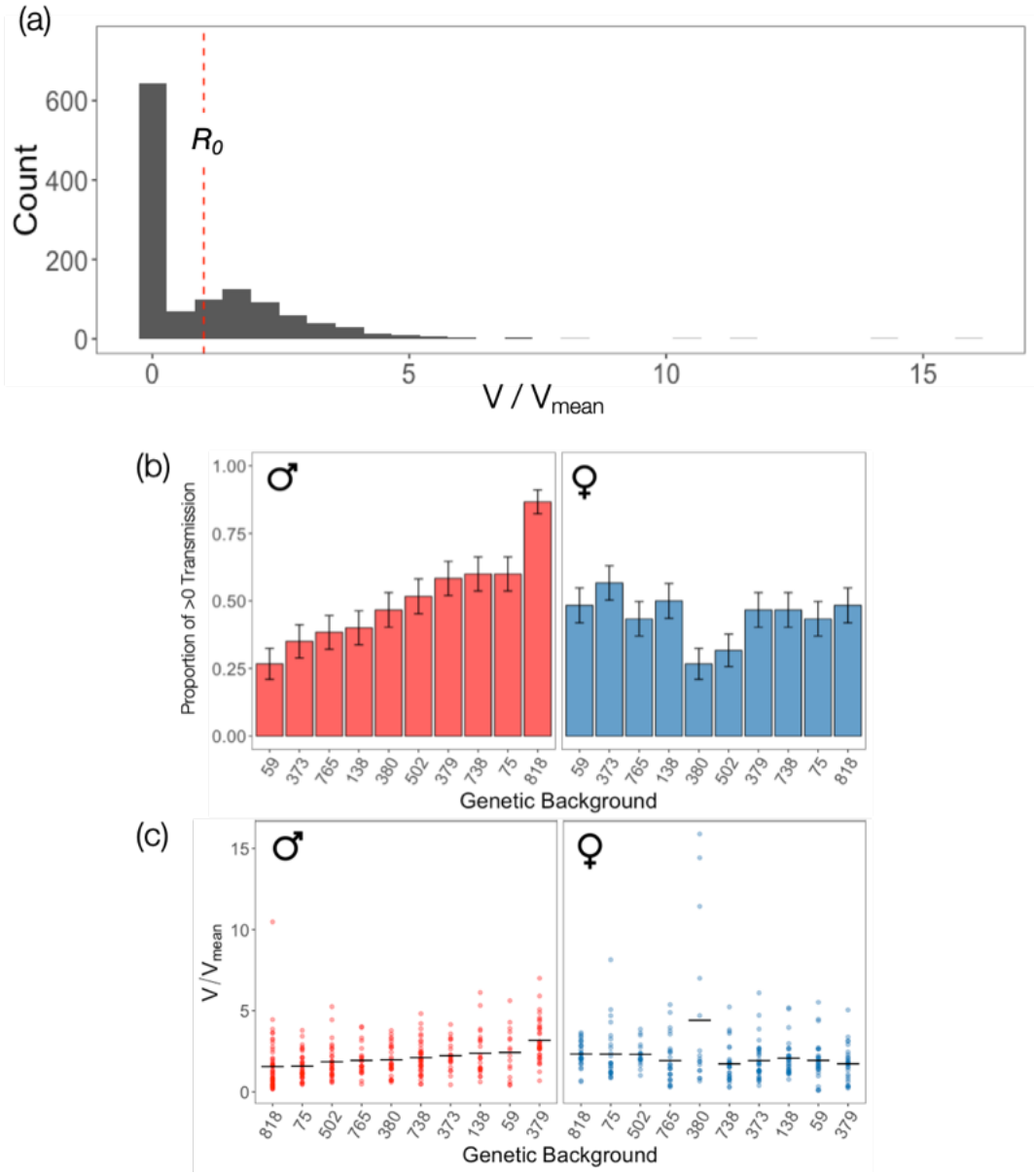
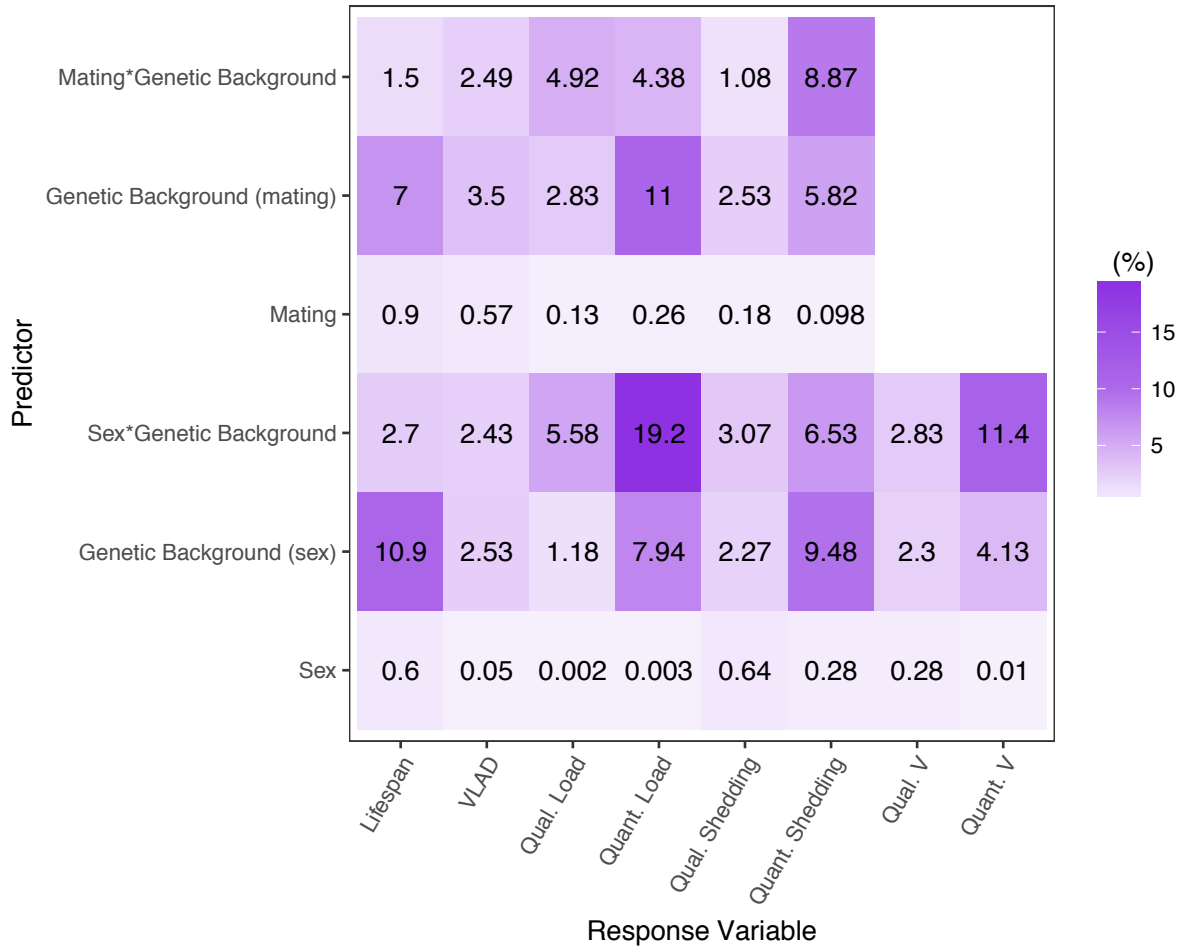


Figure 4. Bootstrap simulation results of transmission potential (*V*) (n=60): (a) the population-level distribution of *V* relative to the mean of the population. The red dashed line demarcates the average transmission potential of the population (similar to R_0), a traditional metric used to describe a population's outbreak risk. The mean±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.

389



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

422 background as part of an interaction with host sex, while this interaction has no
423 significant effect on quantitative DCV shedding (Tables 5 & 6). Similar differences are
424 seen in the amount of deviance and variance genetic background explains in models
425 of qualitative and quantitative variation in DCV shedding. Genetic background
426 accounts for only 2.27% of deviance in qualitative DCV shedding whereas it accounts
427 for 9.48% of the variance in quantitative DCV shedding (Figure 5). Genetic variation
428 therefore appears to play an important role in determining shedding and affects
429 qualitative and quantitative shedding in different ways. Similar effects of genetic
430 backgrounds on parasite shedding have been reported in the Ramshorn snail
431 species, *Biomphlamaria glabrata*, during infection with *Schistosoma mansoni.*
432 Genetic backgrounds differ in how many parasite eggs they shed and how quickly
433 they start shedding after infection (Tavalire et al., 2016). The differences we see in
434 the proportion of flies shedding DCV may be caused by a similar pattern of variation
435 in individual's delaying virus shedding. Delaying the onset of shedding could be
436 affected by a range of DCV infection symptoms. These include paralysis of muscles
437 in the crop organ of the foregut, abdominal swelling, broad nutritional stress and
438 reduced defecation rate (Chtarbanova et al., 2014).
439
440 Genetic background also appears to play a key role in transmission potential, we
441 detected a significant effect on both qualitative and quantitative variation in *V.* The
442 amount of deviance and variance explained by genetic background does not hugely
443 differ (2.3% and 4.13%, respectively). However, when part of an interaction with sex,
444 genetic background accounts for 11.4% of the variance in quantitative variation in
445 DCV shedding, whereas this same interaction only accounts for 2.83% of the
446 deviance in qualitative variation in shedding (Figure 5). Alongside other studies, this
447 highlights the potential significance of genetic variation in pathogen shedding to
448 generating transmission heterogeneity. For example, genetic variation in transmission
449 was demonstrated using families of turbot fish (*Scophthalmus maximus*) which
450 produced outbreaks that differed in how quickly individuals showed symptoms of

451 infection and died (Anacleto et al., 2019). Shedding may underlie this genetic
452 variation in transmission as it was not directly measured and there were no
453 significant differences in infection duration and contact rate (Anacleto et al., 2019).
454 Common garden experiments have revealed shedding dynamics capable of
455 influencing the population-level transmission dynamics of wild populations of the
456 plant, *Plantago lanceolata*. In controlled laboratory settings, multi-strain coinfection
457 was shown to increase the number of spores released of the fungal pathogen,
458 *Podosphaera plantaginis*. Measures of natural populations have also demonstrated
459 outbreak severity increases at higher levels of coinfection (Susi, Barrès, Vale, & Laine,
460 2015). The relationship between spore shedding and coinfection has also been
461 shown to be affected by host genotype, with genotypes significantly differing in the
462 number of spores released over a number of days post-infection (Susi, Vale, & Laine,
463 2015). Genetic variation in transmission potential has also been demonstrated in
464 the freshwater ciliate, *Paramecium caudatum*, following *Holospora undulata* infection
465 (Fellous et al., 2012). The genotype of the first individual to be infected was a key
466 determinant of pathogen transmission as host genotype appears to affect the
467 infectious potential of shed pathogens (Fellous et al., 2012). *H. undulata*
468 infectiousness increases with host population density, as reduced variation in
469 contact rate makes infectiousness the primary determinant of transmission
470 (Magalon et al., 2010).

471

472 <u>The effect of host sex in generating heterogeneity in transmission</u>

473 We also observed clear qualitative and quantitative differences in *V* between males and
474 females, which is suggestive of sex-specific variation in disease transmission. While the
475 extent of any difference between males and females is also determined by genetic
476 background, a greater proportion of males tend to transmit DCV than females across
477 these backgrounds. In DCV shedding, a greater proportion of males from several genetic
478 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

503 shedding being measured during the first three days of infection, whereas mortality
504 occurred much later. We might therefore see sex-differences in shedding during the later
505 stages of infection.
506
507 Both the qualitative and quantitative differences in *V* between males and females were
508 determined alongside genetic background. While sex explained very little deviance and
509 variance in qualitative and quantitative variation in *V* (Figure 6), its interaction with genetic
510 background explained 2.83% and 11.4 %, respectively. Sex could therefore be an
511 important source of variation in individual disease transmission. Sex differences in
512 transmission or virus shedding, lifespan and social aggregation are commonly observed
513 in a wide range of species (Duneau & Ebert, 2012; Ferrari, Cattadori, Nespereira, Rizzoli,
514 & Hudson, 2004; Kaltz & Shykoff, 2001; Sanchez et al., 2011). Sex-specific variation has
515 been relatively well-studied because sexes are easily distinguished in the wild, and
516 examples of sexual dimorphism in physiological and behavioural traits are relatively
517 common (Duneau and Ebert, 2012). Many mammalian hosts exhibit male-biased
518 transmission (Ezenwa et al., 2016; Grear, Luong, & Hudson, 2012; Luong, Grear, &
519 Hudson, 2009; Rhines, 2013), although there are exceptions of female-bias (Sanchez et
520 al., 2011). In the white-footed mouse, *Peromyscus leucopus*, male-biased transmission
521 is thought to be driven by sex differences in contact network connectivity, which has
522 been linked to testosterone production (Foo, Nakagawa, Rhodes, & Simmons, 2017;
523 Grear et al., 2012). Testosterone may be particularly relevant to transmission as its
524 immunosuppressive (Foo et al., 2017) effects may also alter infectiousness and infection
525 duration.
526

527 Female Mating Status in Shedding

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

543

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

551 heterogeneity (Chen, Sanderson, & Lanzas, 2013). This is particularly relevant to our
552 study as virus shedding was only measured at a single time point per fly, and we do
553 not know how shedding, and therefore *V*, may change over time. Within-host, temporal
554 variation in shedding is observed in a range of host-pathogen systems (Chen et al.,
555 2013; Matthews et al., 2006; Mideo, Alizon, & Day, 2008). For example, avian hosts
556 tend to shed more parasites during the late afternoon (Brawner III and Hill, 1999;
557 Martinaud et al., 2009).
558
559 By combining measures of virus shedding, lifespan and social aggregation into a simple
560 framework our work demonstrates that genetic and sex-specific variation can affect
561 individual heterogeneity in disease transmission potential. We also show that genetic and
562 sex-specific variation, as well as mating, can produce variation outcomes of infection.
563 Alongside its interaction with sex, genetic background explains 5.41% of qualitative, and
564 15.54% of quantitative, individual variation in transmission potential. While our results do
565 not implicate a particular genetic background, males generally present a greater
566 transmission risk than females. In addition to highlighting high-risk individuals, our results
567 are congruous with the observation that the majority of infected individuals produce very
568 few, if any, secondary cases of infection. Non-infectious individuals are particularly
569 relevant to predicting outbreaks of infectious disease as they obscure high-risk
570 individuals in traditional, population-wide estimations of outbreak risk. Our findings
571 demonstrate the benefit of using a model laboratory system as well established as *D.*
572 *melanogaster* to study disease transmission. The number of available protocols and
573 methodologies are central to considering multiple traits central to disease transmission
574 and holistically studying their underlying determinants.

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826

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

Table 5. Model outputs for the GLM analysis conducted on qualitative DCV shedding (the proportion of sheddings 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	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	X^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

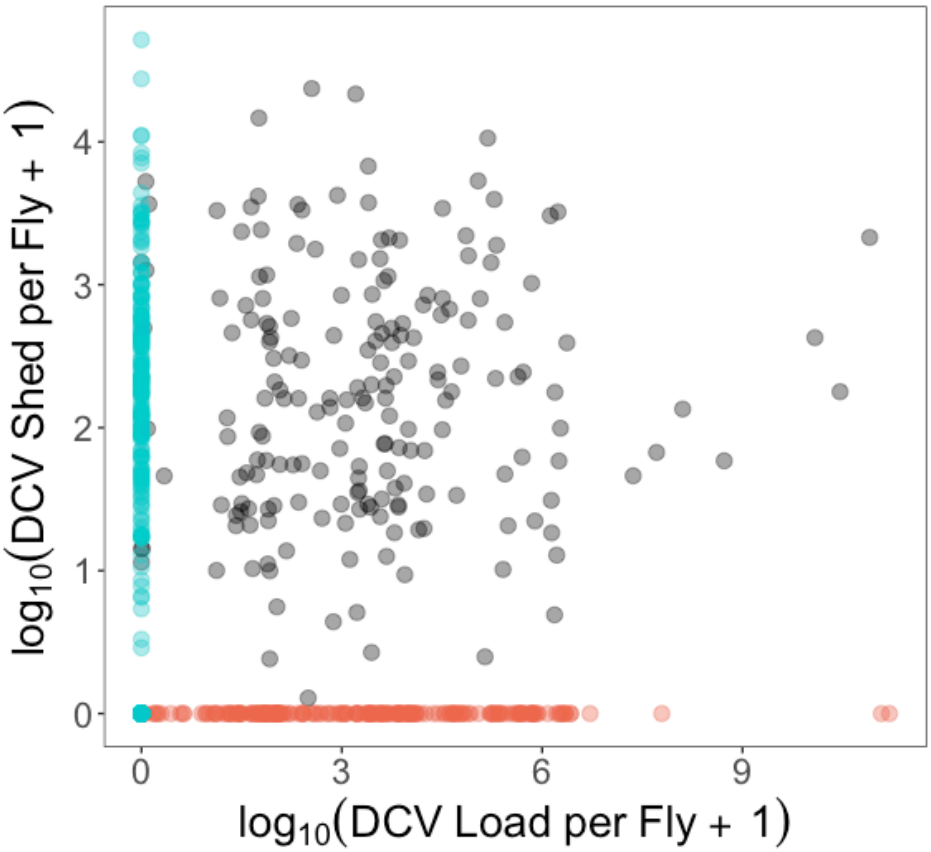
Table 7. Model outputs for the logistic regression analysis conducted on qualitative *V* (the proportion of non-zero *V* values).

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

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

868 Supplementary Figures and Tables

869



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	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	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	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

884

885 Table S3 – The number of non-zero viral load samples for each treatment group (a)

886 1 DPI, (b) 2 DPI and (c) 3 DPI.

887

(a)

	59	75	138	373	379	380	502	738	765	818
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	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	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

888

889 Table S4 – The number of virus shedding samples for each treatment group (a) 1

890 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

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