Sex and tissue differences in virus susceptibility across species

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Abstract

Species vary in their susceptibility to pathogens, and this can alter the ability of a pathogen to infect a novel host. However, many factors can obscure our ability to assess host susceptibility and understand pathogen emergence. Often overlooked is sexual dimorphism in susceptibility, males are often reported to be intrinsically more susceptible, but this can vary by host and pathogen. Further, we know little about whether the tissues infected by a pathogen in one host are the same in another species, and how this relates to the harm a pathogen does to its host. Here, we first take a comparative approach to examine sex differences in susceptibility across 31 species of Drosophilidae infected with Drosophila C Virus (DCV). We found a strong positive inter-specific correlation in viral load between males and females, with a close to 1:1 relationship, suggesting that susceptibility across species is not sex specific. Next, we made comparisons of the tissue tropism of DCV across seven species of fly. We found differences in viral load between the tissues of the seven host species, but no evidence of tissues showing different patterns of susceptibility in different host species. We conclude that, in this system, patterns of viral infectivity across host species are robust between males and females, and susceptibility in a given host is general across tissue types.

Introduction

Emerging pathogens often arise from a host shift event – where a pathogen jumps into and establishes in a novel host species. Species vary in their susceptibility to pathogens, but little is known about the factors underlying these differences, and whether differences between clades are due to the same or different factors [1, 2]. Understanding this is critical for determining which hosts pathogens are likely to jump between, and the harm they cause to their hosts. The host phylogeny has been shown to be an important determinant of host shifts in a range of systems [3-8] as well as being important for understanding how pathogen virulence may change when a pathogen finds itself in a new host [9, 10]. For example, virulence tends to increase, and onward transmission and pathogen load decrease, with greater evolutionary distance between donor and recipient hosts [10-12] . In addition, clades of closely related species tend to have similar levels of susceptibility independent from their distance to the pathogens natural host [4, 9].

46   When looking at patterns of susceptibility, experimental studies typically try and minimise
47   within species or environmental effects. Often overlooked are differences between males
48   and females, as typically only one sex is utilized to remove between sex differences [13].
49   Sexual dimorphism is seen across most animal systems in a range of life history traits from
50   body size, growth rate, reproductive effort and immunity [14-16]. In mammals, males and
51   females often differ in their pathogen burdens and mortality rates [17]. For example, in
52   SARS-COV-2 infection in humans, women have a lower risk of morbidity and mortality than
53   men [18]. In HIV infected individuals, women have up to 40% lower HIV viral RNA in
54   circulation but a greater likelihood of developing AIDS than men with matched viral loads
55   [19]. Sex biases in parasitism rate in mammals have been suggested to be due to males
56   investing in traits that favour their reproductive success. In support of this, sex biased
57   parasitism is positively correlated with sexual size dimorphism [17]. In insects a comparative
58   analysis found the degree of sex biased parasitism and mortality is explained by an
59   interaction between the mating system (polygynous vs non-polygynous) and sexual size
60   dimorphism [20]. This is consistent with parasites having a greater impact on the survival of
61   male insects compared to females (particularly in polygynous species where males are larger
62   than females). However, many experimental studies of host-parasite interactions do not
63   compare differences between sexes [13]. Furthermore, for most pathogens we have little
64   understanding of whether sex differences are consistent across host species, which has
65   important implications for our understanding of pathogen emergence.
66
67   Despite phylogenetic patterns of host susceptibility having been observed in a range of
68   systems [3, 6-8], we know little about why species vary in their susceptibilities. For example,
69   given equal exposure why do we see high mortality in some species but little in others? One
70   factor that appears important in determining the severity of disease is the tissue tropism of
71   a pathogen. In humans, RNA viruses with neural tropism or generalised systemic tropism
72   tend to result in severe disease [21]. In terms of the patterns of susceptibility across species,
73   virulence may be a consequence of a virus getting into a sensitive tissue or organ resulting in
74   damage by the pathogen directly or by autoimmunity. For example, in bacterial meningitis
75   the pathology is a consequence of bacteria infecting the cerebrospinal fluid and resulting in
76   inflammatory autoimmune damage to the central nervous system [22]. Alternatively, it may
77   be due to the pathogen getting into a particularly permissive tissue type and proliferating to
78   high levels. Virus macroevolutionary change is thought to be driven by cross-species
79   transmission or codivergence rather than by acquiring new niches – or tissues – within a
80   host [23]. Likewise, the host specificities of viruses are thought to be more liable than tissue
81   specificities [24].
82
83   Here we use a Drosophila-virus system to examine the factors underlying susceptibility
84   across host species. Across *Drosophila* species many physiological traits show sexual
85   differentiation [14]. Using a comparative approach, we firstly ask if the patterns of infection
86   seen across the host phylogeny [3, 5, 9, 25] differ between males and females. We infected
87   both males and females of a panel of 31 species of Drosophilidae with Drosophila C Virus
88   (DCV), a positive sense RNA virus in the family Dicistroviridae. Males of *Drosophila*
89   *melanogaster* have previously been reported to have higher viral loads than females [26]
90   and show greater rates of shedding, lower clearance and higher transmission potential of

91 DCV, although these traits can interact with host genotype [27]. Viral load has shown to
92 have a strong positive correlation with mortality across host species [9, 25]. DCV has been
93 reported to show tissue tropism in *D. melanogaster*, with high levels of infection in the heart
94 tissue, fat body, visceral muscle cells around the gut (midgut) and food storage organ (crop)
95 [28, 29]. To test if the same patterns of tissue infection were observed across species, we
96 then made comparisons of the tissue tropism of DCV in 7 species of fly.
97
98 **Methods**
99
100 **Viral Infections**
101 Thirty one species of Drosophilidae were used to examine sex differences in viral infection.
102 Stock populations were reared in the laboratory in multi generation populations, in
103 Drosophila stock bottles (Fisherbrand) on 50 ml of their respective food medium (Table S1)
104 at 22˚C and 70% relative humidity with a 12-hour light-dark cycle. Flies were then collected
105 twice a day in order to try and control for age of maturity in an effort to minimize the
106 chances that flies would have reached sexual maturity and mated before the sexes were
107 separated out. Although no effect of mating status on DCV viral load was previously
108 observed in *D. melanogaster*, this can vary by host genotype and mating status is known to
109 affect susceptibility to other pathogens [27]. examine differences in viral load between
110 males and females , two vials of 0-1 day old males flies and two vials of 0-1 day old female
111 flies were collected daily for each species. Flies were tipped onto fresh vials of food every
112 day to minimise differences in the microbiomes of flies (Broderick & Lemaitre, 2012; Blum *et*
113 *al.*, 2013). All vials were kept for 10 days in order to check for larvae, as a sign of successful
114 mating. Only 4 vials from 3 species were found to contain larvae, these were one vial of
115 *D.sturtevanti,* two vials of bilivittata and one vial of *D.tuberculatus.* After 3 days flies were
116 experimentally infected with DCV. Three replicate blocks were carried out, with each block
117 being completed over consecutive days. The order of experimental infection was
118 randomized each day so that both sex and species were randomised. We carried out three
119 biological replicates for each species for each sex at time zero and 2 days post infection.
120 There was a mean of 17 flies per replicate (range across species = 12-20).
121
122 Viral challenge was carried out by needle inoculation of Drosophila C virus (DCV) strain B6A
123 [30], derived from an isolate collected from *D. melanogaster* in Charolles, France [31]. The
124 virus was prepared as described previously [32]. DCV was grown in Schneider's Drosophila
125 line 2 cells and the Tissue Culture Infective Dose 50 (TCID50) per ml was calculated using the
126 Reed-Muench end-point method. Flies were anesthetized on CO_2 and inoculated using a
127 0.0125 mm diameter stainless steel needle bent to a right angle ~0.25mm from the end
128 (Fine Science Tools, CA, USA). The bent tip of the needle was dipped into the DCV solution
129 (TCID50 = 6.32×10^9) and pricked into the anepisternal cleft in the thorax of the flies [9, 33].
130 This mode of infection is used as it creates a more reproducible infection that oral
131 inoculation, which is found to cause stochastic infection outcomes in *D. melanogaster* [28].
132 Both methods of infection have been shown to produce systemic infections with the same
133 tissues ultimately becoming infected [28].
134

135   To control for relative viral dose between species a time point zero sample of one vial of flies
136   was immediately snap frozen in liquid nitrogen as soon as they were inoculated. The second
137   vial of flies were inoculated and placed onto a fresh vial of food, and returned to the
138   incubator. Two days after challenge (+/- 1 hour) these flies were snap frozen in liquid
139   nitrogen. This time point is chosen as the sampling time point as previous studies show a
140   clear increase in viral growth but little mortality at this point in infection [5, 25]. Each
141   experimental block contained a day 0 and day 2 replicate for each sex and each species (31
142   species × 2 sexes × 3 experimental blocks).

143

144   **Measuring the change in viral load**
145   Using quantitative Reverse Transcription PCR (qRT- PCR) we measured the change in viral
146   load in male and female flies from day 0 to day 2 post- infection. Total RNA was extracted
147   from the snap frozen flies by homogenizing them in Trizol reagent (Invitrogen) using a bead
148   homogenizer for 2 pulses of 10 seconds (Bead Ruptor 24; Omni international) and stored at
149   −70°C for later extraction. Samples were defrosted and RNA extracted as described
150   previously [25]. Briefly, Trizol homogenized flies were processed in a chloroform isopropanol
151   extraction, eluted in water and reverse- transcribed with Promega GoScript reverse
152   transcriptase (Sigma) and random hexamer primers. Quantification of the change in viral
153   RNA load was calculated in relation to a host endogenous control, the housekeeping gene
154   *RpL32*. Primers were designed to match the homologous sequence for each of the
155   experimental species that crossed an intron– exon boundary so will only amplify mRNA. qRT-
156   PCR was carried out on 1:10 diluted cDNA using Sensifast Hi-Rox Sybr kit (Bioline). Two qRT-
157   PCR reactions (technical replicates) were carried out per sample with both the viral and
158   endogenous control primers. All melt curves were checked to verify that the correct
159   products were being amplified. All experimental plates had experimental replicates
160   distributed across the plates in a randomized block design to control for between plate
161   differences. Each qRT- PCR plate contained three standard samples. A linear model which
162   included plate ID and biological replicate ID was used to correct the cycle threshold (C_t)
163   values between plates. Any technical replicates had C_t values more than two cycles apart
164   after the plate correction were repeated. Change in viral load was calculated as the mean C_t
165   value of the pairs of technical replicates. We then used these to calculate the ΔC_t as the
166   difference between the cycle thresholds of the viral DCV qRT- PCR and the RpL32
167   endogenous control for each sample. The Ct of the day 2 flies relative to day 0 flies was then
168   calculated as, $2^{-\Delta\Delta Ct}$; where $\Delta\Delta C_t = \Delta C_t$ day0 − ΔC_t day2.

169

170   **Body Size**
171   We measured wing size of the flies to control for between species and sex differences in
172   body size. In Drosophilidae wing length has been shown to be a good proxy for body size
173   (Huey *et al.*, 2006). For measurement wings were removed from a mean of 15 male and
174   females flies of each species (range 10–18), stored in 80% ethanol, and later photographed
175   under a dissecting microscope. The length of the IV longitudinal vein from the tip of the
176   proximal segment to where the distal segment joins vein V was recorded Using ImageJ
177   software (version 1.48), and the mean taken for each sex of each species.

178

179   **Inferring the host phylogeny**

180 We used a previously inferred phylogenetic tree [5] using seven genes (mitochondrial; *COI,*
181 *COII*, ribosomal; *28S* and nuclear; A*dh, SOD, Amyrel, RpL32*). Briefly, we download publicly
182 available sequences from Genbank and where these were not available they were Sanger
183 sequenced from our laboratory stocks. For each gene the sequences were aligned in
184 Geneious (version 9.1.8, www.geneious.com) (Kearse *et al.*, 2012) using the global alignment
185 setting, with free end gaps and 70% similarity IUB cost matrix. The phylogeny was inferred
186 using these genes and the BEAST programme (v1.10.4) (Drummond *et al.*, 2012). Genes
187 were partitioned into three groups; mitochondria, ribosomal and nuclear, each with
188 separate relaxed uncorrelated lognormal molecular clock models using random starting
189 trees. Each of the partitions used a HKY substitution model with a gamma distribution of
190 rate variation with 4 categories and estimated base frequencies. Additionally, the
191 mitochondrial and nuclear data sets were partitioned into codon positions 1+2 and 3, with
192 unlinked substitution rates and base frequencies across codon positions. The tree-shape
193 prior was set to a birth-death process. We ran the BEAST analysis three times to ensure
194 convergence for 1000 million MCMC generations sampled every 10000 steps. On completion
195 the MCMC process was examined by evaluating the model trace files using the program
196 Tracer (version 1.7.1) (Rambaut *et al.*, 2014) to ensure convergence and adequate sampling.
197 The consensus constructed tree was then visualised using FigTree (v1.4.4) (Rambaut, 2006).
198
199 **Tissue Tropism**
200 In order examine patterns of tissue infection across species we infected 7 species of flies
201 used above; *D. melanogaster, D. stuventi, S. lativaitata, D.pseudooscura, D. virilis, D.*
202 *prosaltans* and *D.littoralis*). Male flies were infected with DCV using the same inoculation
203 method as described above. Two days post infection flies were placed on ice to sedate them,
204 they were then surface sterilized in ice-cold 70% ethanol before being dissected. The head,
205 crop, gut (all parts), malpighian tubules, sex organs (testis and accessory glands) and
206 abdominal cuticle including the attached fat body (hereafter referred to as body) were
207 dissected from each male fly and placed into individual tubes on ice. Six individual flies were
208 pooled per replicate and then snap frozen in liquid nitrogen for later RNA extraction. For
209 each species there were six replicate pools of each of the six tissue types. At the same time
210 as the dissections were carried out whole flies were snap frozen for a "whole fly"
211 comparative viral load measure. All samples were processed as per the methods for viral
212 load quantification as described above.
213
214 **Statistical analysis**
215 *Sex differences*
216 Viral load in males and females were analysed using phylogenetic mixed models. We fitted
217 all models using a Bayesian approach in the R package MCMCglmm [34, 35]. We used a
218 multivariate model with viral load of each sex as the response variable.
219
220 The models took the form of:
221   (1) $y_{hit} = \beta_{1:t} + wingsize\beta_{2:t} + u_{p:ht} + u_{s:ht} + e_{hit}$
222   (2) $y_{hit} = \beta_{1:t} + wingsize\beta_{2:t} + u_{p:ht} + e_{hit}$
223

224   where y is the change in viral load of the *i*th biological replicate of host species *h*, for trait *t*
225   (male or female). *β* are the fixed effects, with *β₁* being the intercepts for each trait and *β₂*
226   being the effect of wing size. u_p are the random phylogenetic species effects, and *e* the
227   model residuals. Models were also run which included the mating status of the species as a
228   fixed effect. We included this as a binary response for any species that had offspring in at
229   least one replicate vial (we only found evidence of mating for three species: *D.sturtevanti,*
230   *S.lativittata* and *Z.tuberculatus).* Model (1) also includes a species-specific component
231   independent of the phylogeny $u_{s:ht}$ that allow us to estimate the proportion of variation
232   that is not explained by the host phylogeny v_s (Longdon et al., 2011). However, this was
233   removed from model (2) as model (1) failed to separate the phylogenetic and strain -specific
234   effects. The main model therefore assumes a Brownian motion model of evolution
235   (Felsenstein, 1973). The random effects and the residuals are assumed to follow a
236   multivariate normal distribution with a zero mean and a covariance structure **V$_p$** $\otimes$ **A** for the
237   phylogenetic affects and **V$_e$** $\otimes$ **I** for the residuals, **Vs** $\otimes$ **I** for species-specific effects, ($\otimes$ here
238   represents the Kronecker product). **A** is the phylogenetic relatedness matrix, **I** is an identity
239   matrix and the **V** are 2×2 (co)variance matrices describing the (co)variances between viral
240   load of the two sexes. The phylogenetic covariance matrix, **V$_p$**, describes the phylogenetic
241   inter-specific variances in each trait and the inter-specific covariances between them, **V$_s$**, the
242   non-phylogenetic between-species variances. The residual covariance matrix, **V$_e$**, describes
243   the within-species variance that can be both due to real within-species effects and
244   measurement or experimental errors. The off-diagonal elements of **V$_e$** (the covariances) are
245   not estimable because each vial only contains one sex and therefore no vial has multiple
246   measurements, so were set to zero. The MCMC chain was run for 1,300 million iterations
247   with a burn- in of 30 million iterations and a thinning interval of 1 million. All the models
248   were run with different prior structures (as in [5]) in order to test results for sensitivity to
249   the use of priors, but note they all gave similar results.
250
251   The proportion of between strain variance that can be explained by the phylogeny was
252   calculated from model (1) using the equation $\frac{V_p}{V_p + V_s}$, where V_p and V_s represent the
253   phylogenetic and strain-specific components of between-strain variance respectively, and is
254   equivalent to phylogenetic heritability or Pagel's lambda [36, 37]. The repeatability of
255   susceptibility measurements was calculated from model (2) as $\frac{V_p}{V_p + V_e}$, where V_e is the
256   residual variance. Inter-strain correlations in viral load between each method were
257   calculated from model (2) V_p matrix as $\frac{cov_{x,y}}{\sqrt{var_x + var_y}}$ and the slopes (β) of each relationship as
258    $\frac{cov_{x,y}}{var_x}$. Parameter estimates stated below are means of the posterior density, and 95%
259   credible intervals (CIs) were taken to be the 95% highest posterior density intervals
260   *Tissue tropism*
261
262   Viral load data across species and tissues was analysed using a linear mixed effects model
263   using the lmer function in the lme4 package in R [35, 38] with models compared using the
264   anova function. Tissue type, species and their interaction were included as fixed effects and
265   experimental replicate as a random effect to account for the individual pool that each set of

266   tissues came from. With only seven species there is little power to carry out models
267   controlling for phylogeny which is why species was fitted as a fixed effect.
268
269   **Data availability**
270
271   All data and scripts are available at dx.doi.org/10.6084/m9.figshare.21437223.
272   .
273   **Results**
274
275   **Sex differences in viral load**
276   To examine if the sexes respond the same way to viral infection we infected 31 species of
277   Drosophilidae with DCV and quantified the change in viral load at 2 days post infection using
278   qRT-PCR. In total we infected 6324 flies across 186 biological replicates (biological replicate
279   = change in viral load from day 0 to day 2 post-infection), with a mean of 17 flies per
280   replicate (range across species = 12-20).
281
282   The mean change in viral load across all species was similar between the sexes (females =
283   12.59, 95% CI = 1.16, 23.80; males = 12.93, 95% CI = -0.65, 26.25). We found strong positive
284   interspecific correlation between the viral load of females and males (correlation = 0.92,
285   95% CI = 0.78, 1.00; Figure 1). The estimate of the slope is close to 1 (β = 0.99, 95% CI= 0.58,
286   1.38) suggesting males and females respond similarly to infection.
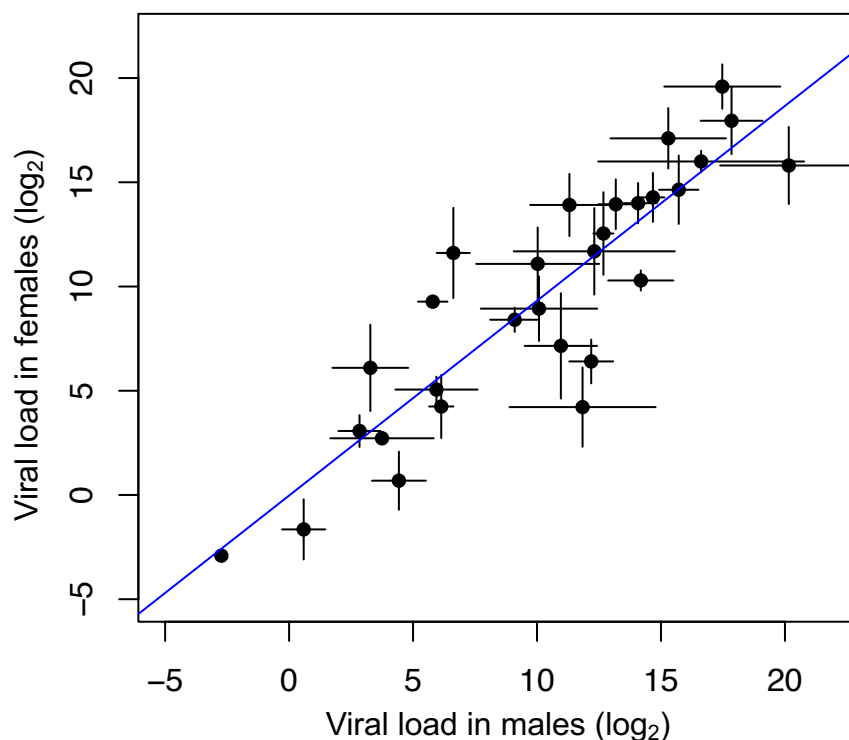
287
288   **Figure 1. Correlation between viral load in males and females.** Each point represents a
289   species mean, error bars show standard errors and the trend line is estimated from a linear
290   model.
291

292 The full model including the species-specific random effect independent of the host
293 phylogeny ($u_{s:ht}$) allowed us to calculate the proportion of the variation between the
294 species that can be explained by the phylogeny ($\frac{V_p}{V_p + V_s}$), equivalent to phylogenetic
295 heritability or Pagel's lambda [36, 37]. The host phylogeny explains a large proportion of the
296 inter-specific variation for both males and females (females = 0.68, 95% CIs: 0.06, 0.99;
297 males = 0.66, 95% CIs: 0.04, 0.99) consistent with previous findings for males [3, 5, 9, 25].
298 However, we note these estimates have broad confidence intervals, due to the model
299 struggling to separate out the phylogenetic and non-phylogenetic components. The
300 repeatability of viral load across species was relatively high for both sexes (females = 0.63,
301 95% CIs = 0.41, 0.80; males = 0.52, 95% CIs = 0.31, 0.74). We found no effect of either body
302 size (-0.05, 95% CI's = -0.28, 0.19) or mating status (0.34, 95% CI's = -5.63, 6.27) on viral load.
303
304 **Tissue Tropism**
305 To look at the how the tissue tropism of DCV varied across host species, we infected seven
306 species of fly with DCV and dissected them into six tissue types. We found large effects of
307 species on viral load (χ^2= 320.65, d.f=6, P<0.001) with >18 million fold difference in viral load
308 between the least and most susceptible species. Tissues differed in their viral loads to a
309 lesser extent, with the maximum difference being seen in *D. pseudobscura* with an
310 approximately 550 fold difference in viral load between the least and most susceptible
311 tissues (χ^2= 15.264, d.f=4, P=0.009). There was no evidence of tissues showing different
312 patterns of susceptibility in different hosts i.e. no evidence for a tissue-by-species
313 interaction (χ^2=41.515, d.f=30, P=0.079).
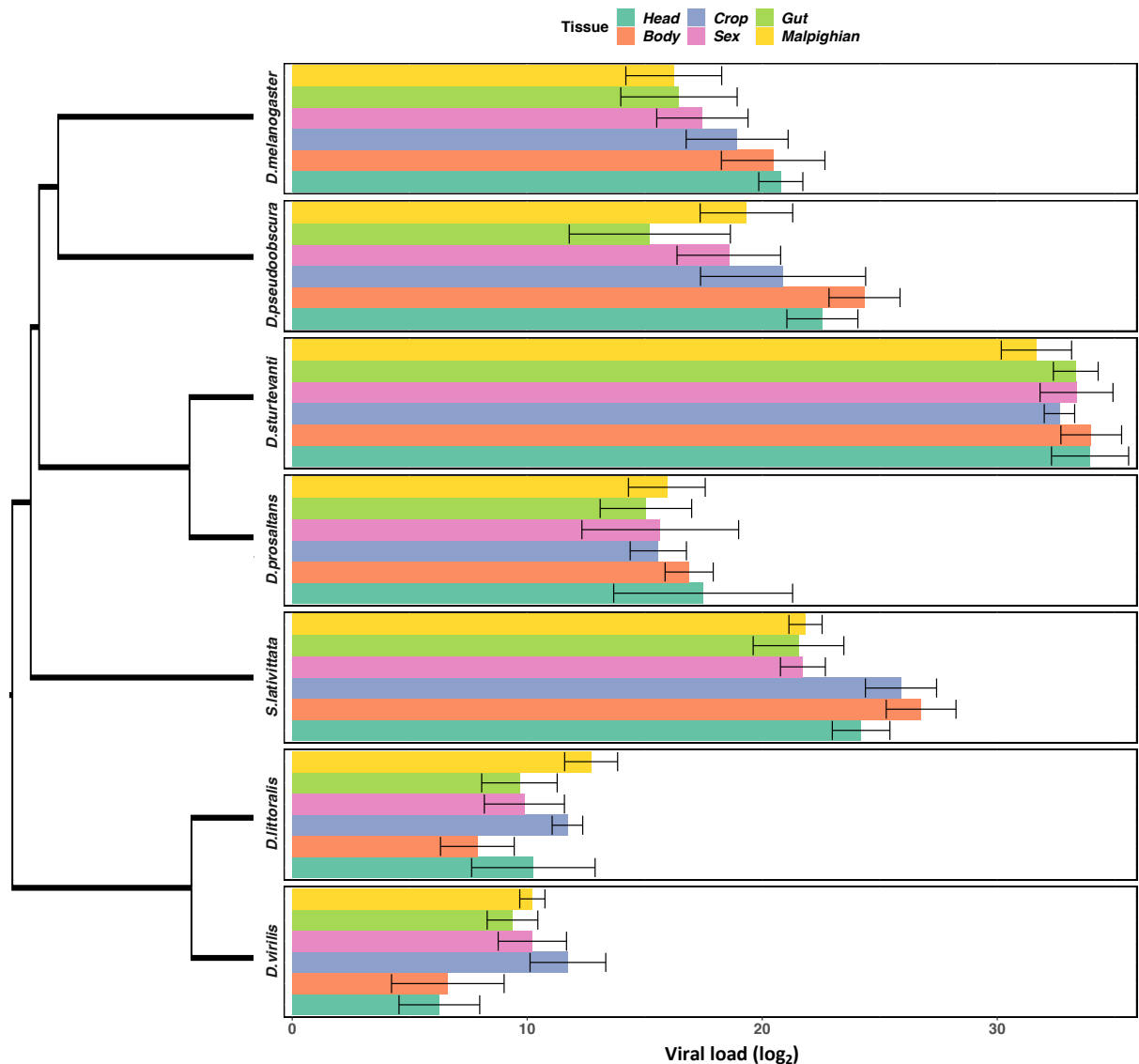
314
315 **Figure 2: Tissue tropism data from DCV infected flies.** Flies were dissected into the six tissue
316 types 2 days post-infection before undergoing RNA extraction and quantification of viral
317 load. On the right is the phylogeny of the host species. The bars of the individual panels are
318 organised following the order of the *D. melanogaster* ranked from the tissue with lowest to
319 highest viral load. Error bars show standard errors.
320
321 **Discussion**
322
323 We found that viral susceptibility between females and males of 31 host species showed a
324 strong positive correlation with a close to 1:1 relationship, suggesting that susceptibility
325 across species is not sex specific. We also found differences in viral load between tissues of
326 seven host species, but no evidence of tissues showing different patterns of susceptibility in
327 different host species.
328
329 A difference between the sexes in immune function and resistance has been in found in a
330 range of studies [15, 39]. However, this is not universal, and interactions between the host,

331   pathogen and environmental factors can alter the outcome of infections [40]. Previous
332   meta-analyses have found mixed results. For example, some studies of arthropods have
333   found little evidence for consistent sex differences in parasite prevalence or intensity [41]
334   (although these were largely from natural infections which may have inherently greater
335   sources of variation). Likewise, a phylogenetically controlled meta-analysis of 105 species
336   (including 30 insect species) of immune responses found no evidence for sex biases. Other
337   studies have reported a small male bias in parasitism rates for polygynous insects, but a
338   significant female bias for non-polygynous species, with the extent of sex bias parasitism
339   increasing with the degree of sexual size dimorphism [20]. However, other studies of insects
340   (with smaller number of species) have reported sex differences in some immune traits [15,
341   20]. In mammals, males have been shown to often have greater pathogen burdens, with
342   parasitism rates positively correlated to male biased sexual size dimorphism [15-17, 20].
343
344   In *Drosophila melanogaster*, some previous studies have reported males have higher DCV
345   viral loads than females [26]. However, others found no effect of sex on viral load, but did
346   find effects of sex on viral shedding, clearance, and transmission potential, with these traits
347   showing interactions with host genotype [27]. Sexual dimorphism in infection avoidance
348   behaviour has also been reported, when female flies previously exposed to DCV were found
349   to prefer a clean food source indicating a potentially important dimorphism in infection
350   avoidance [42]. Here, we used controlled experimental conditions, but in nature sex
351   differences in behaviour, or how the sexes interact with the environment may lead to
352   differences in pathogen load.
353
354   The tissue tropism results here show that susceptibility in a given host is general across
355   tissue types – for example *D. sturtevanti* has a high viral load across all tissues whereas *D.*
356   *virilis* has relatively low viral loads in all tissues (Figure 2). Mortality to DCV infection has
357   previously been shown to show a strong positive correlation with viral load [9, 25]. The data
358   presented here show the susceptibility of a given species is general across all tissue types.
359   This does not exclude the possibility that pathology is due to high viral loads in a given
360   tissue, but does suggest that the mechanism restricting viral load is general across tissues.
361   This may be linked to the ability of the virus to bind to or enter hosts cells, utilise the hosts
362   cellular components for replication or to avoid or supress the host immune response [43].
363   Comparative studies of human viruses have identified the tissue tropism of viruses to be a
364   significant determinant of virulence; viruses that cause systemic infections (across multiple
365   organs) or that have neural or renal tropisms are most likely to cause severe virulence [21].
366   It has been suggested that high levels of non-adaptive virulence can be the result of
367   pathogens infecting tissues that do not contribute to onward transmission [22]. Other
368   studies have shown differences in host physiology can be important in determining the
369   virulence of a novel pathogen [10]. However, further understanding of how infection results
370   in pathology (i.e. in which tissue the disease tropism occurs [44]) and how virulence is
371   correlated with transmission potential in infections, is needed to explore this further.
372
373   In summary, our results demonstrate that in this system there is little evidence for sexual
374   dimorphism in susceptibility to viral infection across species. As such susceptibility in one sex
375   is predictive of that in the other. We find that susceptibility of a species is general across

376   tissue types, suggesting virulence is not due to species specific differences in viral tropism.
377   Further work is needed to explore how sex differences can vary with the environment and
378   pathogen type, and the underlying mechanisms as to why species vary in their susceptibility.
379
380

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398

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543
544   **Supplementary information**
545
546   **Table S1**: Full list of species used in the sex difference experiment and their rearing food for
547   stock populations. All cornmeal and proprionic medium have dried yeast sprinkled onto the
548   surface of the food, other food types do not unless stated below. The recipes for the food
549   types are described here https://doi.org/10.1371/journal.ppat.1004728.s001
550

Species	Food
D.affinis	Malt
D.americana	Malt
D.ananassae	Cornmeal
D.arizonae	Banana
D.buzzatii	Malt
D.erecta	Malt + yeast
D.flavomontana	Malt + yeast
D.hydei	Cornmeal
D.immigrans	Malt + yeast
D.lacicola	Malt
D.littoralis	Banana
D.mauritiana	Proprionic
D.melanogaster	Cornmeal

D.montana	Malt + yeast
D.novamexicana	Banana
D.obscura	Proprionic
D.persimilis	Malt
D.prosaltans	Proprionic
D.pseudoobscura	Malt
D.putrida	Proprionic
D.santomea	Cornmeal
D.sturtevanti	Cornmeal
D.takahashii	Cornmeal
D.teissieri	Cornmeal
D.virilis	Proprionic
H.duncani	Proprionic
S. lativittata	Banana
S.lebanonensis	Proprionic
Z. inermis	Banana
Z. taronus	Banana
Z. tuberculatus	Banana

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