Diet has no effect on susceptibility to an RNA virus across host species

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Abstract

The likelihood of a successful host shift of a parasite to a novel host species can be influenced by environmental factors that can act on both the host and parasite. Factors such as resource availability or temperature can drive changes in species distributions and abundances making encounters between infected and susceptible individuals more likely. They can also act as stressors that can change the physiology of the interacting parties, including altering the host immune response or varying parasite virulence. Changes in nutritional resource availability have been shown to alter pathogen susceptibility and the outcome of infection in a range of systems. Here we infected 27 species of Drosophilidae with an RNA virus on three different food types of differing protein to carbohydrate ratios. We then measured how viral load and mortality across the species was affected by changes in diet. We found that changes in the ratio of protein to carbohydrate in the diet did not alter the outcomes of infection, with strong positive inter-species correlations in both viral load and mortality across diets, and a strong positive correlation between mortality and viral load.

Introduction

A key driver of pathogen host shifts – where a pathogen jumps from one host species to another – is environmental change (Carlson et al., 2020; Hoberg & Brooks, 2015). Ecological factors can influence the likelihood of host shifts by altering species distributions and abundances making encounters more likely, or by acting as stressors that alter physiological factors including immunity or virulence. The main ecological factor studied has been temperature, which can have asymmetrical impacts on hosts and parasites and potentially altering the likelihood of host shifts (Brooks & Hoberg, 2007; Hoberg & Brooks, 2015; Kirk et al., 2018; Roberts, Hadfield, Sharma, & Longdon, 2018). The role of other ecological traits such as resource availability, humidity, population density and geographical range, or within host ecological traits such as metabolic rate, have been less well studied in explaining the outcomes of host shifts, despite an increasing understanding of the role such factors play in effecting the outcomes of more simple host parasite interactions (Blanford & Thomas, 1999; Cumnock et al., 2018; Harvell et al., 2002; Hayman, Pulliam, Marshall, Cryan, & Webb, 2016; Ponton et al., 2013).

Nutrition can shape the outcome of host-parasite interactions through its ability to moderate both parasite virulence and host resistance (Pike, Lythgoe, & King, 2019; Ponton, Wilson, Cotter, Raubenheimer, & Simpson, 2011; Ponton et al., 2013). The nutritional resources of a host can impact its ability to resist infection as immune responses are thought to be costly to both maintain and activate (Sheena C. Cotter, Simpson, Raubenheimer, & Wilson, 2011; Knutie, Wilkinson, Wu, Ortega, & Rohr, 2017; Kraaijeveld & Godfray, 1997; Kutzer & Armitage,

2016a; Lochmiller & Deerenberg, 2000; McKean, Yourth, Lazzaro, & Clark, 2008). Nutrition is known to have long term consequences, with developmental nutritional status been known to have latent or even trans-generational effects on immune responses (Fellous & Lazzaro, 2010; Grueber, Gray, Morris, Simpson, & Senior, 2018; Savola, Vale, & Walling, 2020). Hosts can also show behavioural modifications in feeding upon infection; parasite-induced anorexia is thought to be an adaptive host response (Ayres & Schneider, 2009; Rao et al., 2017) and in some cases hosts actively increase the consumption of certain nutrients in their diet (Povey, Cotter, Simpson, Lee, & Wilson, 2009). Nutrition may also constrain the amount of investment that a host can allocate to an energetically demanding immune response (Sheena C. Cotter et al., 2011; Knutie et al., 2017; Kraaijeveld & Godfray, 1997; Lochmiller & Deerenberg, 2000), and subsequent ability to cope with costs associated with a parasite burden if infection does become established (Sheldon & Verhulst, 1996). A suboptimal nutritional status may lead a host to be unable to suppress or tolerate a parasite challenge they may otherwise have been able to resist; or suffer reduced fitness imposed by trade-offs with resources from life history traits (Sheena C. Cotter et al., 2011; Knutie et al., 2017; Kraaijeveld & Godfray, 1997; Lochmiller & Deerenberg, 2000).

From a parasite perspective infecting a host of suboptimal nutritional status may mean they encounter a weaker immune response and therefore infection and establishment is easier. However, once established the parasite may encounter its own resource limitations due to competition with an already depleted host, causing suboptimal growth and potential onward transmission. Therefore, predicting the outcome of the interaction between nutrition, host immunity and

subsequent resistance is complex as the effects on the two parties may be divergent.

Multiple life history traits are moderated by resource availability, with condition dependency across reproductive traits, aging and lifespan (Camus, Fowler, Piper, & Reuter, 2017; Y. Henry, Overgaard, & Colinet, 2020; Youn Henry, 2018; Lee, Simpson, & Wilson, 2008; Maklakov et al., 2009). Laboratory experiments on dietary restriction, where individuals experienced a reduction in nutrition without inducing malnutrition (differentiated from Calorie Restriction) have been found to extend life span in a range of organisms (Anderson et al., 2003; Klass, 1983; Nakagawa, Lagisz, Hector, & Spencer, 2012; Weindruch & Walford, 1982). The effects of dietary restriction appear to be explained by resource-mediated trade-offs between longevity and reproductive effort (but see review by Moatt *et al.*, 2020). Geometric frameworks – the use of artificial diets with known compositions of specific nutrients that develop an understanding of dimensional nutrient space – have been used to examine the consequences of different ratios of macronutrients across a range of organisms (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Jensen, McClure, Priest, & Hunt, 2015; Le Couteur et al., 2016; Lee et al., 2008; Raubenheimer & Simpson, 1999; S. J. Simpson & Raubenheimer, 1995; Stephen J. Simpson & Raubenheimer, 2011; Skorupa, Dervisefendic, Zwiener, & Pletcher, 2008). Across multiple species low protein to carbohydrate ratios reduce reproductive rates but appear to maximise lifespan (Nakagawa et al., 2012). However, individuals with diets higher in protein and lower in carbohydrates have greater reproductive rates but shorter

life spans. Additionally when given a choice of diet, individuals have been shown to optimise their reproduction over lifespan (Bunning et al., 2016).

To investigate the effect that host diet has on the likelihood of pathogen host shifts we infected 27 species of Drosophilidae with Drosophila C Virus (DCV; Dicistroviridae) fed on three diets with varying ratios of protein to carbohydrates but comparable calorie content. We then measured the change in viral load and host mortality across these different diets. DCV can cause nutritional stress in the midgut of flies causing similar pathologies to those seen in starvation (Chtarbanova et al., 2014). DCV shows specific tissue tropism of infection in the visceral muscle cells around the midgut and food storage organ (crop) causing reduced defecation, food blockage and dehydration/starvation (Arnold, Johnson, & White, 2013; Chtarbanova et al., 2014). If hosts are in a nutritional environment that allows for investment in immune function, they may be more able to resist establishment off, or potentially tolerate, a novel infection. This could then lead to different outcomes following a host shift depending on resource environment. Either the host could manage to avoid infection entirely or could become infected and minimise parasite damage, tolerating infection whilst withstanding a level of parasite growth. Alternatively hosts may be fully susceptible to infection, and enriched resources may act to enhance pathogen virulence by enabling within host pathogen growth. We therefore examined whether changes in nutritional environment alter the susceptibility to DCV following a host shift, measuring both viral load and host mortality, and establishing whether patterns are consistent across species and diet.

125

126 **Methods**

127 **Diet preparation**

128 Three different cornmeal diets were used in order to establish experimental
129 diets (Table 1). The standard cornmeal diet used in our lab comprised a 1:10
130 protein to carbohydrate ratio and became our "Medium" - protein: carbohydrate
131 ratio diet treatment. We also developed two further diets that were
132 approximately isocaloric (Table 1), a low protein: carbohydrate food (1: 20
133 protein to carbohydrate) and a high protein: carbohydrate food (1: 5 protein
134 carbohydrate). These were based around previous findings that suggested that
135 in *D. melanogaster* lifespan was maximized on a protein: carbohydrate ratio of
136 around 1:16, and fitness - measured as lifetime egg production at an
137 intermediate ratio of 1:4 (Lee et al., 2008). All diets were manipulated by altering
138 the dextrose and yeast amounts whilst maintaining as close as possible the same
139 calorie content at 142 Calories g/100 m. Yeast was manipulated as it provides
140 the majority of the protein as well as other non-caloric nutritional requirements
141 (Piper, 2017). Values were confirmed using the Drosophila Dietary Composition
142 Calculator (Lesperance & Broderick, 2020).

143

144 **Viral Infections**

145 Twenty-seven different species of Drosophilidae were maintained in multi
146 generation populations, in Drosophila stock bottles (Dutscher Scientific) on 50
147 ml of their respective food medium at 22˚C and 70% relative humidity with a 12-
148 hour light-dark cycle (See *Supplementary for species and food*). Each day, two
149 vials of 0-1 day old male flies (15-22 flies per vial) were randomly assigned to

150 one of three potential food types; low, medium or high, protein: carbohydrate
151 ratio (Table 1). Flies were kept on their respective food treatments for 5 days,
152 and tipped onto fresh vials of food every day (Blum, Fischer, Miles, &
153 Handelsman, 2013; Broderick & Lemaitre, 2012). After 5 days of acclimatisation
154 on their food treatment flies were experimentally infected with DCV. These
155 collections and inoculations were carried out over three replicate blocks, with
156 each block being completed over consecutive days. The order that the species
157 were infected was randomized each day, as was food treatment for each species.
158
159 We used Drosophila C virus (DCV) strain B6A (Longdon et al., 2018), which is
160 derived from an isolate collected from *D. melanogaster* in Charolles, France
161 (Jousset, Plus, Croizier, & Thomas, 1972). The virus was prepared as described
162 previously (Longdon, Cao, Martinez, & Jiggins, 2013). Briefly, DCV was grown in
163 Schneider's Drosophila line 2 cells and the Tissue Culture Infective Dose 50
164 (TCID50) per ml was calculated using the Reed-Muench end-point method. Flies
165 were anesthetized on CO_2 and inoculated using a 0.0125 mm diameter stainless
166 steel needle that was bent to a right angle ~0.25mm from the end (Fine Science
167 Tools, CA, USA). The bent tip of the needle was dipped into the DCV solution
168 (TCID50 = 6.32×10^9) and pricked into the pleural suture on the thorax of the flies
169 in order to standardize infection (Longdon et al., 2015). We selected this route of
170 infection as oral inoculation has been shown to lead to stochastic infection
171 outcomes in *D. melanogaster*, and injection has been show to follow a similar
172 course of infection (Ferreira et al., 2014). One vial of inoculated flies was
173 immediately snap frozen in liquid nitrogen to provide time point zero samples to
174 be used as a reference and therefore control for relative viral dose. The second

175 vial of flies were infected and then placed back into a new vial of their respective
176 food treatment. After 2 days (+/- 1 hour) flies were snap frozen in liquid
177 nitrogen. This time point was chosen based on previous studies that show a clear
178 increase in viral growth but little mortality at 2 days post infection (Longdon et
179 al., 2015; Roberts et al., 2018). Each experimental block contained a day 0 and
180 day 2 replicate for each species, at each diet (27 species × 3 diet treatments × 3
181 experimental blocks). In total, we quantified viral load in 7580 flies in 474
182 biological replicates (biological replicate = change in viral load from day 0 to day
183 2 post-infection), with a mean of 16 flies per replicate (range across species = 8-
184 28).
185
186 **Survival**
187 In order to measure the effect of diet on virulence we also carried out a survival
188 assay where mortality was recorded following infection. The same infection
189 protocol was carried out as above; one vial of flies was infected with DCV whilst
190 another was injected using a clean virus free needle dipped in *Drosophila*
191 Ringer's solution as a control treatment (Sullivan, Ashburner, & Hawley, 2000).
192 Flies were maintained in vials as described above and tipped onto their
193 respective fresh food every 2 days. The number of dead flies was counted every
194 day for 20 days. The survival assay was carried out across three blocks with
195 infections carried out over consecutive days, to obtain a control and virus
196 challenged vial per species each day. Treatment (virus challenged or control
197 sham infected) and the order in which fly species were inoculated were
198 randomized between blocks. Diet was randomized across days, so for a given
199 food type a control and viral challenged vial was completed each day, and this

200 was repeated over consequent days until there was a control and challenged for
201 each species on each food type (27 species × 2 treatments (control / challenged)
202 × 3 diet treatments × 3 experimental blocks). In total, we measured mortality in
203 9222 flies with a mean of 20 flies per replicate (range across species: 6–30 flies).
204

205 **Measuring the change in viral load**

206 The change in RNA viral load from day 0 to day 2-post infection was measured
207 using quantitative Reverse Transcription PCR (qRT-PCR). Frozen flies were
208 homogenised in Trizol reagent (Invitrogen) using a bead homogeniser for 30
209 seconds (Bead Ruptor 24; Omni international) and stored at -80˚C for later
210 extraction. Total RNA was extracted from the Trizol homogenised flies in a
211 chloroform isopropanol extraction, reverse-transcribed with Promega GoScript
212 reverse transcriptase and random hexamer primers. Viral RNA load was
213 expressed relative to the endogenous control housekeeping gene *RpL32*. Primers
214 were designed to match the homologous sequence in each species and crossed
215 an intron-exon boundary so will only amplify mRNA. The primers in *D.*
216 *melanogaster* were *RpL32* qRT-PCR F (5'-TGCTAAGCTGTCGCACAAATGG -3') and
217 *RpL32* qRT-PCR R (5'- TGCGCTTGTTCGATCCGTAAC -3'). DCV primers were 599F
218 (5'-GACACTGCCTTT GATTAG-3') and 733R (5'CCCTCTGGGAACTAAATG-3') as
219 previously described (Longdon et al., 2015). Two qRT-PCR reactions (technical
220 replicates) were carried out per sample with both the viral and endogenous
221 control primers, with replicates distributed across plates in a randomised block
222 design. qRT-PCR was performed on an Applied Biosystems StepOnePlus system
223 using Sensifast Hi-Rox Sybr kit (Bioline) with the following PCR cycle: 95˚C for 2
224 min followed by 40 cycles of: 95˚C for 5 sec followed by 60˚C for 30 sec. Each

225 qRT-PCR plate contained four standard samples. A linear model was used to
226 correct the cycle threshold (Ct) values for differences between qRT-PCR plates.
227 Samples where the technical replicates had Ct values more than 2 cycles apart
228 after plate correction were repeated. To estimate the change in viral load, we
229 first calculated ΔCt as the difference between the cycle thresholds of the DCV
230 qRT-PCR and the RpL32 endogenous control. For each species the viral load of
231 day 2 flies relative to day 0 flies was calculated as $2^{-\Delta\Delta Ct}$; where ΔΔCt = ΔCt day0 –
232 ΔCt day2. The ΔCt day 0 and ΔCt day 2 is a pair of ΔCt values from a day 0
233 biological replicate and a day 2 replicate.
234

235

Diet	Ratio Protein: Carb	Cornmeal (g)	Dextrose (g)	Yeast (g)	Agar (g)	Nipagin (ml)	dH20 (L) Calories	Calories per 100ml
High	**1:5**	**176**	**131.2**	**84**	**22**	**29**	**1**	**142.60**
Normal	**1:10**	**176**	**176**	**38**	**22**	**29**	**1**	**142.30**
Low	**1:20**	**176**	**203**	**10**	**22**	**29**	**1**	**142.02**

236 **Table 1: Ingredients for the experimental diet treatments**. Amounts given
237 are enough to produce ~100 vials of food, with calculated calories per 100ml.
238
239 **Effect of Body Size**
240 To account for any potential differences in body size between species, we
241 measured wing length as a proxy for body size (Huey et al., 2006). During the
242 collections for the viral load assay males of each species were collected and
243 immediately stored in ethanol. Subsequently, wings were removed and
244 photographed under a dissecting microscope. Using ImageJ software (version
245 1.48) the length of the IV longitudinal vein from the tip of the proximal segment
246 to where the distal segment joins vein V was recorded, and the mean taken for
247 each species, overall there was a mean of 28 wings measured per species (range
248 20–35).
249
250 **Host phylogeny**
251 The host phylogeny was inferred as described in Longdon *et al.* (Longdon et al.,
252 2015) using seven genes (mitochondrial; *COI, COII*, ribosomal; *28S* and nuclear;
253 *Adh, SOD, Amyrel, RpL32*). Publicly available sequences were downloaded from

254 Genbank or were Sanger sequenced. In total we had *RpL32* sequences for all 27
255 species, *28S* from 24 species, *Adh* from 24 species, *Amyrel* from 15 species, *COI*
256 from 27 species, *COII* from 27 species and *SOD* from 12 species. For each gene
257 the sequences were aligned in Geneious (version 9.1.8) (Kearse et al., 2012)
258 using the global alignment setting, with free end gaps and a cost matrix of 70%
259 similarity (*See supplementary for table of sequences used*). The phylogeny was
260 inferred using BEAST (v1.10.4) (Drummond, Suchard, Xi, & Rambaut, 2012),
261 with genes partitioned into three groups; mitochondria, ribosomal and nuclear,
262 with their own molecular clock models. A random starting tree was used, with a
263 relaxed uncorrelated lognormal molecular clock. Each of the partitions used a
264 HKY substitution model with a gamma distribution of rate variation with 4
265 categories and estimated base frequencies. Additionally, the mitochondrial and
266 nuclear data sets were partitioned into codon positions 1+2 and 3, with unlinked
267 substitution rates and base frequencies across codon positions. The tree-shape
268 prior was set to a birth-death process. The BEAST analysis was run twice to
269 ensure convergence for 1000 million MCMC generations sampled every 10000
270 steps. On completion the MCMC process was examined using the program
271 Tracer (version 1.7.1) (Rambaut, Suchard, Xie, & Drummond, 2014) to ensure
272 convergence and adequate sampling, and the constructed tree was then
273 visualised using FigTree (v1.4.4) (Rambaut, 2006).
274
275 **Statistical analysis**
276 We used phylogenetic mixed models to look at the effects of host relatedness on
277 mortality and viral load across the three diet treatments. We fitted all models
278 using a Bayesian approach in the R package MCMCglmm version 2.29 (Hadfield,

2010) in RStudio (R version 3.5.1) (R Development Core Team, 2005). We used a multivariate model with mortality of the controls, mortality of the virus infected flies and viral load at each of the diets as the response variables. Mortality was calculated as the mean portion of flies alive each day for each vial. The model took the following form:

$$y_{hit} = \beta_{1:t} + wingsize\beta_{2:t} + u_{p:ht} + e_{hit}$$

Where y is the change in viral load of the ith biological replicate of host species h, for trait t. β are the fixed effects, with β_1 being the intercepts for each trait and β_2 being the effect of wing size. u_p are the random phylogenetic species effects and e the model residuals. The models were also run with a non-phylogenetic random species effect ($u_{s:ht}$) that allow us to estimate the proportion of variation the host phylogeny explains (Longdon, Hadfield, Webster, Obbard, & Jiggins, 2011). The main model was run without this term as it struggled to separate the phylogenetic and non-phylogenetic terms. Our main model therefore assumes a Brownian motion model of evolution (Felsenstein, 1973). The random effects and the residuals are assumed to be multivariate normal with a zero mean and a covariance structure $\mathbf{V_p} \otimes \mathbf{A}$ for the phylogenetic affects and $\mathbf{V_e} \otimes \mathbf{I}$ for the residuals ($\otimes$ here is the Kronecker product). $\mathbf{A}$ is the phylogenetic relatedness matrix, $\mathbf{I}$ is an identity matrix and the $\mathbf{V}$ are 9×9 (co)variance matrices describing the (co)variances between viral load, mortality in virus infected, and mortality in controls each at the 3 different diet levels. The phylogenetic covariance matrix, $\mathbf{V_p}$ - describes the inter-specific variances in each trait and the inter-specific covariances between them. The residual covariance matrix, $\mathbf{V_e}$ describes the

304 within-species variance that includes both any actual within-species effects and
305 also any measurement or experimental error. The off-diagonal elements in $\mathbf{V_e}$
306 (the covariances) are unable to be estimated because no vial has multiple
307 measurements - so were set to zero. The MCMC chain was run for 1300 million
308 iterations with a burn-in of 30 million iterations and a thinning interval of 1
309 million. Results were tested for sensitivity to the use of different priors by being
310 run with different prior structures (see supplementary R code), which gave
311 qualitatively similar results. We also ran models with the data subset into viral
312 load and mortality that gave similar results. All confidence intervals (CI's)
313 reported are 95% highest posterior density intervals.
314

315 **Results**

316 In order to investigate the effect that host diet may have on the likelihood of
317 virus host shifts we quantified DCV viral load in 27 species of Drosophilidae that
318 had been housed on three different diets (Figure 1). Viral loads differed between
319 species, with a thousand million times more virus in the most susceptible
320 compared to the least susceptible species, consistent with previous studies
321 (Longdon et al., 2015; Roberts et al., 2018). Viral loads were highly repeatable,
322 with the inter-specific phylogenetic component (v_p), explaining a high proportion
323 of the variation in viral load across diets (Repeatability = v_p /(v_p + v_r); Low =
324 0.92, (95% CI: 0.86, 0.96); Medium = 0.90, (95% CI: 0.84,0.96); High = 0.83, (95%
325 CI: 0.75, 0.92).

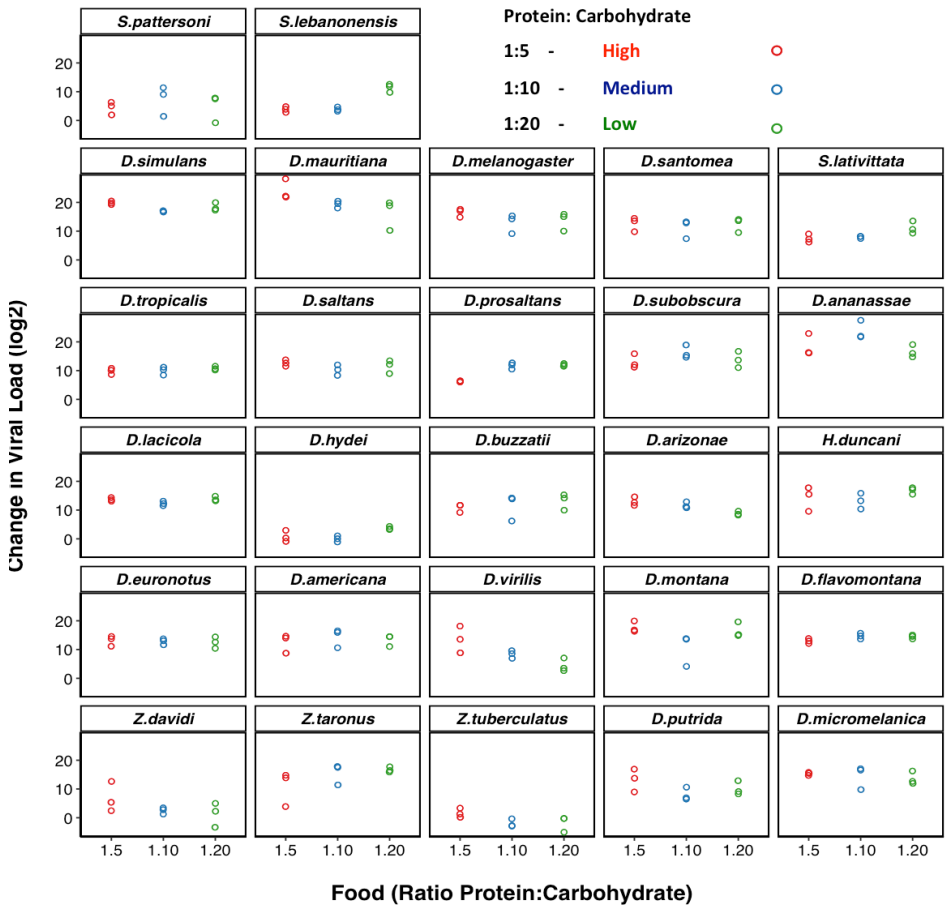

Figure 1. Change in RNA viral load (log_2) for 27 Drosophilidae species infected with DCV across the three different diets of differing protein: carbohydrate ratios. Individual points are plotted with a small x-axis jitter for clarity of points and represent the change in viral load between day 0 and day 2-post infection.

The proportion of the between species variance that can be explained by the phylogeny can also be calculated, using $v_p/(v_p + v_s)$ (Freckleton, Harvey, & Pagel, 2002), and can be equated to the phylogenetic heritability or Pagel's lambda (Housworth, Martins, & Lynch, 2004; Pagel, 1999). We found that the host phylogeny explained a modest amount of the inter-specific variation in viral loads across diets, however these estimates had broad confidence intervals (Low = 0.20, (95% CI: 3.5 x10^{-6}, 0.63); Medium = 0.34, (95% CI: 2.0 x 10^{-6}, 0.80); High =

0.51, (95% CI: 3.2 x 10^{-6}, 0.88), due to the model struggling to separate out the phylogenetic and non-phylogenetic components.

In order to examine if the susceptibility of species responded in the same or different ways to the changes in diet we examined viral load across the different protein: carbohydrate ratios. We found strong positive inter-specific correlations between viral loads across the diet treatments, suggesting the species are responding in similar ways to the changes in dietary ratios (Table 2). There was a decline in the between species variance in the high diet compared to low and medium – but this was not found to be significantly different– (v_p: Low =77.13 (95% CI: 35.09, 125.50); Medium =82.33 (95% CI: 37.55, 135.26); High =45.59 (95% CI: 19.61, 76.39) and mean viral loads were similar across the diets (Low = 11.4 (95% CI: 5.3, 17.7); Medium = 10.6 (95% CI: 3.6, 16.6); High = 10.6, (95% CI: 3.6, 16.7). Residual variance did not differ significantly between treatments (Low = 6.45 (95% CI: 4.87, 8.04); Medium = 8.23 (95% CI: 6.45, 10.35); High = 8.32, (95% CI: 6.54, 10.6).

		Interspecific Correlation	**95% CIs**
Viral load	Low - Medium	0.93	0.85, 0.98
	Medium - High	0.83	0.66, 0.96
	Low - High	0.80	0.63, 0.96
Survival in virus challenged	Low - Medium	0.93	0.81, 0.99
	Medium - High	0.88	0.71, 0.99
	Low - High	0.90	0.73, 0.99

357 **Table 2. Interspecific correlations between viral load and mortality**
358 **measures across the different diet treatments.**
359

360 As similar pathogen loads can cause different levels of harm to their hosts
361 (tolerance rather than resistance) (Boots, 2008; Råberg, Graham, & Read, 2009;
362 Roy & Kirchner, 2000) we examined if virus induced mortality differed across
363 diets over a 20 day period after viral challenge (Figure 2). We found differences
364 in the virulence (mortality) caused by DCV between host species, with some
365 species seeing no apparent increase in mortality over the experimental period
366 compared to sham infected controls, (e.g. *S. pattersoni* and *D. saltans*), whilst
367 other species show higher susceptibility with up to 50% of flies dead by day 10
368 post infection (e.g. *D. simulans* and *D. melanogaster*). As with the viral load data
369 we calculated the repeatability of survival in these virus challenged flies which
370 was high in all cases (Repeatability = v_p /(v_p + v_r); Low = 0.90, (95% CI: 0.78,
371 0.98); Medium = 0.87, (95% CI: 0.71,0.97); High = 0.98, (95% CI: 0.85, 1.00). We
372 also calculated the proportion of between species variance that can be explained
373 by the phylogeny for the virus infected flies (Low = 0.16, (95% CI: 2.58 x10^{-6},
374 0.62); Medium = 0.18, (95% CI: 5.75 x 10^{-7}, 0.78); High = 0.32, (95% CI: 7.75 x 10^{-}
375 ^{8}, 0.87), which – like the viral load data – had broad confidence intervals due to
376 the model struggling to separate the phylogenetic and non-phylogenetic
377 components.
378

379 We found strong positive inter-specific correlations between the survival of
380 virus challenged flies across the diets, suggesting the species are responding in
381 similar ways to the dietary changes (Table 2). Among species variance in

mortality of virus infected flies was consistent across diets (Low = 0. 18 (95% CI: 0.07, 0.31); Medium = 0.16 (95% CI: 0.04, 0.30); High = 0.12 (95% CI: 0.04, 0.23) as was the mean mortality (Low = 0.64 (95% CI: 0.47, 0.82); Medium = 0.58 (95% CI: 0.38, 0.75); High = 0.65, (95% CI: 0.47, 0.82). The residual variance was also consistent across the diets (Low = 0.02 (95% CI: 0.01, 0.03); Medium = 0.02 (95% CI: 0.01, 0.04); High = 0.02, (95% CI: 0.01, 0.03).

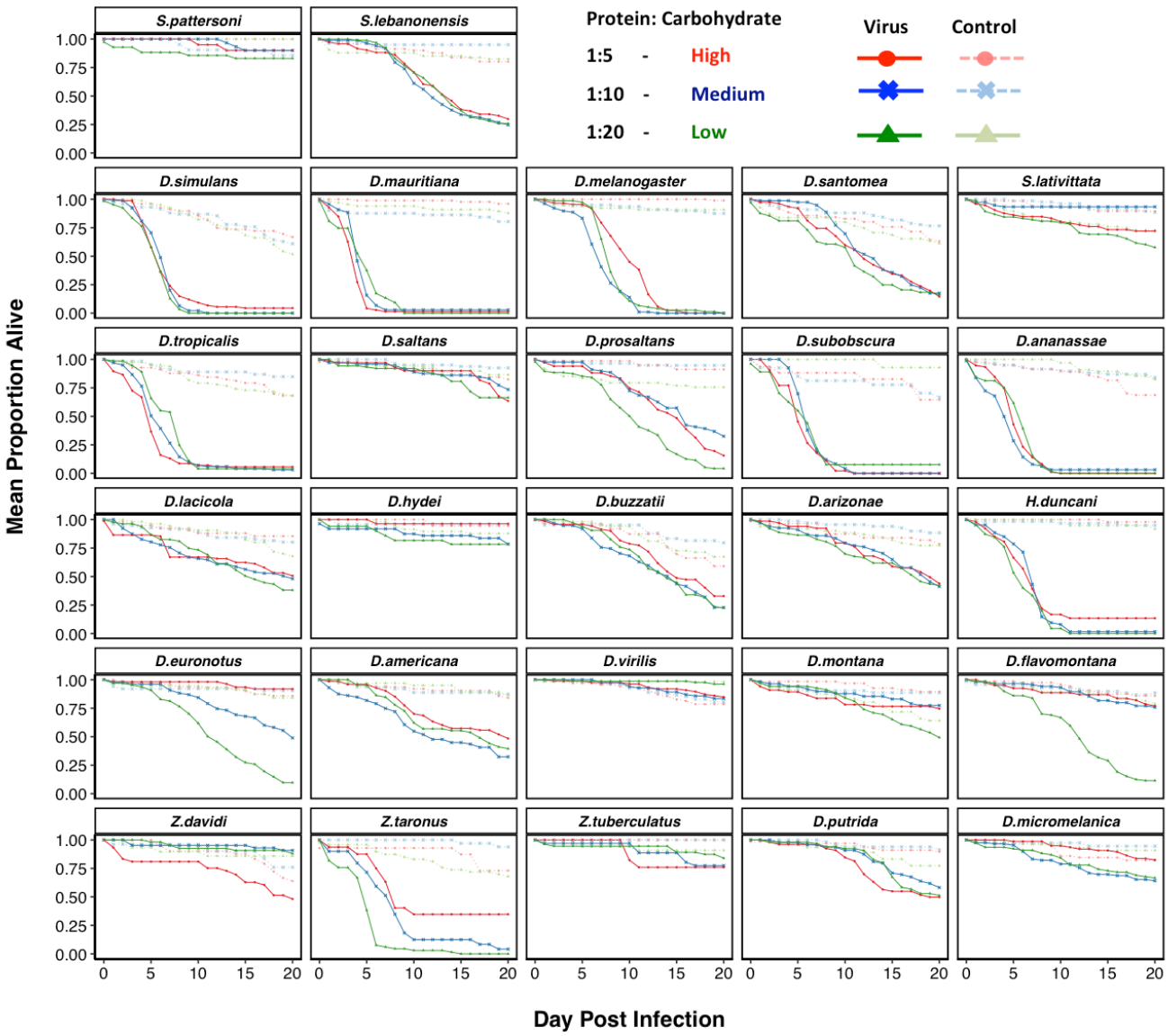

Figure 2. Mortality in 27 species of Drosophilidae housed on three different diets of varying protein: carbohydrate ratios. High- red circles,

392 Medium - blue crosses and Low- green triangles and either control sham infected
393 (dashed line) or virally challenged with DCV (solid lines).
394

395 We found that there were strong positive correlations between mortality and
396 RNA viral load (interspecific correlations between viral load and survival of virus
397 infected flies: Low = 0.89 (95% CI: 0.78, 0.98); Medium = 0.85 (95% CI: 0.67,
398 0.97); High = 0.67, (95% CI: 0.35, 0.90). To confirm that these differences are due
399 to changes in mortality caused by the virus rather than intrinsic differences in
400 the survivorship of the different species, we also inoculated flies with a control
401 solution. There was far less mortality in the controls than the virus infected flies
402 (Figure. 2 dashed lines). There was inter-specific variation in control mortality
403 (Low = 0.18, (95% CI: 0.01, 0.59); Medium = 0.43, (95% CI: 0.01,0.76); High =
404 0.55, (95% CI: -0.72, 1.00) but this was not significantly correlated with survival
405 of the virus infected flies (survival of control versus virus infected on: Low = -
406 0.11 (95% CI: -0.92, 0.75); Medium = 0.34 (95% CI: -0.48 0.97); High = 0.12,
407 (95% CI: -0.82, 0.89). We found no effect of wing length as a proxy for body size,
408 (mean: -0.05, 95% CI: -0.13, 0.05).
409

410 **Discussion**

411 We found dietary treatments of differing protein to carbohydrate ratios did not
412 alter the outcome of infection in 27 species of Drosophilidae infected with DCV.
413 We found strong positive inter-specific correlations across diets in both viral
414 load and mortality, suggesting that the species are responding in similar ways to
415 nutritional changes. Despite there being among species variation in
416 susceptibility, changes in diet did not affect viral loads. Nor did it appear to alter

the likelihood of tolerating or surviving an infection, as we found strong positive correlations between mortality and viral load on each of the diets.

Both mounting and maintaining an immune response requires energy and nutrients. During an acute immune challenge the provisioning of nutrients may become more demanding for a host, with pathogen induced malabsorption through damage to, or obstruction of, digestive tissues (Lochmiller & Deerenberg, 2000). DCV is known to cause severe pathology of the tissues of the digestive tract with subsequent accumulation of food in the crop (food storage organ) and obstruction in the intestine (Chtarbanova et al., 2014). These physical symptoms alter an infected hosts energy stores with infected flies showing significantly reduced glycogen and triglyceride levels three to four days post infection (Chtarbanova et al., 2014). DCV infected flies also increase in body mass, with a reduced food intake and reduced metabolism, suggesting that they may be experiencing increased water retention (Arnold et al., 2013; Chtarbanova et al., 2014; Thomas-Orillard, 1984). We therefore hypothesised that changing the ratio of protein to carbohydrate in the diet may alter outcome of infection, and as species may all have their own “optimal diet”, that species may respond in different ways to such changes. However our current study does not appear to support this.

Geometric frameworks for nutrition were developed in response to the fact that what is “optimal” will depend on a balance of particular nutrients in the organism and trait being investigated (Archer, Royle, South, Selman, & Hunt, 2009; Sheena C. Cotter et al., 2019; S. J. Simpson & Raubenheimer, 1995). For

example mice infected with *Salmonella* were found to survive better on diets containing a higher ratio of protein to carbohydrate (Peck, Babcock, & Alexander, 1992) as were bacterially infected army worm caterpillars - with survival increasing with dietary protein: carbohydrate, suggesting high protein requirements are associated with bacterial resistance (Povey et al., 2009). A recent study using 10 different protein: carbohydrate diets challenged flies with *P. entomophila* bacteria (Savola, Montgomery, et al., 2020). Survival on low protein diets was found to be very low in infected flies suggesting protein was key for tolerating a bacterial challenge. This study also monitored lifespan and reproduction in flies and found that regardless of injury and infection, dietary restriction extended lifespan and reduced reproductive output (Savola, Montgomery, et al., 2020). However a potential mechanism of the interaction of diet and infection has been suggested in research using a different model system with *in vivo* and *in vitro* elements (Wilson et al., 2020). Caterpillars of *S. littoralis* challenged with the bacteria *X. nematophila in vivo* and on high dietary protein had slower bacterial growth and higher survival. When this was combined with *in vitro* experiments the results suggested this was being driven by the osmolality of the hosts' blood (hemolymph) being altered by an increase in solutes in the high protein diets that acted to slow bacterial growth (Wilson et al., 2020). Although these results are using a bacterial system whilst we use a viral host shift, it highlights a significant area for further mechanistic studies of the interaction of diet and immunity.

Resources are critical in determining immune investment and the level of response to a pathogen challenge in invertebrates (Traniello, Rosengaus, & Savoie, 2002) and development time, longevity, feeding behaviour and

467 reproduction are all affected by dietary modifications (Moatt et al., 2020). In
468 particular protein is fundamental for producing amino acid rich immunological
469 components needed for resistance, particularly to viral pathogens (S. C. Cotter &
470 Kilner, 2010; Lee et al., 2008; Ponton, Lalubin, et al., 2011) whilst carbohydrates
471 are essential for energetically costly immune responses (Arnold et al., 2013;
472 Kutzer & Armitage, 2016b). A recent study of bacterial infection in *D.*
473 *melanogaster* found that a low protein: carbohydrate diet can improve survival
474 upon infection, and the expression levels of genes associated with antimicrobial
475 peptides were significantly affected by diet (Mondotte & Saleh, 2017; Xu &
476 Cherry, 2014). This study also found that infected flies choose to actively
477 decrease their protein intake whilst maintaining their carbohydrate
478 consumption, highlighting the importance of the correct balance of key nutrients.
479 Flies can respond to the absence of just one essential amino acid from their diet;
480 increasing their appetite for foods rich in such amino acids (Kay et al., 2014;
481 Keaton Wilson, Ruiz, & Davidowitz, 2019; Lochmiller & Deerenberg, 2000;
482 McKean et al., 2008), suggesting they that they are constantly modifying their
483 intake in response to internal cues. This mirrors the result of an experiment in
484 rats, upon immune stimulation the rats actively ate more carbohydrates than fat,
485 suggesting they wanted a direct source of energy and not just increased calories
486 (Ponton et al., 2020). Whereas caterpillars infected with a nucleopolyhedrovirus,
487 actively choose to feed on higher protein foods and as a result had stronger
488 immune responses, including encapsulation, lysozyme-like antimicrobial activity
489 and phenol-oxidase activity and decreased levels of infection (Leitão-Gonçalves
490 et al., 2017).
491

492 Other nutrients may also interact with the ability of a host to respond to
493 infection. For example, lipid and fats have been associated with *D. melanogaster*
494 response to DCV viral infection; peroxisomes were found to be required for host
495 defense to infection, through their primary function in lipid metabolism (Aubert,
496 Goodall, & Dantzer, 1995). Indeed recent understanding of the lipidome in
497 mosquito cells co-infected with *Wolbachia* and Dengue or Zika virus pointed to
498 the importance of fatty acids in the mechanism underlying *Wolbachia* -mediated
499 pathogen blocking (Manokaran et al., 2020). The lipid levels across our
500 experimental diets were however held constant and this is therefore a potential
501 area for further study.
502 There has been an increased use of a chemically defined (holidic) diet in order
503 to manipulate individual nutrients present in fly diets (Lee, Cory, Wilson,
504 Raubenheimer, & Simpson, 2006). Exome matched diets have been shown to
505 alleviate trade-offs in fecundity and longevity (Merkling, Riahi, Overheul,
506 Schenck, & van Rij, 2019). A possible extension of this would be to look at the
507 effect of matching diets to transcriptional changes during infection, and seeing if
508 this alleviates (or exacerbates) pathology.
509
510 Changes in diet have been shown to alter pathogen susceptibility in a number of
511 systems. We hypothesised that changes in diet could alter the potential outcomes
512 of virus host shifts. We found that changes in the ratio of protein to carbohydrate
513 did not alter susceptibility to DCV across host species in this instance.

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