Costs and benefits of sub-lethal Drosophila C virus infection

Pedro F. Vale[1,2*] Vanika Gupta[1], Charlotte Stewart[1], Samuel S.C Rund[2] Katy Monteith[1]

Affiliations

[1]Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh. Edinburgh EH9 3FL

[2] Centre for Immunity, Infection and Evolution, University of Edinburgh. Edinburgh EH9 3FL

* author for correspondence: pedro.vale@ed.ac.uk

Abstract word count: 187

Main text Word count: 4890

Running title: Costs and benefits of infection

ABSTRACT

Viruses are major evolutionary drivers of insect immune systems. Much of our knowledge of insect immune responses derives from experimental infections using the fruit fly *Drosophila melanogaster*. Most experiments, however, employ lethal pathogen doses through septic injury, frequently overwhelming host physiology. While this approach has revealed a number of immune mechanisms, it is less informative about the fitness costs hosts may experience during infection in the wild. Using both systemic and oral infection routes we find that even apparently benign, sub-lethal infections with the horizontally transmitted Drosophila C Virus (DCV) can cause significant physiological and behavioral morbidity that is relevant for host fitness. We describe DCV-induced effects on fly reproductive output, digestive health, and locomotor activity, and we find that viral morbidity varies according to the concentration of pathogen inoculum, host genetic background and sex. Notably, sub-lethal DCV infection resulted in a significant increase in fly reproduction, but this effect depended on host genotype. We discuss the relevance of sub-lethal morbidity for *Drosophila* ecology and evolution, and more broadly, we remark on the implications of deleterious and beneficial infections for the evolution of insect immunity.

Key-words: Sub-lethal infection; systemic infection; oral infection; fecundity; locomotor activity; fecal excretion; fitness.

INTRODUCTION

Viral infections are pervasive throughout the living world (Suttle, 2005; Rosario & Breitbart, 2011). Viruses of insects have attracted considerable interest (Miller & Ball, eds, 1998), in part due to their potential role in the bio-control of insect pests (Lacey *et al.*, 2015), and also because insects are vectors of many viral pathogens of plants (Whitfield *et al.*, 2015), animals and humans (Conway *et al.*, 2014). The abundance and diversity of insect viruses, combined with the extensive morbidity and mortality they cause, make viral infections potentially powerful determinants of insect population dynamics and evolution (Dwyer *et al.*, 2004; Obbard *et al.*, 2006; Wilfert *et al.*, 2016).

Much of our knowledge of insect immune responses to viral infections has come from work using the fruit fly *Drosophila melanogaster*, where the focus has been on elucidating the genetics underlying antiviral immunity (Dostert *et al.*, 2005; Huszar & Imler, 2008; Kemp & Imler, 2009; Sabin *et al.*, 2010; Magwire *et al.*, 2012). Several RNA viruses have been described and investigated in this context, including Nora virus (Habayeb *et al.*, 2009), Drosophila A virus (DAV)(Ambrose *et al.*, 2009), Flock House Virus (FHV) (Scotti *et al.*, 1983) and Drosophila C Virus (DCV) (Jousset *et al.*, 1977), a horizontally transmitted ssRNA virus in the *Dicistroviridae* family (Huszar & Imler, 2008). Initial investigations of DCV infection found that it replicates in the fly's reproductive and digestive tissues (Lautié-Harivel & Thomas-Orillard, 1990) and that infection results in accelerated larval development but also causes mortality (Thomas-Orillard, 1984; Gomariz-Zilber *et al.*, 1995). More recent work has shown that systemic infection with elevated concentrations of DCV causes pathology within the fly's

food storage organ, the crop, leading to intestinal obstruction, lower metabolic rate and reduced locomotor activity (Arnold *et al.*, 2013; Chtarbanova *et al.*, 2014). There is also considerable genetic variation in fly survival when challenged systemically with DCV, which appears to be controlled by few genes of large effect (Magwire *et al.*, 2012).

While this level of detail concerning the physiological consequences and the underlying genetics of infection is remarkable, it is important to recognize that our knowledge of viral infections comes almost entirely from experimental infections that challenge model systems, such as *Drosophila*, with artificially high viral concentrations during systemic infections. Even in cases where natural routes of infection have been investigated (Gomariz-Zilber *et al.*, 1995; Ferreira *et al.*, 2014; Stevanovic & Johnson, 2015; Vale & Jardine, 2015), these have often been achieved by using much higher doses than flies are likely to encounter in the wild in order to cause significant mortality. Highly lethal systemic or oral infections have been useful in unravelling broad antiviral immune mechanisms (Dostert *et al.*, 2005; Wang *et al.*, 2006; Kemp & Imler, 2009; Nayak *et al.*, 2013; Karlikow *et al.*, 2014), but it is unlikely that the morbidity and mortality they cause is an accurate reflection of the level of disease experienced by flies in the wild, where viral infections appear to be widespread among many species of *Drosophila* as low level persistent infections with apparently little pathology (Kapun *et al.*, 2010; Webster *et al.*, 2015). Our understanding of the fitness costs of viral infection in *Drosophila* is therefore severely limited, which is striking given the evidence from population genetic data that viruses are major drivers of

92 adaptive evolution in *Drosophila* immune genes (Obbard *et al.*, 2006, 2009; Early
93 *et al.*, 2016).
94
95 To gain a better understanding of the potential fitness costs of DCV infection, we
96 measured the physiological and behavioural responses of flies challenged with
97 either a low, sub-lethal concentration of DCV through the oral route of infection
98 or when exposed to a range of sub-lethal viral concentrations systemically
99 through intra-thoracic injury. We focused on traits that have been previously
100 shown to be affected by DCV infection such as survival, fecal excretion, and
101 locomotor activity, as well as female reproductive output, which is ultimately
102 important for evolutionary fitness. We find that even apparently benign, sub-
103 lethal infections can cause significant physiological and behavioural morbidity
104 that is relevant to fly fitness, and that these effects vary according to viral
105 concentration, host genetic background and sex.
106
107

MATERIAL AND METHODS

Fly lines and rearing conditions

In experiment 1 (systemic DCV infection) we used *Drosophila melanogaster* line *G9a*$^{+/+}$ described previously (Merkling *et al.*, 2015), kindly provided by R. van Rij (Radboud University, Nijmegen, NL). This line was maintained on standard Lewis Cornmeal medium (Lewis, 2014) under standard laboratory conditions at 25°C, 12h: 12h Light:Dark cycle. Experimental flies were generated by setting up 20 replicate Lewis vials with 15 males and 15 females to mate and lay eggs for 24 hours. Three-to-four-day-old adults that eclosed from the eggs laid during this period were infected systemically (see below) and then followed individually for health measures.

In experiment 2 (oral DCV exposure) we used ten *D. melanogaster* lines from the Drosophila Genetic Reference Panel (DGRP): RAL-83, RAL-91, RAL-158, RAL-237, RAL-287, RAL-317, RAL-358, RAL-491, RAL-732, and RAL-821. Given we had no prior knowledge of how the DGRP panel vary in response to oral DCV infection, these lines were chosen randomly. All lines were previously cleared of *Wolbachia* and have been maintained *Wolbachia*-free for at least 3 years. Fly stocks were kept at a density of 30 individuals in bottles on standard Lewis medium at 24.5± 0.5°C. Flies were allowed to mate and lay eggs for three days and then removed. When eggs had developed into three-day old imagoes, we picked 16 male and 16 female flies at random from each DGRP line (320 flies in total). Half of these flies (n=8 replicates) were individually exposed to DCV through the oral route of infection (see details below) and the other half were exposed to a sterile Ringers solution (7.2 g/L NaCl; 0.17 g/L $CaCl_2$; 0.37 g/L KCl,

diluted in sterile water, pH 7.4) as a control (n=8 replicates). Following infection, all flies were kept individually in vials kept in incubators at 24.5°C ± 0.5 with a 12h:12h light:dark cycle for the remainder of the experiment. Vials were randomized within trays to reduce any positional effects within incubators.

DCV stock and culturing

The Drosophila C Virus (DCV) isolate used in both experiments was originally isolated in Charolles, France (Jousset *et al.*, 1977), and was produced in Drosophila line 2 (DL2) cells as described previously (Longdon *et al.*, 2013; Vale & Jardine, 2015). Infectivity of the virus was calculated by measuring cytopathic effects in DL2 cells using the Reed-Muench end-point method to calculate the Tissue Culture Infective Dose 50 ($TCID_{50}$) (Reed & Muench, 1938). The DCV stock used in this experiment had an infectivity of approximately $4x10^9$ DCV infectious units (IU)/mL. This stock culture was serially diluted to achieve the desired concentrations (approximately 10^2 10^3 and 10^5 DCV IU/mL for systemic infection and 10^5 DCV IU/mL for oral infection) and kept at -80°C until needed.

Systemic DCV infection and viral titers

We exposed 20 individual male and female flies to each of 4 viral concentrations (160 flies in total)– 0 (control), 10^2, 10^3 and 10^5 DCV IU/ml, obtained by serial diluting the viral stock with 10mM Tris-HCl (pH 7.3). Flies were infected systemically by intra-thoracic pricking with a needle immersed in DCV suspension under light CO_2 anesthesia. Control flies were pricked with a needle dipped in sterile10mM Tris-HCl (pH 7.3). An additional five individuals for each sex/dose combination were infected as described above to quantify DCV within

158 flies following infection, using the expression of DCV RNA. Flies were individually
159 placed in TRI reagent (Ambion) following five days of infection (5 DPI),
160 homogenized total RNA was extracted using Direct-zol RNA miniprep kit, which
161 includes a DNAse step (Zymo Research), reverse-transcribed with M-MLV
162 reverse transcriptase (Promega) and random hexamer primers, and then diluted
163 1:2 with nuclease-free water. qRT-PCR was performed on an Applied Biosystems
164 StepOnePlus system using Fast SYBR Green Master Mix (Applied Biosystems)
165 and DCV primers, which include 5'-AT rich flaps to improve RT-PCR fluorescent
166 signal (Afonina *et al.*, 2007) (DCV_Forward: 5'
167 AATAAATCATAAGCCACTGTGATTGATACAACAGAC 3'; DCV_Reverse:
168 AATAAATCATAAGAAGCACGATACTTCTTCCAAACC). We measured the relative
169 fold change in DCV RNA relative to *rp49*, (Dmel_rp49 Forward: 5'
170 ATGCTAAGCTGTCGCACAAATG 3' ; Dmel_rp49 Reverse: 5'
171 GTTCGATCCGTAACCGATGT
172 3'). an internal *Drosophila* control gene, calculated as $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen,
173 2001).
174
175 *Oral DCV exposure*
176 In separate pilot infections, we determined that a DCV culture diluted to contain
177 approximately 10^5 DCV RNA copies was enough to establish a viable infection
178 (Figure S1), but did not cause noticeable mortality, and we used this dilution of
179 DCV stock to inoculate all ten DGRP lines. Individual flies were exposed to DCV in
180 vials containing Agar (5% sugar) using 3mL plastic atomizer spray bottles
181 containing 2mL of the sub-lethal DCV dilution. One spray, releasing roughly 50µL
182 of DCV dilution (or sterile Ringer's solution), was deployed into each vial. Flies

were left in the these 'exposure vials' for three days to allow them to ingest the viral solution during feeding and grooming, and then tipped into vials containing clean, blue-dyed Lewis medium (see below).

Survival following infection

Both systemically and orally infected flies were housed individually following infection in vials containing Lewis medium. In the systemic infection experiment, flies were monitored daily for mortality for 38 days post-infection and were transferred to fresh food vials once a week. In the oral infection experiment, flies were transferred to fresh food vials every 3-4 days, and mortality was recorded at this point for the first 32 days post infection and then daily until 40 DPI (oral infection).

Fecal excretion following oral DCV exposure

Following the exposure period, flies were tipped into vials containing blue-dyed Lewis medium. Blue medium was prepared by adding 0.5g/L FIORI COLORI brilliant blue FCF E133 granules to standard Lewis medium. Flies remained on blue Lewis food for the remainder of the experiment and were tipped to new blue Lewis vials every three to four days. When flies were tipped to new vials, the old vials were kept for fecal spot counts (measured immediately) and fecundity measures (see below). Fecal spots were recorded by photographing vials with a Leica S8APO microscope. A slip of white printer paper (2.5cm x 8.5cm) was inserted into each vial to ensure only spots on one side of the vial were being photographed. These images were then analyzed with ICY image software (Version 1.6.1.1 ICY - Bio Imaging Analysis) and fecal spots were

208 counted using 'spot detection' analysis on a 2cm x 4cm region of interest. Each
209 image was checked individually for miscounts, and miscounted spots were
210 removed. Fecal excretion was recorded for 30 days following infection.
211
212 *Fecundity*
213 The fecundity of individual flies was measured by counting viable offspring
214 emerging in the vials they were reared in, which happed weekly until day 30 post
215 infection in the systemically infected flies, and every 3-4 days in the orally
216 infected flies, for 28 days following exposure to DCV. Short-term fecundity
217 estimates have been shown to be well correlated with lifetime reproduction in
218 Drosophila (Nguyen & Moehring, 2015) Vials that individuals were tipped from
219 (and following the recording of fecal shedding in the oral infection experiment),
220 were placed in the incubators at 24.5°C ± 0.5 with a 12h:12h light:dark cycle to
221 allow any offspring to develop. After 14 days, the total number of living emerged
222 adult offspring within each vial was recorded as a measure of female fecundity.
223
224 *Activity*
225 Locomotor activity was measured using the Drosophila Activity Monitor (DAM2,
226 Trikinetics) as described previously (Pfeiffenberger *et al.*, 2010; Vale & Jardine,
227 2015). In the DAM, individual fly activity is recorded when individually housed
228 flies break an infrared beam passing through a transparent plastic tube placed
229 symmetrically inside a DAM unit. Activity was measured in a separate
230 experiment on flies reared and exposed to DCV as described above. In
231 systemically infected flies, activity was measured on 10 replicate male flies for
232 each DCV dose the day following septic injury (40 flies in total), and measured

for 2 weeks following infection. In the oral infection experiment, activity was recorded for 24 hours, fourteen days after the initial oral exposure. These differences in the timing of activity measurements arise from the faster and more severe effects of systemic infections on locomotor behavior, while we have found that effects on activity following oral infection take longer to manifest, and become apparent 10-15 days after DCV ingestion(Vale & Jardine, 2015). Four replicate flies for each DGRP (10 lines) / sex (M/F) / infection (DCV/Control) combination were tested (160 flies in total). In both experiments, flies were placed individually in a single DAM tube containing a small agar plug on one end, and allocated a slot in one of five DAM unit (each unit can house a maximum of 32 tubes). At least one slot in each DAM unit was filled with an empty tube and at least two slots were left empty as negative controls. All DAM units were placed in the incubator (25 °C 12:12 light:dark cycle) and continuous activity data was collected every minute for 24 hours. Raw activity data was processed using the DAM System File Scan Software (www.trikinetics.com) and the resulting data was manipulated using R v. 3.1.3 (The R Foundation for Statistical Computing, Vienna, Austria). Flies that died during the DAM assay (6/40 flies in the systemic infection experiment; 25/160 in the oral infection experiment) were removed from the analysis because they would wrongly bias the estimate of activity.

Data analysis

All analyses were carried out in JMP 12 (SAS). Survival data was analyzed on the 'day of death' using a Cox Proportional Hazards models in with 'fly sex' and 'DCV exposure' and their interaction as fixed effects (systemic infection experiment) or 'fly sex', fly 'line' and 'DCV dose' and their interactions as fixed effects (oral

infection experiment). In the systemic infection, DCV titers were Log_{10}-transformed and analyzed in a linear model with 'DCV Dose' and 'Sex' and their interaction as fixed effects. Fecundity following systemic infection was calculated on the cumulative number of emerged offspring in a model containing 'DCV dose' as a fixed effect. In the oral exposure experiment, the cumulative number of offspring was analyzed in a model including 'Fly line' and 'DCV exposure' and their interaction as fixed effects. Total excretion per fly was analyzed using a linear model with 'Fly line', 'DCV exposure', and 'sex' as categorical fixed effects, 'Time' as a continuous covariate, and all pair-wise interactions. Activity was analyzed as the total number of DAM beam breaks recorded per day. Activity following systemic infection was analyzed in a linear model with 'DCV dose' and 'Time' as fixed effects. Activity following oral infection was measured for 24h and analyzed in a linear model with 'Fly line', 'Sex' and 'DCV exposure' as fixed effects. In all analyses, individual replicate was included as a random factor, and in all cases accounted for only 2-5% of the total variance.

RESULTS

Experiment 1: Sub-lethal systemic infection

In a first experiment, we tested how systemic infection with very low sub-lethal doses of DCV affected fly health. The survival of both female and male flies exposed to doses of 10^2 and 10^3 DCV IU/ ml did not differ from control flies that had been pricked with sterile buffer solution (Figure 1a). In females, 100% flies exposed to these doses survived infection during the 38-day survival assay, while roughly 20% of males died during this period (Figure 1a). However, this difference in survival between sexes ('sex' effect, Table 1), was also observed in control flies and therefore is likely to reflect sex-specific responses to injury during intra-thoracic pricking than to infection. Flies infected with a slightly higher concentration of 10^5 DCV IU/ ml died significantly faster than control flies. This virus concentration-specific pattern of mortality was generally consistent with the observed DCV titers measured 5 days following infection, (Table 2, 'dose' effect) which were generally higher in male flies across all DCV concentrations (Table 2, 'sex' effect, Figure 1b). Our experiment therefore spanned the range of sub-lethal viral doses, with 10^5 DCV IU/ ml being the lowest virus concentration with lethality in the experiment (Figure 1a).

Fecundity following systemic DCV infection

We used mated females, which allowed us to quantify fly reproductive health during systemic infection by following the number of adult offspring produced by individual females for 30 days following infection. The total fecundity measured during this period varied according to the dose females had received ($F_{3,66} = 10.32$, $p<0.0001$) and we observed that the total reproduction of infected

299 flies was higher than control flies, and increased in a dose-specific manner
300 (Figure 1c).
301
302 *Activity following systemic DCV infection*
303 The locomotor activity of individual male flies infected systemically with all sub-
304 lethal concentrations of DCV was measured during 18 days after infection in a
305 Trikinetics® Drosophila Activity Monitor (DAM). All flies included in the analysis
306 remained alive for the whole period, so changes in activity were not confounded
307 with potential death of individual flies. We found that flies in all treatments,
308 including uninfected controls, showed a reduction in activity over the course of
309 the activity assay (Figure 1d, Table time effect). This general effect is not
310 especially surprising given the constrained environment experienced by flies in
311 the DAM tubes, and that the only source of nutrition and hydration is small agar
312 plug. However, our analysis showed that the temporal reduction in activity
313 depended on the dose that flies had received ('time x dose' interaction, Table 1).
314 In the early stages of infection flies receiving the higher of the 4 doses (10^3 and
315 10^5 DCV copies) showed a reduction in activity relative to control flies and those
316 receiving the lowest dose. Over time, a reduction in locomotor activity was most
317 apparent in flies infected with the highest dose of 10^5 DCV copies (Figure 1d).
318
319 ***Experiment 2: Sub-lethal gut infection***
320 In a separate experiment, we tested how exposure to a single sub-lethal dose of
321 DCV through the oral route of infection impacted upon fly health. We conducted
322 the experiment on ten fly lines from the DGRP panel (Mackay *et al.*, 2012) and we
323 included both male and female flies to test for the effects of host genetic

background and sex in response to sub-lethal oral infection. While DGRP lines differ in their lifespan in the absence of infection (Durham *et al.*, 2014), we did not detect any difference between DGRP lines or between sexes in their survival during oral DCV infection compared to control flies (Table S1) which, as expected, was generally non-lethal across all lines.

Fecundity following oral exposure to DCV

Despite not observing any effects on fly survival during infection, we detected significant variation in reproductive health following exposure to DCV. The total fecundity of females during the 28 days following oral exposure to DCV (or a control inoculum) varied significantly between DGRP lines (Figure 2; Table 2), reflecting well-known genetic differences in the lifetime reproductive output of these lines (Durham *et al.*, 2014). In addition, we found line-specific fecundity responses to DCV infection ('infection status x line', Table 2, see also Table S2 for pairwise contrasts). In some lines (158, 491, 317) low-level oral infection resulted in a decrease in fecundity; in other lines (821, 358) there was no detectable effect of DCV exposure; while in 2 lines we detected significant increases in fecundity in DCV infected flies compared to uninfected control flies of the same genetic background (Figure 2; see Table S2 for least-square pairwise contrasts).

Locomotor activity following oral exposure to DCV

348 Overall, DGRP lines differed in their activity in a sex specific way ('Fly line x Sex'
349 effect Table 2), but these differences were not altered by infection. While we
350 detected a reduction in locomotor activity following systemic infection (Figure
351 1d), we did not detect any effect of oral DCV exposure on the overall activity of
352 flies (Table 2, Figure 3).
353
354 *Fecal excretion following oral exposure to DCV*
355 We quantified fecal excretion for 30 days following DCV exposure as a proxy for
356 gut health, by counting fecal spots excreted into vials after ingestion of blue-dyed
357 food. Overall we found that males showed higher levels of fecal excretion
358 compared to females (Table 2, 'sex' effect; Figure 4) and that DCV infection was
359 associated with a general reduction in fecal excretion throughout the 30-day
360 observation period ('Infection status' effect, Figure 4). However, we found that
361 males and females differed in the overall severity of this reduction ('sex x
362 infection status' effect), with males showing a greater reduction in defecation
363 overall (Figure 4). Furthermore, we found significant variation among the DGRP
364 lines in the magnitude of the effect of DCV on fecal excretion ('fly line x infection
365 status' effect).

DISCUSSION

We find that sub-lethal infections with DCV can cause measurable morbidity that is relevant for the fitness costs experienced by *D. melanogaster* during DCV infection. In systemic and oral infections with doses that did not cause mortality we observed DCV-induced effects on fly reproductive output, digestive health, and locomotor activity.

Systemically infected flies increase reproductive output

We found that the fly line used in the systemic infection experiment showed an increase in reproductive output when infected with sub-lethal doses of DCV. There are numerous examples from both invertebrates and vertebrates of fecundity increases following infection (Bonneaud *et al.*, 2004; Vale & Little, 2012; Leventhal *et al.*, 2014; Vézilier *et al.*, 2015). In addition, earlier work reported that DCV infection could increase ovariole number and decrease development time in *D. melanogaster* (Thomas-Orillard, 1984; Gomariz-Zilber & Thomas-Orillard, 1993). However, a subsequent re-analysis of these data showed very weak support for the beneficial effects of DCV infection (Longdon, 2015). It is notable however that neither of the earlier studies measured the number of viable offspring of infected flies compared to healthy ones. The fecundity data we report therefore suggests that DCV may indeed result in increased reproductive output.

A dose-dependent increase in fecundity could suggest a direct effect of DCV infecting fly ovaries, but it is unclear why such a strategy would be adaptive for

391 the virus. An alternative hypothesis may instead involve more complex
392 interactions between the allocation of resources during DCV infection, and how
393 they relate to fly nutritional stress and reproductive investment. For example, *D.*
394 *melanogaster* females selected under conditions of nutritional stress were found
395 to produce a greater number of ovarioles, while the offspring of starved mothers
396 also exhibited greater investment in reproduction (Wayne *et al.*, 2006). Similar
397 to the studies cited above, this work also focused on ovariole number and egg
398 production, and did not quantify female lifetime fecundity. Given that DCV
399 infection is known to lead to intestinal obstruction, one possibility for the
400 increase in the number of adult offspring we observed in infected flies is that
401 DCV-induced nutritional stress leads to a greater production of ovarioles, and
402 consequently, an increased number of offspring. Given we only tested a single fly
403 line however, it important to note that this response may not be universal. As we
404 discuss below fecundity responses to infection have generally been found to
405 differ between host genotypes (Vale & Little, 2012; Parker *et al.*, 2014)
406
407 *Fecundity costs and benefits of DCV infection are genotype-specific*
408 Similar to systemically infected flies (Figure 1c), we also find evidence for
409 fecundity benefits in orally exposed flies, but these benefits were only revealed
410 in two out of the ten genetic backgrounds we tested. Indeed, in three of the
411 tested lines, DCV infection resulted in lower reproductive output. Taking
412 fecundity as a proxy for evolutionary fitness, the existence of genotype specific
413 fitness costs and benefits means that DCV could be a potentially powerful driver
414 of *D. melanogaster* evolutionary dynamics. Previous analyses of *Drosophila* spp.
415 population genetic data have shown that the fastest evolving *D. melanogaster*

416 genes are those involved in RNAi-based antiviral defense (Obbard *et al.*, 2006,
417 2009; Early *et al.*, 2016), but the DCV-induced fitness costs that drive this rapid
418 evolution in wild-infected flies (where infections are persistent and often non-
419 lethal), has remained obscure. These data suggest that genotype-specific
420 fecundity costs and benefits of DCV infection could potentially mediate the arms-
421 race between flies and viruses.
422
423 *Systemically infected flies show a dose-dependent decline in activity over time*
424 Reduced activity, or lethargy, following infection is a common response to
425 infection across a range of taxa (Hart, 1988; Adelman & Martin, 2009; Sullivan *et*
426 *al.*, 2016). The most obvious explanation for reduced activity is simply that
427 infected individuals are sick, and lethargy reflects the underlying pathology of
428 infection (Moore, 2013). A popular alternative explanation is that infection-
429 induced lethargy evolved as an adaptive host strategy that conserves energy,
430 which may then be allocated to other physiological tasks such as mounting an
431 immune response (Hart, 1988; Adelman & Martin, 2009).
432
433 Support for the adaptive nature of these 'sickness behaviours' has come mainly
434 from vertebrate species challenged with deactivated pathogens or their derived
435 components, which are sufficient to stimulate an immune response without
436 causing pathology (Adelman & Martin, 2009; Lopes *et al.*, 2016). In addition to
437 vertebrates, sickness behaviors including lethargy and anorexia have also been
438 described in insect hosts (Ayres & Schneider, 2009; Kazlauskas *et al.*, 2016;
439 Sullivan *et al.*, 2016). However, in the current experiment it is not possible to
440 disentangle the effect of an adaptive sickness behavior from the direct effect of

441 pathology caused by replicating DCV. Regardless of the underlying cause of
442 reduced activity, it is likely to come at an additional cost of lower involvement in
443 fitness-enhancing activities such as foraging, competing for resources with
444 conspecifics, or courtship and mating (Adelman & Martin, 2009; Adamo *et al.*,
445 2015; Vale & Jardine, 2016). Further, reduced activity following infection can
446 also reduce the potential for disease spread (Lopes *et al.*, 2016). In the context of
447 understanding sub-lethal DCV infection in an ecological setting, reduced activity
448 may therefore be a potentially important source of DCV-induced fitness costs
449 and benefits.
450
451 We did not find an effect of oral DCV exposure on fly activity. Previous work has
452 shown that *Drosophila*, especially females, show a reduction in activity following
453 oral infection with DCV (Vale & Jardine, 2015). However, the viral concentration
454 that flies were exposed to in that experiment was at least 1000x higher, so it is
455 likely that in the current experiment flies did not ingest virus in quantities large
456 enough to affect locomotor activity.
457
458 *The severity of DCV-induced digestive dysfunction is sex-specific*
459 Previous work has shown that DCV infection results in digestive dysfunction,
460 leading to increased body mass due to the inability to excrete digested food
461 (Arnold *et al.*, 2013; Chtarbanova *et al.*, 2014). We found that this measure of gut
462 health varied between genotypes and also between sexes. Extensive genetic
463 variation for gut immune-competence has previously been reported in the DGRP
464 panel (Bou Sleiman *et al.*, 2015), which could underlie some of the variation we
465 observe in DCV-associated digestive dysfunction in some lines. Although that

study focused on enteric infection with entomopathogenic bacteria, the mechanisms that mediate variation in gut health during infection include general processes of gut damage and repair, such as the production of reactive oxygen species (ROS) and the production of intestinal stem cells during epithelial repair (Buchon *et al.*, 2013). It is plausible that these mechanisms also mediate disease severity during enteric virus infection, but we are unaware of any systematic study of genetic variation in gut immune-competence during viral infection.

The mechanistic basis of the observed sex differences in fecal excretion is less clear. The Malpighian tubules are the main organ involved in osmoregulation and excretion of waste matter in insects (Dow & Davies, 2001). *D. melanogaster* male and female Malpighian tubules have been shown to differ at the transcriptional level with over 18% of genes (2308 genes) showing sex-specific expression (Huylmans & Parsch, 2014). We measured fecal excretion by quantifying fecal spots on the sides of the vials. Given that females are known to also spend more time feeding (Wong *et al.*, 2009), it is possible that females also defecate more on the surface of the food compared to males, and therefore spend less time on the sides of the vials. Only a few studies have investigated sex differences in fecal excretion in *D. melanogaster*, finding inconsistent patterns of excretion between sexes (Zeng *et al.*, 2011; Urquhart-Cronish & Sokolowski, 2014). The link between fecal excretion and fitness is not as clear as with fecundity or locomotor activity, but it is relevant in the context of disease transmission of fecal-orally transmitted pathogens such as DCV. The study of temporal trends in fecal excretion and how they vary with host sex and genetic background may

490 therefore be used as a useful model to understand the sources of heterogeneity
491 in pathogen shedding (Vale *et al.*, 2013).
492
493 *Concluding remarks*
494 Altogether, these measures of sub-lethal morbidity give insight into the potential
495 fitness costs of low-level, persistent DCV infection in *Drosophila*. More generally,
496 the combination of both positive and negative effects on fly fitness effects
497 according to the specific host genetic background presents a non-trivial
498 evolutionary scenario for host immune defense (Gandon & Vale, 2014). For
499 instance, frequent encounters between beneficial symbionts and detrimental
500 pathogens are hypothesized to have played a role in the evolution of aphid
501 immune systems, which lack several components of the IMD immune pathway
502 critical for the recognition and elimination of Gram-negative bacteria (Gerardo *et*
503 *al.*, 2010). The combination of fitness costs and benefits of infection, such as
504 those incurred during DCV infection, may therefore have driven the evolution of
505 immune defense across a wide range of host taxa, from insects to mammals
506 (Elsik, 2010; Gerardo *et al.*, 2010; Lee & Mazmanian, 2010; Gandon & Vale,
507 2014).
508
509 **Acknowledgements**
510 We are grateful to D. Obbard and lab members (Edinburgh) for general technical
511 support and for advice about DCV infection. We also thank H. Cowan and H.
512 Borthwick for help with media preparation. This work was supported by a
513 strategic award from the Wellcome Trust for the Centre for Immunity, Infection
514 and Evolution (http://ciie.bio.ed.ac.uk; grant reference no. 095831), and by a

515 Society in Science – Branco Weiss fellowship (http://www.society-in-
516 science.org), both awarded to P. Vale. All authors declare no conflict of interest.

REFERENCES

Adamo, S.A., Gomez-Juliano, A., LeDue, E.E., Little, S.N. & Sullivan, K. 2015. Effect of immune challenge on aggressive behaviour: how to fight two battles at once. *Anim. Behav.* **105**: 153–161.

Adelman, J.S. & Martin, L.B. 2009. Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integr. Comp. Biol.* **49**: 202–214.

Afonina, I., Ankoudinova, I., Mills, A., Lokhov, S., Huynh, P. & Mahoney, W. 2007. Primers with 5' flaps improve real-time PCR. *BioTechniques* **43**: 770, 772, 774.

Ambrose, R.L., Lander, G.C., Maaty, W.S., Bothner, B., Johnson, J.E. & Johnson, K.N. 2009. Drosophila A virus is an unusual RNA virus with a T=3 icosahedral core and permuted RNA-dependent RNA polymerase. *J. Gen. Virol.* **90**: 2191–2200.

Arnold, P.A., Johnson, K.N. & White, C.R. 2013. Physiological and metabolic consequences of viral infection in Drosophila melanogaster. *J. Exp. Biol.* **216**: 3350–3357.

Ayres, J.S. & Schneider, D.S. 2009. The Role of Anorexia in Resistance and Tolerance to Infections in Drosophila. *PLoS Biol.* **7**: e1000150.

Bonneaud, C., Mazuc, J., Chastel, O., Westerdahl, H. & Sorci, G. 2004. Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the house sparrow. *Evol. Int. J. Org. Evol.* **58**: 2823–2830.

Bou Sleiman, M.S., Osman, D., Massouras, A., Hoffmann, A.A., Lemaitre, B. & Deplancke, B. 2015. Genetic, molecular and physiological basis of variation in Drosophila gut immunocompetence. *Nat. Commun.* **6**.

Buchon, N., Broderick, N.A. & Lemaitre, B. 2013. Gut homeostasis in a microbial world: insights from Drosophila melanogaster. *Nat. Rev. Microbiol.* **11**: 615–626.

Chtarbanova, S., Lamiable, O., Lee, K.-Z., Galiana, D., Troxler, L., Meignin, C., *et al.* 2014. Drosophila C virus systemic infection leads to intestinal obstruction. *J. Virol.*, doi: 10.1128/JVI.02320-14.

Conway, M.J., Colpitts, T.M. & Fikrig, E. 2014. Role of the Vector in Arbovirus Transmission. *Annu. Rev. Virol.* **1**: 71–88.

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., *et al.* 2005. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. *Nat. Immunol.* **6**: 946–953.

Dow, J.A.T. & Davies, S.A. 2001. The Drosophila melanogaster malpighian tubule. In: (B.-A. in I. Physiology, ed), pp. 1–83. Academic Press.

556 Durham, M.F., Magwire, M.M., Stone, E.A. & Leips, J. 2014. Genome-wide analysis
557 in Drosophila reveals age-specific effects of SNPs on fitness traits. *Nat.*
558 *Commun.* **5**.

559 Dwyer, G., Dushoff, J. & Yee, S.H. 2004. The combined effects of pathogens and
560 predators on insect outbreaks. *Nature* **430**: 341–345.

561 Early, A.M., Arguello, J.R., Cardoso-Moreira, M., Gottipati, S., Grenier, J.K. &
562 Clark, A.G. 2016. Survey of Global Genetic Diversity Within the Drosophila
563 Immune System. *Genetics*, doi: 10.1534/genetics.116.195016.

564 Elsik, C.G. 2010. The pea aphid genome sequence brings theories of insect defense
565 into question. *Genome Biol.* **11**: 106.

566 Ferreira, Á.G., Naylor, H., Esteves, S.S., Pais, I.S., Martins, N.E. & Teixeira, L. 2014.
567 The Toll-Dorsal Pathway Is Required for Resistance to Viral Oral Infection in
568 Drosophila. *PLoS Pathog.* **10**.

569 Gandon, S. & Vale, P.F. 2014. The evolution of resistance against good and bad
570 infections. *J. Evol. Biol.* **27**: 303–312.

571 Gerardo, N.M., Altincicek, B., Anselme, C., Atamian, H., Barribeau, S.M., Vos, M.
572 de, *et al.* 2010. Immunity and other defenses in pea aphids, Acyrthosiphon
573 pisum. *Genome Biol.* **11**: R21.

574 Gomariz-Zilber, E., Poras, M. & Thomas-Orillard, M. 1995. Drosophila C virus:
575 experimental study of infectious yields and underlying pathology in
576 Drosophila melanogaster laboratory populations. *J. Invertebr. Pathol.* **65**:
577 243–247.

578 Gomariz-Zilber, E. & Thomas-Orillard, M. 1993. Drosophila C virus and Drosophila
579 hosts: a good association in various environments. *J. Evol. Biol.* **6**: 677–689.

580 Habayeb, M.S., Cantera, R., Casanova, G., Ekström, J.-O., Albright, S. & Hultmark,
581 D. 2009. The Drosophila Nora virus is an enteric virus, transmitted via feces.
582 *J. Invertebr. Pathol.* **101**: 29–33.

583 Hart, B.L. 1988. Biological basis of the behavior of sick animals. *Neurosci. Biobehav.*
584 *Rev.* **12**: 123–137.

585 Huszar, T. & Imler, J. 2008. Drosophila Viruses and the Study of Antiviral Host-
586 Defense. In: *Advances in Virus Research*, pp. 227–265. Academic Press.

587 Huylmans, A.K. & Parsch, J. 2014. Population- and Sex-Biased Gene Expression in
588 the Excretion Organs of Drosophila melanogaster. *G3 GenesGenomesGenetics*
589 **4**: 2307–2315.

590 Jousset, F.-X., Bergoin, M. & Revet, B. 1977. Characterization of the Drosophila C
591 Virus. *J. Gen. Virol.* **34**: 269–283.

592 Kapun, M., Nolte, V., Flatt, T. & Schlötterer, C. 2010. Host Range and Specificity of
593 the Drosophila C Virus. *PLoS ONE* **5**: e12421.

Karlikow, M., Goic, B. & Saleh, M.-C. 2014. RNAi and antiviral defense in Drosophila: Setting up a systemic immune response. *Dev. Comp. Immunol.* **42**: 85–92.

Kazlauskas, N., Klappenbach, M., Depino, A.M. & Locatelli, F.F. 2016. Sickness Behavior in Honey Bees. *Front. Physiol.* **7**.

Kemp, C. & Imler, J.-L. 2009. Antiviral immunity in drosophila. *Curr. Opin. Immunol.* **21**: 3–9.

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M. & Goettel, M.S. 2015. Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol.* **132**: 1–41.

Lautié-Harivel, N. & Thomas-Orillard, M. 1990. Location of Drosophila C virus target organs in Drosophila host population by an immunofluorescence technique. *Biol. Cell* **69**: 35–39.

Lee, Y.K. & Mazmanian, S.K. 2010. Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System? *Science* **330**: 1768–1773.

Leventhal, G.E., Dünner, R.P. & Barribeau, S.M. 2014. Delayed virulence and limited costs promote fecundity compensation upon infection. *Am. Nat.* **183**: 480–493.

Lewis, E. 2014. A new standard food medium. 1960 Drosophila Information Service. in. *Cold Spring Harb. Protoc.* **2014**: pdb.rec081414.

Livak, K.J. & Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods San Diego Calif* **25**: 402–408.

Longdon, B. 2015. Examination of data claiming Drosophila C virus is beneficial do not support this claim. *Figshare*, doi: http://dx.doi.org/10.6084/m9.figshare.1297064.

Longdon, B., Cao, C., Martinez, J. & Jiggins, F.M. 2013. Previous Exposure to an RNA Virus Does Not Protect against Subsequent Infection in Drosophila melanogaster. *PLoS ONE* **8**: e73833.

Lopes, P.C., Block, P. & König, B. 2016. Infection-induced behavioural changes reduce connectivity and the potential for disease spread in wild mice contact networks. *Sci. Rep.* **6**: 31790.

Mackay, T.F.C., Richards, S., Stone, E.A., Barbadilla, A., Ayroles, J.F., Zhu, D., *et al.* 2012. The Drosophila melanogaster Genetic Reference Panel. *Nature* **482**: 173–178.

Magwire, M.M., Fabian, D.K., Schweyen, H., Cao, C., Longdon, B., Bayer, F., *et al.* 2012. Genome-Wide Association Studies Reveal a Simple Genetic Basis of Resistance to Naturally Coevolving Viruses in Drosophila melanogaster. *PLoS Genet* **8**: e1003057.

632 Merkling, S.H., Bronkhorst, A.W., Kramer, J.M., Overheul, G.J., Schenck, A. & Van
633 Rij, R.P. 2015. The Epigenetic Regulator G9a Mediates Tolerance to RNA
634 Virus Infection in Drosophila. *PLoS Pathog* **11**: e1004692.

635 Miller, L.K. & Ball, L.A. (eds). 1998. *The Insect Viruses*. Springer US, Boston, MA.

636 Moore, J. 2013. An overview of parasite-induced behavioral alterations – and some
637 lessons from bats. *J. Exp. Biol.* **216**: 11–17.

638 Nayak, A., Tassetto, M., Kunitomi, M. & Andino, R. 2013. RNA Interference-
639 Mediated Intrinsic Antiviral Immunity in Invertebrates. In: *Intrinsic Immunity*
640 (B. R. Cullen, ed), pp. 183–200. Springer Berlin Heidelberg.

641 Nguyen, T.T.X. & Moehring, A.J. 2015. Accurate Alternative Measurements for
642 Female Lifetime Reproductive Success in Drosophila melanogaster. *PLOS*
643 *ONE* **10**: e0116679.

644 Obbard, D.J., Jiggins, F.M., Halligan, D.L. & Little, T.J. 2006. Natural selection
645 drives extremely rapid evolution in antiviral RNAi genes. *Curr. Biol. CB* **16**:
646 580–585.

647 Obbard, D.J., Welch, J.J., Kim, K.-W. & Jiggins, F.M. 2009. Quantifying Adaptive
648 Evolution in the Drosophila Immune System. *PLoS Genet.* **5**: e1000698.

649 Parker, B.J., Garcia, J.R. & Gerardo, N.M. 2014. Genetic variation in resistance and
650 fecundity tolerance in a natural host-pathogen interaction. *Evol. Int. J. Org.*
651 *Evol.* **68**: 2421–2429.

652 Pfeiffenberger, C., Lear, B.C., Keegan, K.P. & Allada, R. 2010. Locomotor Activity
653 Level Monitoring Using the Drosophila Activity Monitoring (DAM) System.
654 *Cold Spring Harb. Protoc.* **2010**: pdb.prot5518.

655 Reed, L.J. & Muench, H. 1938. A Simple Method of Estimating Fifty Per Cent
656 Endpoints,. *Am. J. Epidemiol.* **27**: 493–497.

657 Rosario, K. & Breitbart, M. 2011. Exploring the viral world through metagenomics.
658 *Curr. Opin. Virol.* **1**: 289–297.

659 Sabin, L.R., Hanna, S.L. & Cherry, S. 2010. Innate antiviral immunity in Drosophila.
660 *Curr. Opin. Immunol.* **22**: 4–9.

661 Scotti, P.D., Dearing, S. & Mossop, D.W. 1983. Flock House virus: a nodavirus
662 isolated from Costelytra zealandica (White) (Coleoptera: Scarabaeidae). *Arch.*
663 *Virol.* **75**: 181–189.

664 Stevanovic, A. & Johnson, K.N. 2015. Infectivity of Drosophila C virus following
665 oral delivery in Drosophila larvae. *J. Gen. Virol.*, doi: 10.1099/vir.0.000068.

666 Sullivan, K., Fairn, E. & Adamo, S.A. 2016. Sickness behaviour in the cricket Gryllus
667 texensis: Comparison with animals across phyla. *Behav. Processes* **128**: 134–
668 143.

669 Suttle, C.A. 2005. Viruses in the sea. *Nature* **437**: 356–361.

670 Thomas-Orillard, M. 1984. Modifications of Mean Ovariole Number, Fresh Weight of
671 Adult Females and Developmental Time in DROSOPHILA
672 MELANOGASTER Induced by Drosophila C Virus. *Genetics* **107**: 635–644.

673 Urquhart-Cronish, M. & Sokolowski, M.B. 2014. Gene-environment interplay in
674 Drosophila melanogaster: Chronic nutritional deprivation in larval life affects
675 adult fecal output. *J. Insect Physiol.* **69**: 95–100.

676 Vale, P.F., Choisy, M. & Little, T.J. 2013. Host nutrition alters the variance in
677 parasite transmission potential. *Biol. Lett.* **9**.

678 Vale, P.F. & Jardine, M.D. 2015. Sex-specific behavioural symptoms of viral gut
679 infection and Wolbachia in Drosophila melanogaster. *J. Insect Physiol.* **82**:
680 28–32.

681 Vale, P.F. & Jardine, M.D. 2016. Viral exposure reduces the motivation to forage in
682 female Drosophila melanogaster. *Fly (Austin)* 1–7.

683 Vale, P.F. & Little, T.J. 2012. Fecundity compensation and tolerance to a sterilizing
684 pathogen in Daphnia. *J. Evol. Biol.* **25**: 1888–1896.

685 Vézilier, J., Nicot, A., Gandon, S. & Rivero, A. 2015. Plasmodium infection brings
686 forward mosquito oviposition. *Biol. Lett.* **11**.

687 Wang, X.-H., Aliyari, R., Li, W.-X., Li, H.-W., Kim, K., Carthew, R., *et al.* 2006.
688 RNA Interference Directs Innate Immunity Against Viruses in Adult
689 Drosophila. *Science* **312**: 452–454.

690 Wayne, M.L., Soundararajan, U. & Harshman, L.G. 2006. Environmental stress and
691 reproduction in Drosophila melanogaster: starvation resistance, ovariole
692 numbers and early age egg production. *BMC Evol. Biol.* **6**: 57.

693 Webster, C.L., Waldron, F.M., Robertson, S., Crowson, D., Ferrari, G., Quintana, J.F.,
694 *et al.* 2015. The Discovery, Distribution, and Evolution of Viruses Associated
695 with Drosophila melanogaster. *PLOS Biol* **13**: e1002210.

696 Whitfield, A.E., Falk, B.W. & Rotenberg, D. 2015. Insect vector-mediated
697 transmission of plant viruses. *Virology* **479–480**: 278–289.

698 Wilfert, L., Long, G., Leggett, H.C., Schmid-Hempel, P., Butlin, R., Martin, S.J.M., *et*
699 *al.* 2016. Deformed wing virus is a recent global epidemic in honeybees driven
700 by Varroa mites. *Science* **351**: 594–597.

701 Wong, R., Piper, M.D.W., Wertheim, B. & Partridge, L. 2009. Quantification of Food
702 Intake in Drosophila. *PLOS ONE* **4**: e6063.

703 Zeng, C., Du, Y., Alberico, T., Seeberger, J., Sun, X. & Zou, S. 2011. Gender-specific
704 prandial response to dietary restriction and oxidative stress in Drosophila
705 melanogaster. *Fly (Austin)* **5**: 174–180.

706

707 **Tables**

Table 1 - Systemic infection

Survival	**DF**	**χ^2**	**p-value**
DCV concentration	4	45.24	0.0001
Sex	1	8.37	0.0038
DCV concentration × Sex	2	8.26	0.0161
Viral titer	**DF**	**F Ratio**	**p-value**
DCV concentration	3	3.14	0.0399
Sex	1	7.34	0.0111
DCV concentration × Sex	3	1.35	0.2776
Activity per day			
Time (DPI)	1	290.68	0.0001
DCV concentration	3	5.17	0.0016
Time (DPI) × DCV concentration	3	5.51	0.001

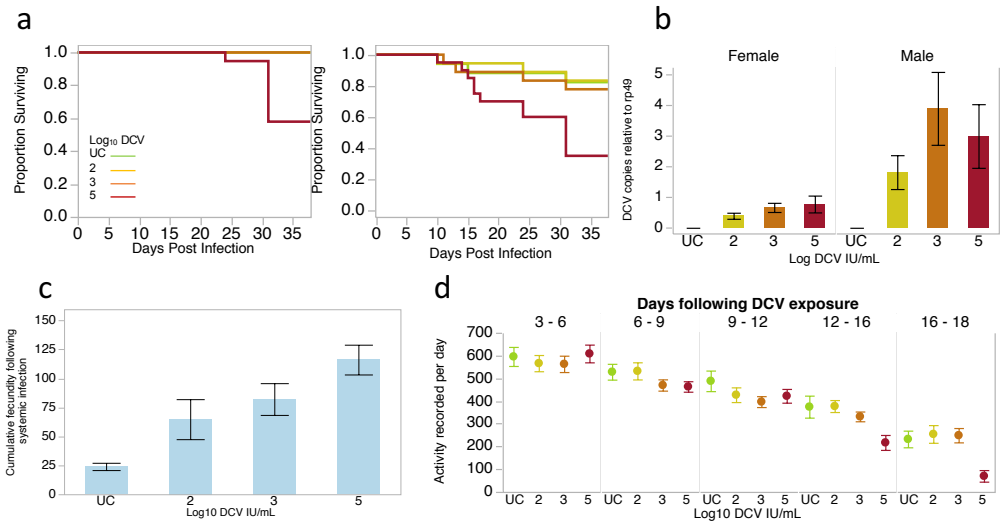
708

Table 2 - Oral infection

Fecundity	**DF**	**F Ratio**	**p-value**
DGRP Line	9	16.17	<.0001
DCV exposure	1	0.99	0.3186
DGRP Line × DCV exposure	9	2.59	0.0076
Activity per day			
DGRP Line	9	2.91	0.0037
Sex	1	0.02	0.8947
DCV exposure	1	1.45	0.2315
DGRP Line × Sex	9	2.18	0.0277
DGRP Line × DCV exposure	9	0.67	0.7352
Sex × DCV exposure	1	0.12	0.7244
Fecal excretion			
DGRP Line	9	32.17	0.0001
Sex	1	212.66	0.0001
Time (DPI)	1	29.95	0.0001
DCV exposure	1	72.83	0.0001
DGRP Line × DCV exposure	9	4.46	0.0001
Sex × DCV exposure	1	13.45	0.0003
Time (DPI) × DCV exposure	1	0.23	0.6295
DGRP Line × Sex	9	31.22	0.0001
DGRP Line × Time (DPI)	9	1.28	0.2405
Sex × Time (DPI)	1	0.06	0.806

709
710

Figure 1

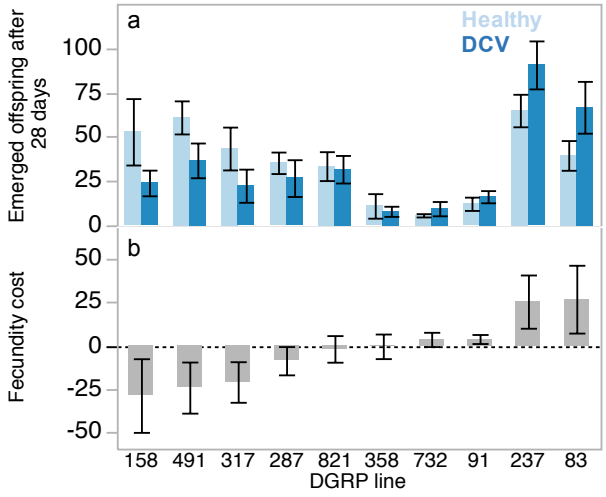


711

712 **Figure 1.** Sub-lethal systemic infection. 1a. Kaplan-Meier curves showing the
713 survival of 20 replicate flies exposed systemically to sub-lethal concentrations of
714 DCV. 1b. DCV titers measured in male and female flies relative to an internal
715 control gene (rp49), following 3 days of systemic infection with sub-lethal
716 concentrations of DCV. For each DCV concentration, data are the average of
717 duplicate qPCR reactions for 5 individual flies. 1c. Total fecundity recorded for
718 30 days following systemic infection in mated females. Data are the means ± SE
719 of 18-19 replicate females flies. 1d. Daily locomotor activity of male flies
720 following systemic infection with DCV. Data are 3 day averages of 7-10 replicate
721 flies for each inoculation concentration. UC are uninfecred controls.

722

723



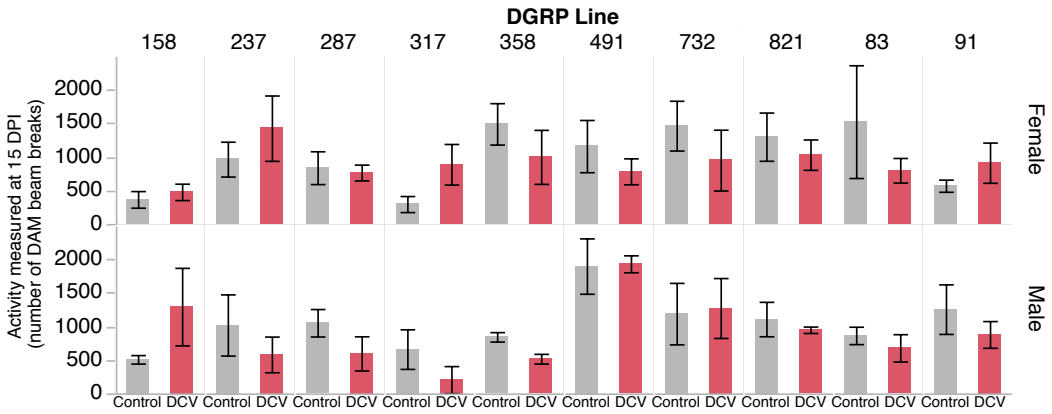
724

725 **Figure 2.** Fecundity following oral DCV exposure. 2a. The cumulative number of
726 adult offspring from healthy (light bars) or DCV-exposed (dark bars) single
727 female flies over the course of the 28-day experiment. 2b. Shows the fecundity
728 difference between healthy and infected flies for the same 10 DRGP lines. In both
729 plots, DGRP lines are ordered from the greatest decrease to the highest fecundity
730 increase. Significant pairwise contrasts (reported in Table S2) are indicated by
731 asterisks. Data are the mean ± SE of eight individual replicate females.

732

733

734



735

736

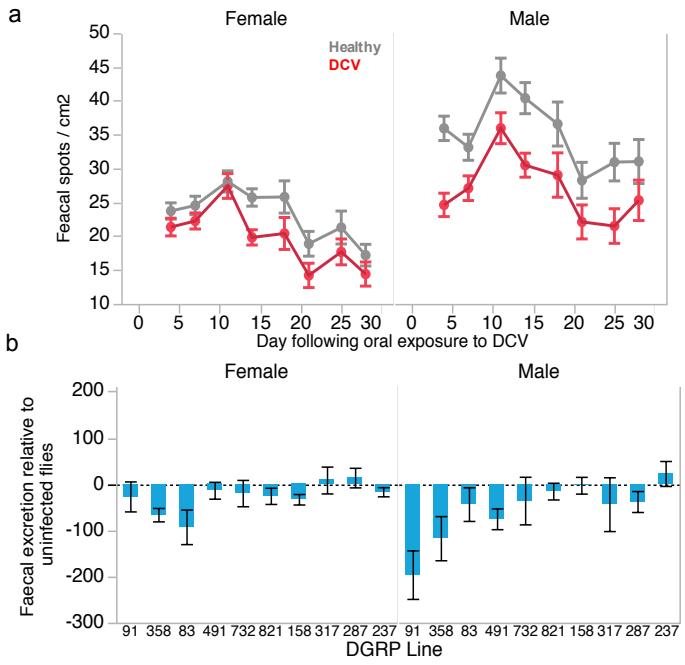
737 **Figure 3.** Locomotor activity following oral DCV exposure. Data show mean ± SE

738 activity of four replicate flies per sex and DGRP line, measured for 24 hours 14

739 days following exposure to DCV (red) or uninfecred controls (grey).

740

741



742

743 **Figure 4.** Fecal excretion following oral DCV exposure. 4a. The general effect of
744 DCV exposure (red) or a control inoculum (grey) on the number of fecal spots
745 shed over time. Data are plotted separately for males and females. Each time
746 point is the mean ± SE of 8 replicate individual flies averaged across all 10 DGRP
747 lines. 4b. Shows the difference between control and infected flies for each DRGP
748 line. Data are the mean ± SE of eight individual replicate flies for each sex and
749 line combination.

750

Supplementary File for

Costs and benefits of sub-lethal Drosophila C Virus infection

This file contains:

- Table S1. Cox proportional hazards analysis of survival following oral exposure to DCV.
- Table S2. Least Square Means Student's t pairwise contrasts between exposed and control fecundity following oral DCV exposure.
- Figure S1. DCV increases in titer following oral exposure to approximately 10^5 DCV copies.

Table S1. Output of Cox proportional hazard model testing variation in survival following oral infection.

Survival during oral infection	DF	χ^2	p-value
DGRP Line	9	3.87084122	0.9197
Sex	1	3.82E-07	0.9995
DGRP Line*Sex	9	2.85864198	0.9696
Infection status	1	4.73E-08	0.9998
DGRP Line* Infection status	9	0.74383375	0.9998
Sex* Infection status	1	1.07E-06	0.9992
DGRP Line*Sex* Infection status	9	0.25051421	1

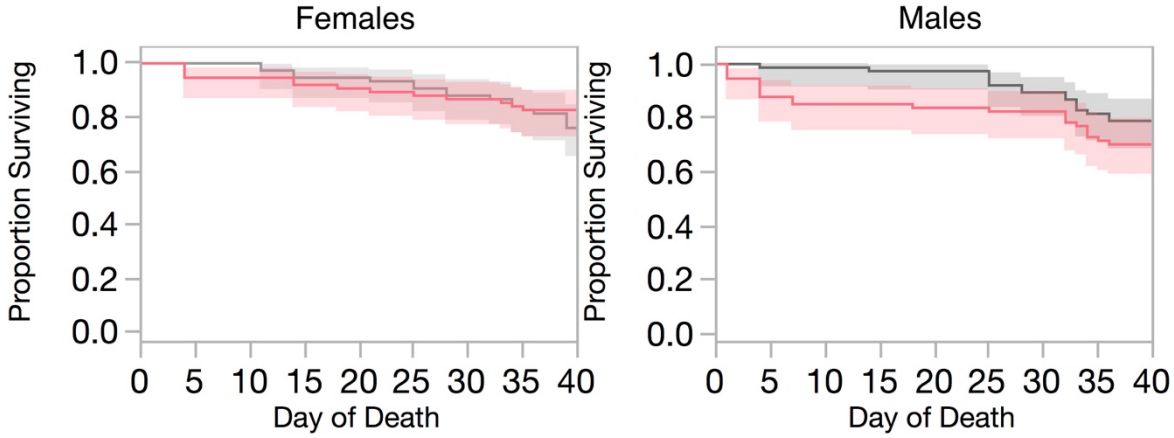
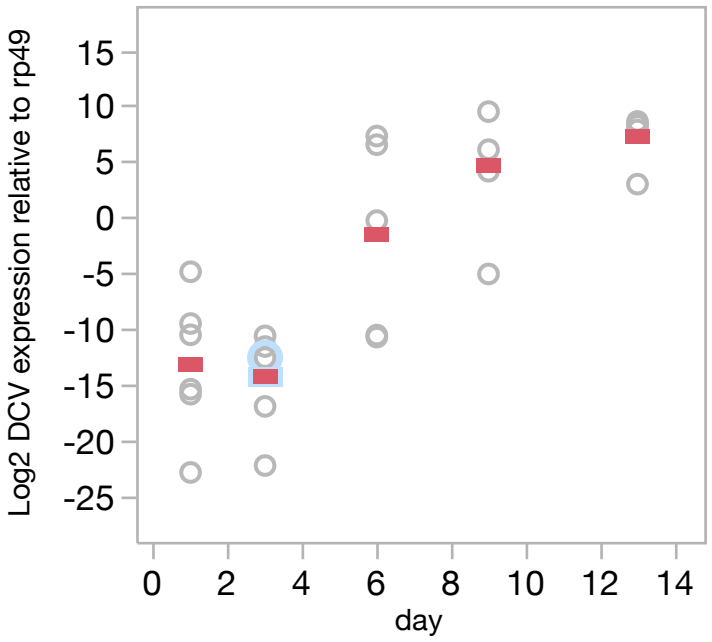


Table S2. Least Square Means Student's t pairwise contrasts between exposed and control fecundity following oral DCV exposure

DGRP line	NumDF	F Ratio	p-value
83	1	5.0178	0.036
91	1	0.2916	0.590
158	1	7.4368	0.007
237	1	5.6287	0.019
287	1	1.0515	0.306
317	1	4.6993	0.042
358	1	0.0525	0.819
491	1	4.7059	0.031
732	1	0.3253	0.569
821	1	0.0813	0.776



794
795
796

797 **Figure S1.** DCV increases in titer following oral exposure to with approximately
798 10^5 DCV copies ($F_{1,27} = 57.97$, $p < 0.001$). This experiment was carried out in *D.*
799 *melanogaster* OreR. Data show the Log2 DCV expression relative to an internal
800 Drosophila control gene (rp49), measured in six individual female flies at each
801 time point following exposure. Oral exposure to DCV was carried out as
802 described in the main text.