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Costs and benefits of sub-lethal Drosophila C Virus infection

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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 several 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

adaptive evolution in *Drosophila* immune genes (Obbard *et al.*, 2006, 2009; Early *et al.*, 2016).

To gain a better understanding of the potential fitness costs of DCV infection, we measured the physiological and behavioural responses of flies challenged with DCV. We carried out two separate experiments, either challenging flies with a range of sub-lethal viral concentrations systemically through intra-thoracic injury (experiment 1) or exposing flies through the oral route of infection to a low, sub-lethal concentration of DCV (experiment 2). Our aim was not to compare the two routes of infection, but to address sub-lethal infections using both infection routes, as these are commonly employed in experimental infections. We focused on traits that have been previously shown to be affected by DCV infection such as survival, fecal excretion, and locomotor activity, as well as female reproductive output, which is ultimately important for evolutionary fitness. We find that even apparently benign, sub-lethal infections can cause significant physiological and behavioural morbidity that is relevant to fly fitness, and that these effects vary according to viral concentration, host genetic background and sex.

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

flies following infection, using the expression of DCV RNA. Flies were individually placed in TRI reagent (Ambion) following five days of infection (5 DPI), homogenized total RNA was extracted using Direct-zol RNA miniprep kit, which includes a DNAse step (Zymo Research), reverse-transcribed with M-MLV reverse transcriptase (Promega) and random hexamer primers, and then diluted 1:2 with nuclease-free water. qRT-PCR was performed on an Applied Biosystems StepOnePlus system using Fast SYBR Green Master Mix (Applied Biosystems) and DCV primers, which include 5'-AT rich flaps to improve RT-PCR fluorescent signal (Afonina *et al.*, 2007) (DCV_Forward: 5' AATAAATCATAAGCCACTGTGATTGATACAACAGAC 3'; DCV_Reverse: AATAAATCATAAGAAGCACGATACTTCTTCCAAACC). We measured the relative fold change in DCV RNA relative to *rp49*, (Dmel_rp49 Forward: 5' ATGCTAAGCTGTCGCACAAATG 3' ; Dmel_rp49 Reverse: 5' GTTCGATCCGTAACCGATGT 3'). an internal *Drosophila* control gene, calculated as $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen, 2001).

Oral DCV exposure

In separate pilot infections, we determined that a DCV culture diluted to contain approximately 10^5 DCV RNA copies was enough to establish a viable infection (Figure S1), but did not cause noticeable mortality, and we used this dilution of DCV stock to inoculate all ten DGRP lines. Individual flies were exposed to DCV in vials containing Agar (5% sugar) using 3mL plastic atomizer spray bottles containing 2mL of the sub-lethal DCV dilution. One spray, releasing roughly 50µL 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

counted using 'spot detection' analysis on a 2cm x 4cm region of interest. Each image was checked individually for miscounts, and miscounted spots were removed. Fecal excretion was recorded for 30 days following infection.

Fecundity

All fecundity estimates are based upon mating that occurred before infection during the first 3-4 days after eclosion. The fecundity of individual flies was measured by counting viable offspring emerging in the vials they were reared in, which happened weekly until day 30 post infection in the systemically infected flies, and every 3-4 days in the orally infected flies, for 28 days following exposure to DCV. Short-term fecundity estimates have been shown to be well correlated with lifetime reproduction in *D. melanogaster* (Nguyen & Moehring, 2015). Vials that individuals were tipped from (and following the recording of fecal shedding in the oral infection experiment), were placed in the incubators at 24.5°C ± 0.5 with a 12h:12h light:dark cycle to allow any offspring to develop. After 14 days, the total number of living emerged adult offspring within each vial was recorded as a measure of female fecundity.

Activity

Locomotor activity was measured using the Drosophila Activity Monitor (DAM2, Trikinetics) as described previously (Pfeiffenberger *et al.*, 2010; Vale & Jardine, 2015). In the DAM, individual fly activity is recorded when individually housed flies break an infrared beam passing through a transparent plastic tube placed symmetrically inside a DAM unit. In systemically infected flies, as we used females to measure fecundity (see above), activity was measured on 10 replicate

male flies for each DCV dose (40 flies in total), starting the day following septic injury, 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 concentrations of DCV (10^2, 10^3 and 10^5 DCV IU/ ml) affected fly health. We have previously observed that DCV is able to establish and grow when inoculated into flies at these low doses (Figure S2). 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

309 measured during this period varied according to the dose females had received
310 ($F_{3,66}$ = 10.32, p<0.0001) and we observed that the total reproduction of infected
311 flies was higher than control flies, and increased in a dose-specific manner
312 (Figure 1c).
313
314 *Activity following systemic DCV infection*
315 The locomotor activity of individual male flies infected systemically with all sub-
316 lethal concentrations of DCV was measured during 18 days after infection in a
317 Trikinetics® Drosophila Activity Monitor (DAM). All flies included in the analysis
318 remained alive for the whole period, so changes in activity were not confounded
319 with potential death of individual flies. We found that flies in all treatments,
320 including uninfected controls, showed a reduction in activity over the course of
321 the activity assay (Figure 1d, Table time effect). This general effect is not
322 especially surprising given the constrained environment experienced by flies in
323 the DAM tubes, and that the only source of nutrition and hydration is small agar
324 plug. However, our analysis showed that the temporal reduction in activity
325 depended on the dose that flies had received ('time x dose' interaction, Table 1).
326 In the early stages of infection flies receiving the higher of the 4 doses (10^3 and
327 10^5 DCV copies) showed a reduction in activity relative to control flies and those
328 receiving the lowest dose. Over time, a reduction in locomotor activity was most
329 apparent in flies infected with the highest dose of 10^5 DCV copies (Figure 1d).
330
331 ***Experiment 2: Sub-lethal gut infection***
332 In a separate experiment, we tested how exposure to a single sub-lethal dose of
333 DCV through the oral route of infection impacted upon fly health. We conducted

334 the experiment on ten fly lines from the DGRP panel (Mackay *et al.*, 2012) and we
335 included both male and female flies to test for the effects of host genetic
336 background and sex in response to sub-lethal oral infection. While DGRP lines
337 differ in their lifespan in the absence of infection (Durham *et al.*, 2014), we did
338 not detect any difference between DGRP lines or between sexes in their survival
339 during oral DCV infection compared to control flies (Table S1) which, as
340 expected, was generally non-lethal across all lines.
341
342 *Fecundity following oral exposure to DCV*
343 Despite not observing any effects on fly survival during infection, we detected
344 significant variation in reproductive health following exposure to DCV. The total
345 fecundity of females during the 28 days following oral exposure to DCV (or a
346 control inoculum) varied significantly between DGRP lines (Figure 2; Table 2),
347 reflecting well-known genetic differences in the lifetime reproductive output of
348 these lines (Durham *et al.*, 2014). In addition, we found line-specific fecundity
349 responses to DCV infection ('infection status x line', Table 2, see also Table S2 for
350 pairwise contrasts). In some lines (158, 491, 317) low-level oral infection
351 resulted in a decrease in fecundity; in other lines (821, 358) there was no
352 detectable effect of DCV exposure; while in 2 lines we detected significant
353 increases in fecundity in DCV infected flies compared to uninfected control flies
354 of the same genetic background (Figure 2; see Table S2 for least-square pairwise
355 contrasts).
356
357 *Locomotor activity following oral exposure to DCV*

Overall, DGRP lines differed in their activity in a sex specific way ('Fly line x Sex' effect Table 2), but these differences were not altered by infection. While we detected a reduction in locomotor activity following systemic infection (Figure 1d), we did not detect any effect of oral DCV exposure on the overall activity of flies (Table 2, Figure 3).

Fecal excretion following oral exposure to DCV

We quantified fecal excretion for 30 days following DCV exposure as a proxy for gut health, by counting fecal spots excreted into vials after ingestion of blue-dyed food. Overall we found that males showed higher levels of fecal excretion compared to females (Table 2, 'sex' effect; Figure 4) and that DCV infection was associated with a general reduction in fecal excretion throughout the 30-day observation period ('Infection status' effect, Figure 4). However, we found that males and females differed in the overall severity of this reduction ('sex x infection status' effect), with males showing a greater reduction in defecation overall (Figure 4). Furthermore, we found significant variation among the DGRP lines in the magnitude of the effect of DCV on fecal excretion ('fly line x infection 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 two independent experiments using sub-lethal concentrations of either systemic or oral DCV infections, we observed 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

401 the virus. An alternative hypothesis may instead involve more complex
402 interactions between the allocation of resources during DCV infection, and how
403 they relate to fly nutritional stress and reproductive investment. For example, *D.*
404 *melanogaster* females selected under conditions of nutritional stress were found
405 to produce a greater number of ovarioles, while the offspring of starved mothers
406 also exhibited greater investment in reproduction (Wayne *et al.*, 2006). Similar
407 to the studies described above (Thomas-Orillard, 1984; Gomariz-Zilber &
408 Thomas-Orillard, 1993), this work also focused on ovariole number and egg
409 production, and did not quantify female lifetime fecundity. Given that DCV
410 infection is known to lead to intestinal obstruction, one possibility for the
411 increase in the number of adult offspring we observed in infected flies is that
412 DCV-induced nutritional stress leads to a greater production of ovarioles, and
413 consequently, an increased number of offspring. Given we only tested a single fly
414 line however, it important to note that this response may not be universal. As we
415 discuss below fecundity responses to infection have generally been found to
416 differ between host genotypes (Vale & Little, 2012; Parker *et al.*, 2014)
417
418 *Fecundity costs and benefits of DCV infection are genotype-specific*
419 Similar to systemically infected flies (Figure 1c), we also find evidence for
420 fecundity benefits in orally exposed flies, but these benefits were only revealed
421 in two out of the ten genetic backgrounds we tested. Indeed, in three of the
422 tested lines, DCV infection resulted in lower reproductive output. Taking
423 fecundity as a proxy for evolutionary fitness, the existence of genotype specific
424 fitness costs and benefits means that DCV could be a potentially powerful driver
425 of *D. melanogaster* evolutionary dynamics. Previous analyses of *Drosophila* spp.

426 population genetic data have shown that the fastest evolving *D. melanogaster*
427 genes are those involved in RNAi-based antiviral defense (Obbard *et al.*, 2006,
428 2009; Early *et al.*, 2016), but the DCV-induced fitness costs that drive this rapid
429 evolution in wild-infected flies (where infections are persistent and often non-
430 lethal), has remained obscure. These data suggest that genotype-specific
431 fecundity costs and benefits of DCV infection could potentially mediate the arms-
432 race between flies and viruses.
433
434 *Systemically infected flies show a dose-dependent decline in activity over time*
435 Reduced activity, or lethargy, following infection is a common response to
436 infection across a range of taxa (Hart, 1988; Adelman & Martin, 2009; Sullivan *et*
437 *al.*, 2016). The most obvious explanation for reduced activity is simply that
438 infected individuals are sick, and lethargy reflects the underlying pathology of
439 infection (Moore, 2013). A popular alternative explanation is that infection-
440 induced lethargy evolved as an adaptive host strategy that conserves energy,
441 which may then be allocated to other physiological tasks such as mounting an
442 immune response (Hart, 1988; Adelman & Martin, 2009).
443
444 Support for the adaptive nature of these 'sickness behaviours' has come mainly
445 from vertebrate species challenged with deactivated pathogens or their derived
446 components, which are sufficient to stimulate an immune response without
447 causing pathology (Adelman & Martin, 2009; Lopes *et al.*, 2016). In addition to
448 vertebrates, sickness behaviors including lethargy and anorexia have also been
449 described in insect hosts (Ayres & Schneider, 2009; Kazlauskas *et al.*, 2016;
450 Sullivan *et al.*, 2016). However, in the current experiment it is not possible to

451 disentangle the effect of an adaptive sickness behavior from the direct effect of
452 pathology caused by replicating DCV. Regardless of the underlying cause of
453 reduced activity, it is likely to come at an additional cost of lower involvement in
454 fitness-enhancing activities such as foraging, competing for resources with
455 conspecifics, or courtship and mating (Adelman & Martin, 2009; Adamo *et al.*,
456 2015; Vale & Jardine, 2016). Further, reduced activity following infection can
457 also reduce the potential for disease spread (Lopes *et al.*, 2016). In the context of
458 understanding sub-lethal DCV infection in an ecological setting, reduced activity
459 may therefore be a potentially important source of DCV-induced fitness costs
460 and benefits.
461
462 We did not find an effect of oral DCV exposure on fly activity. Previous work has
463 shown that *Drosophila*, especially females, show a reduction in activity following
464 oral infection with DCV (Vale & Jardine, 2015). However, the viral concentration
465 that flies were exposed to in that experiment was at least 1000x higher, so it is
466 likely that in the current experiment flies did not ingest virus in quantities large
467 enough to affect locomotor activity.
468
469 *The severity of DCV-induced digestive dysfunction is sex-specific*
470 Previous work has shown that DCV infection results in digestive dysfunction,
471 leading to increased body mass due to the inability to excrete digested food
472 (Arnold *et al.*, 2013; Chtarbanova *et al.*, 2014). We found that this measure of gut
473 health varied between genotypes and also between sexes. Extensive genetic
474 variation for gut immune-competence has previously been reported in the DGRP
475 panel (Bou Sleiman *et al.*, 2015), which could underlie some of the variation we

476 observe in DCV-associated digestive dysfunction in some lines. Although that
477 study focused on enteric infection with entomopathogenic bacteria, the
478 mechanisms that mediate variation in gut health during infection include general
479 processes of gut damage and repair, such as the production of reactive oxygen
480 species (ROS) and the production of intestinal stem cells during epithelial repair
481 (Buchon *et al.*, 2013). It is plausible that these mechanisms also mediate disease
482 severity during enteric virus infection, but we are unaware of any systematic
483 study of genetic variation in gut immune-competence during viral infection.
484
485 The mechanistic basis of the observed sex differences in fecal excretion is less
486 clear. The Malpighian tubules are the main organ involved in osmoregulation and
487 excretion of waste matter in insects (Dow & Davies, 2001). *D. melanogaster* male
488 and female Malpighian tubules have been shown to differ at the transcriptional
489 level with over 18% of genes (2308 genes) showing sex-specific expression
490 (Huylmans & Parsch, 2014). We measured fecal excretion by quantifying fecal
491 spots on the sides of the vials. Given that females are known to also spend more
492 time feeding (Wong *et al.*, 2009), it is possible that females also defecate more
493 on the surface of the food compared to males, and therefore spend less time on
494 the sides of the vials. Only a few studies have investigated sex differences in fecal
495 excretion in *D. melanogaster*, finding inconsistent patterns of excretion between
496 sexes (Zeng *et al.*, 2011; Urquhart-Cronish & Sokolowski, 2014). The link
497 between fecal excretion and fitness is not as clear as with fecundity or locomotor
498 activity, but it is relevant in the context of disease transmission of fecal-orally
499 transmitted pathogens such as DCV. The study of temporal trends in fecal
500 excretion and how they vary with host sex and genetic background may

501 therefore be used as a useful model to understand the sources of heterogeneity
502 in pathogen shedding (Vale *et al.*, 2013).
503
504 *Concluding remarks*
505 Altogether, these measures of sub-lethal morbidity give insight into the potential
506 fitness costs of low-level, persistent DCV infection in *Drosophila*. More generally,
507 the combination of both positive and negative effects on fly fitness effects
508 according to the specific host genetic background presents a non-trivial
509 evolutionary scenario for host immune defense (Gandon & Vale, 2014). For
510 instance, frequent encounters between beneficial symbionts and detrimental
511 pathogens are hypothesized to have played a role in the evolution of aphid
512 immune systems, which lack several components of the IMD immune pathway
513 critical for the recognition and elimination of Gram-negative bacteria (Gerardo *et*
514 *al.*, 2010). The combination of fitness costs and benefits of infection, such as
515 those incurred during DCV infection, may therefore have driven the evolution of
516 immune defense across a wide range of host taxa, from insects to mammals
517 (Elsik, 2010; Gerardo *et al.*, 2010; Lee & Mazmanian, 2010; Gandon & Vale,
518 2014).
519
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528

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.

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

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

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

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

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

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

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

586 Gomariz-Zilber, E., Poras, M. & Thomas-Orillard, M. 1995. Drosophila C virus:
587 experimental study of infectious yields and underlying pathology in
588 Drosophila melanogaster laboratory populations. *J. Invertebr. Pathol.* **65**:
589 243–247.

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

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

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

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

599 Huylmans, A.K. & Parsch, J. 2014. Population- and Sex-Biased Gene Expression in
600 the Excretion Organs of Drosophila melanogaster. *G3 GenesGenomesGenetics*
601 **4**: 2307–2315.

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

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

606 Karlikow, M., Goic, B. & Saleh, M.-C. 2014. RNAi and antiviral defense in
607 Drosophila: Setting up a systemic immune response. *Dev. Comp. Immunol.* **42**:
608 85–92.

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

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

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

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

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

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

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

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

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

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

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

637 Mackay, T.F.C., Richards, S., Stone, E.A., Barbadilla, A., Ayroles, J.F., Zhu, D., *et al.*
638 2012. The Drosophila melanogaster Genetic Reference Panel. *Nature* **482**:
639 173–178.

640 Magwire, M.M., Fabian, D.K., Schweyen, H., Cao, C., Longdon, B., Bayer, F., *et al.*
641 2012. Genome-Wide Association Studies Reveal a Simple Genetic Basis of
642 Resistance to Naturally Coevolving Viruses in Drosophila melanogaster. *PLoS*
643 *Genet* **8**: e1003057.

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

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

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

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

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

656 Obbard, D.J., Jiggins, F.M., Halligan, D.L. & Little, T.J. 2006. Natural selection
657 drives extremely rapid evolution in antiviral RNAi genes. *Curr. Biol. CB* **16**:
658 580–585.

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

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

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

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

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

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

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

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

678 Sullivan, K., Fairn, E. & Adamo, S.A. 2016. Sickness behaviour in the cricket Gryllus
679 texensis: Comparison with animals across phyla. *Behav. Processes* **128**: 134–
680 143.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure legends

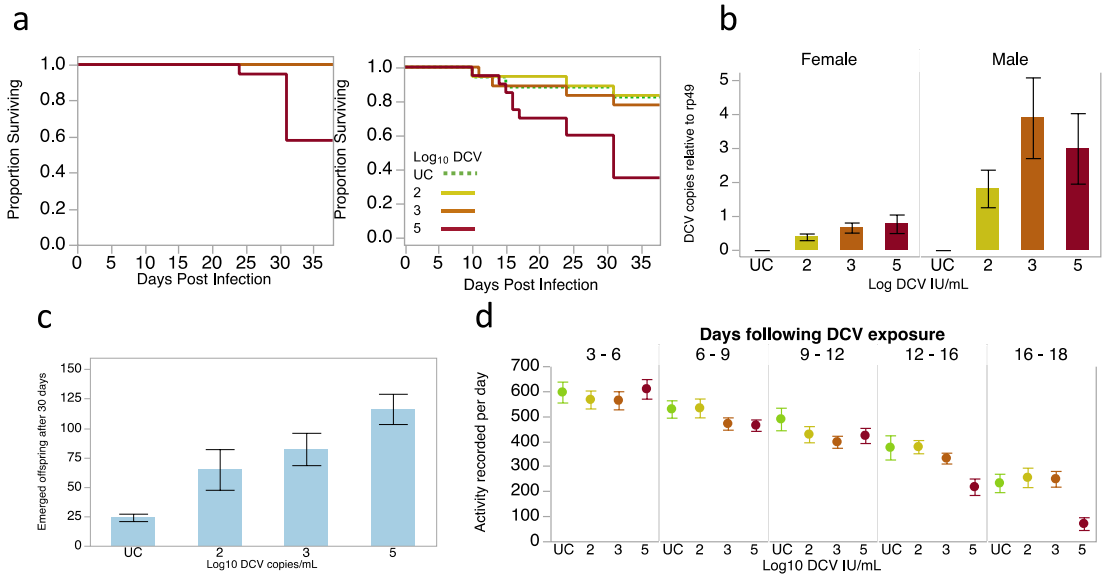


Figure 1. Sub-lethal systemic infection. 1a. Kaplan-Meier curves showing the survival of 20 replicate flies exposed systemically to sub-lethal concentrations of DCV. 1b. DCV titers measured in male and female flies relative to an internal control gene (*rp49*), following 3 days of systemic infection with sub-lethal concentrations of DCV. For each DCV concentration, data are the average of duplicate qPCR reactions for 5 individual flies. 1c. The total number of emerged adult offspring recorded for 30 days following systemic infection based on mating that occurred before infection during the first 3-4 days after eclosion. Data are the means ± SE of 18-19 replicate female flies. 1d. Daily locomotor activity of male flies following systemic infection with DCV. Data are 3 day averages of 7-10 replicate flies for each inoculation concentration. UC are uninfected controls.

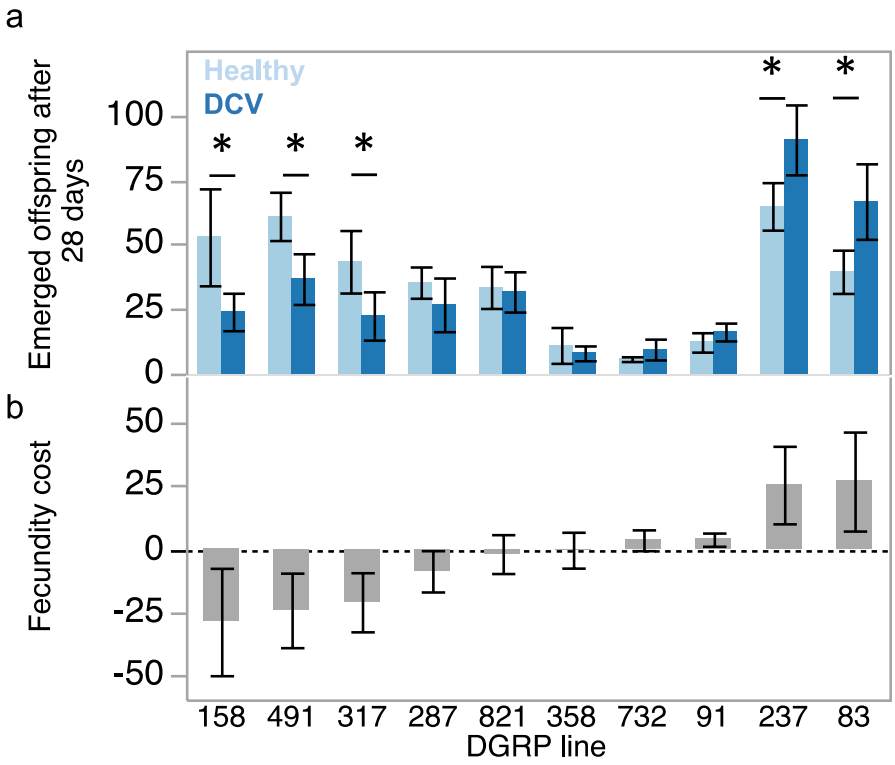
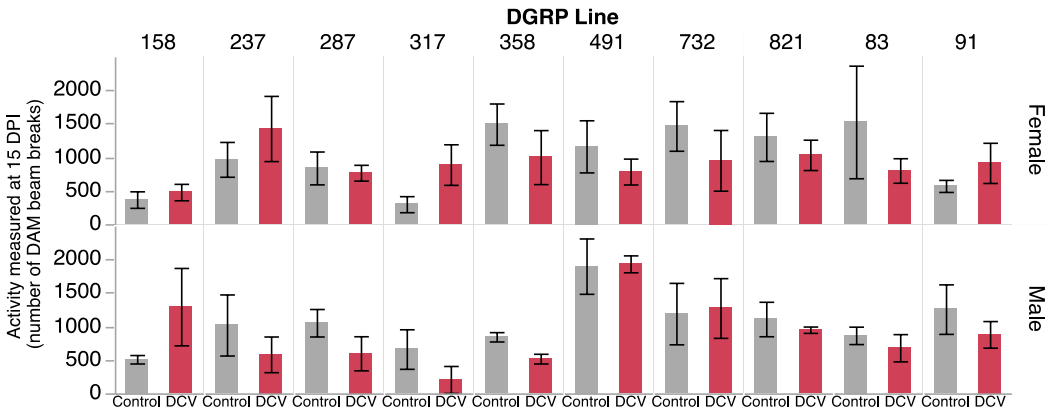


Figure 2. Fecundity following oral DCV exposure. 2a. The cumulative number of adult offspring from healthy (light bars) or DCV-exposed (dark bars) single female flies over the course of the 28-day experiment. 2b. Shows the fecundity difference between healthy and infected flies for the same 10 DRGP lines.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



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

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

746 days following exposure to DCV (red) or uninfected controls (grey).

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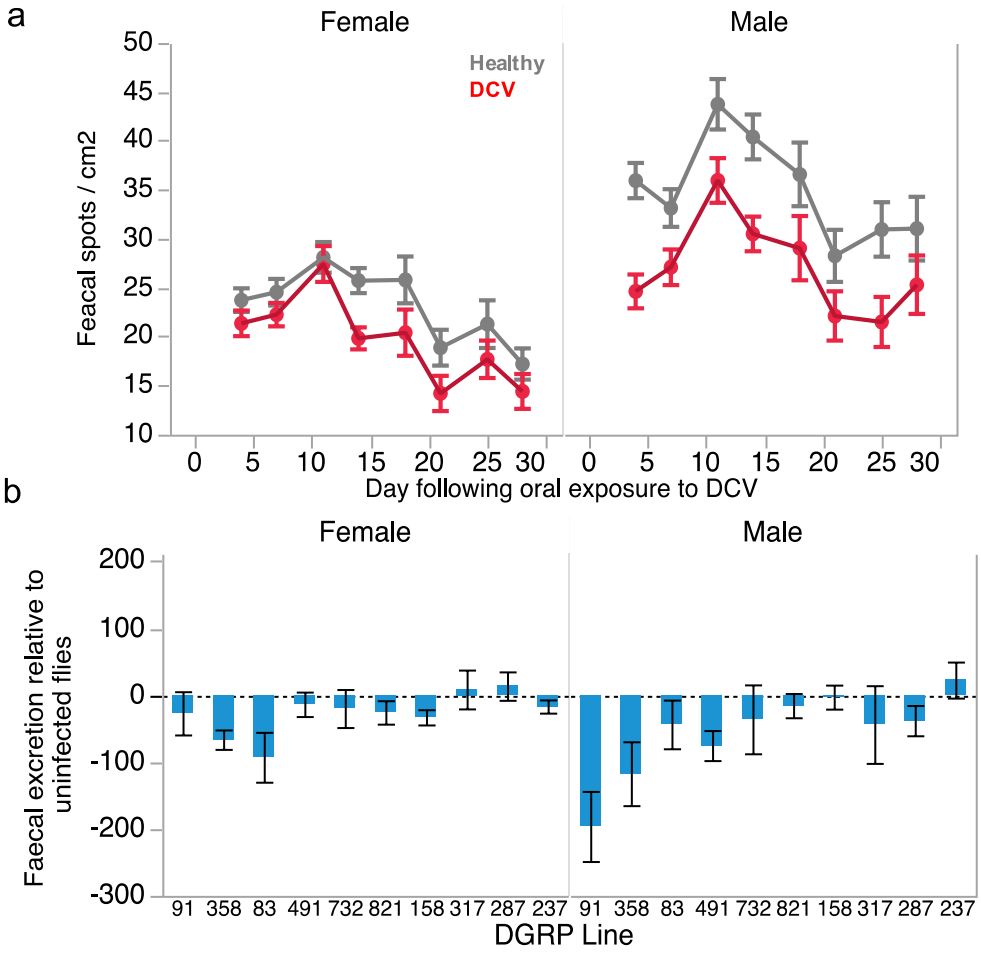


749
750 **Figure 4.** Fecal excretion following oral DCV exposure. 4a. The general effect of
751 DCV exposure (red) or a control inoculum (grey) on the number of fecal spots
752 shed over time. Data are plotted separately for males and females. Each time
753 point is the mean ± SE of 8 replicate individual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

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

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

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.

- Figure S2. DCV increases in titer following systemic challenge with 10^2, 10^3 and 10^5 DCV IU/ ml.

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

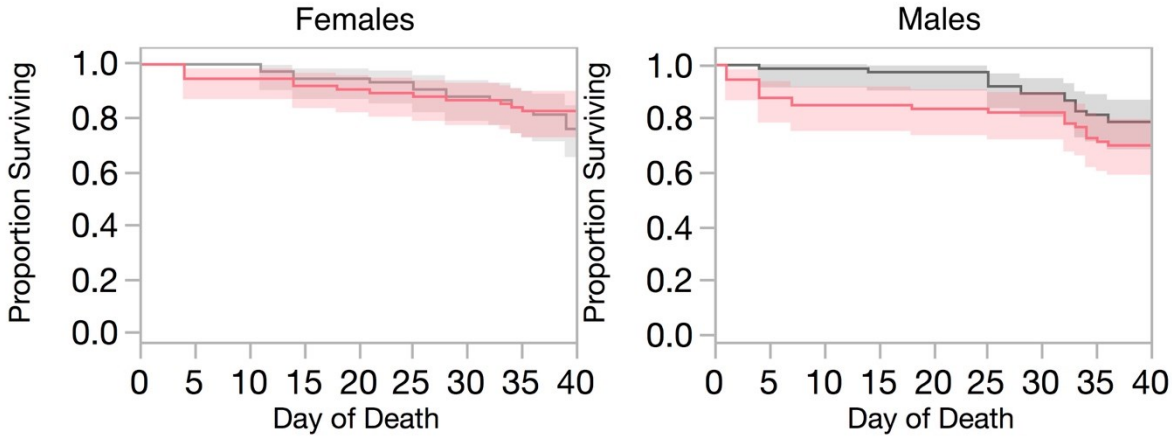


Table S2. Least Square Means Student's t pairwise contrasts between exposed and control fecundity following oral DCV exposure

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

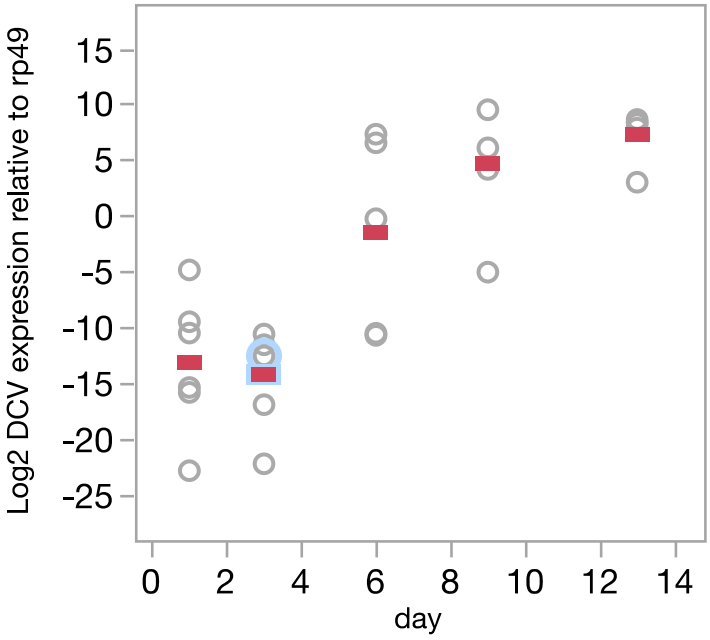
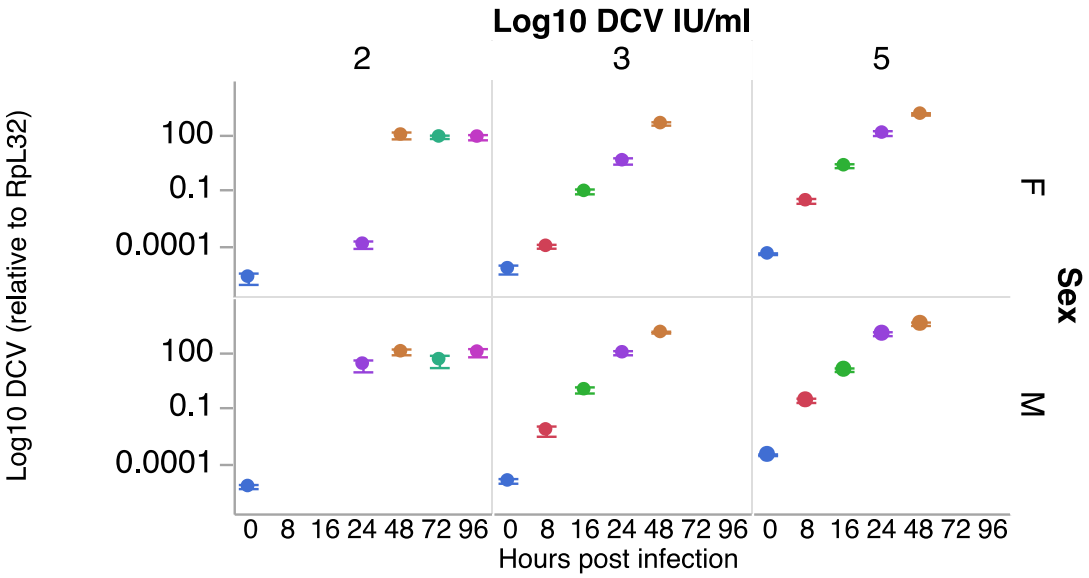


Figure S1. DCV increases in titer following oral exposure to with approximately 10^5 DCV copies ($F_{1,27} = 57.97$, $p < 0.001$). This experiment was carried out in *D. melanogaster* OreR. Data show the Log2 DCV expression relative to an internal Drosophila control gene (rp49), measured in six individual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



819
820 **Figure S2.** These data show DCV expression relative to the internal control gene
821 RpL32 measured at roughly 8-hour intervals. Male (M) of female (F) *D.*
822 *melanogaster* (Oregon R, Wolbachia-negative) were challenged with 2, 3 or 5
823 Log10 DCV IU/ml. Data show means ± SE of duplicate qPCRs on 3 replicate
824 groups of 5 flies per sex/DCV concentration.
825
826