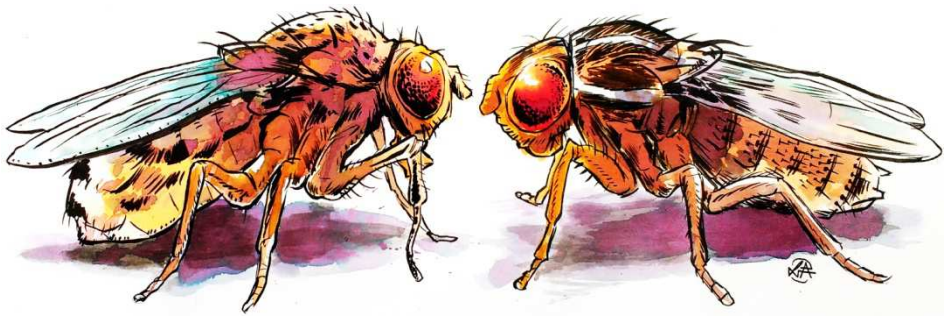
Variação na susceptibilidade do hospedeiro a diferentes patógenos: um estudo experimental e filogenético de *Drosophila*-vírus



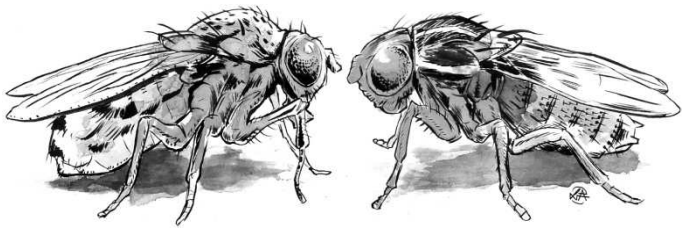
CAMILA SOUZA BERALDO

São Paulo

2018

Variação na susceptibilidade do hospedeiro a diferentes patógenos: um estudo experimental e filogenético de *Drosophila*-vírus

Variation in host susceptibility to different pathogens: an experimental and phylogenetic study of *Drosophila*-viruses



CAMILA SOUZA BERALDO

São Paulo
Junho, 2018

Universidade de São Paulo
Instituto de Biociências
Programa de Pós-Graduação em Ecologia

Variação na susceptibilidade do hospedeiro a diferentes patógenos: um estudo experimental e filogenético de *Drosophila*-vírus

Variation in host susceptibility to different pathogens: an experimental and phylogenetic study of *Drosophila*-viruses

Camila Souza Beraldo

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para obtenção de Título de Mestre em Ciências, na área de Ecologia de Ecossistemas Terrestres e Aquáticos.
Orientador: Rodrigo Cogni

São Paulo
Junho, 2018

Ficha Catalográfica

Beraldo, Camila Souza

Variação na susceptibilidade do hospedeiro a diferentes patógenos: um estudo experimental e filogenético de *Drosophila*-vírus. Versão em inglês: Variation in host susceptibility to different pathogens: an experimental and phylogenetic study of *Drosophila*-viruses/ Camila S. Beraldo; orientador Rodrigo Cogni — São Paulo, 2018.

59p.

Dissertação (Mestrado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Ecologia.

Palavras-chave: 1. troca de hospedeiro. 2. virulência. 3. carga viral. 4. tolerância. 5. interação parasita-hospedeiro.

Universidade de São Paulo. Instituto de Biociências. Departamento de Ecologia.

Comissão Julgadora:

_____
Prof(a). Dr(a)

_____
Prof(a). Dr(a).

_____
Prof(a). Dr(a)

_____
Prof. Dr. Rodrigo Cogni
Orientador

Mosca dependurada na beira de um ralo –
Acho mais importante do que uma jóia pendente.

Os pequenos invólucros para múmias de passarinhos
que os antigos egípcios faziam
Acho mais importante do que o sarcófago de Tutancâmon.

O homem que deixou a vida por se sentir um esgoto –
Acho mais importante do que uma Usina Nuclear.
Aliás, o cu de uma formiga é também muito mais
importante do que uma Usina Nuclear.

As coisas que não têm dimensões são muito importantes.
Assim, o pássaro *tu-you-you* é mais importante por seus
pronomes do que por seu tamanho de crescer.

É no ínfimo que eu vejo a exuberância.

Manoel de Barros

AGRADECIMENTOS

Agradeço ao Rodrigo Cogni pela orientação, dedicação e paciência durante os anos do mestrado. Muito obrigada por sempre incentivar o meu crescimento pessoal e profissional e por, no fim, ter feito com que eu gostasse ainda mais das mosquinhas. Aos meus queridos *lab mates* Murillo, André e Marcos, agradeço por toda a ajuda com a manutenção das linhagens e coleta de dados durante os experimentos. Agradeço também pelas discussões acadêmicas e conversas pessoais — eu não teria concluído esse projeto se não fosse a colaboração e o apoio de vocês.

Agradeço ao Ben Longdon pela orientação e por todas as conversas esclarecedoras durante o intercâmbio. Obrigada também por me ensinar a respeitar os finais de semana e por me inspirar como divulgadora científica. A Katherine, agradeço pela ajuda com as análises dos dados e por ser um grande exemplo de mulher cientista. Ao Ryan, muito obrigada pela amizade, parceria e todas as risadas durante os meus meses em Penryn.

Agradeço ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e à Fundação de Amparo e Pesquisa (FAPESP) pelas bolsas de estudo, viabilizando o projeto no país e o estágio na Inglaterra (Reino Unido).

Obrigada aos professores e demais funcionários do Instituto de Biociências (USP) e do College of Life and Environmental Sciences (University of Exeter - Penryn). Em especial, agradeço aos membros do meu comitê Miúdo e Edu — vocês contribuíram muito para o projeto. Agradeço também aos professores Vilela e Maria Vibranovski pelo espaço no laboratório, discussões e auxílio com identificação de espécies. Ao Glauco e ao Billy, agradeço pelas dicas sobre redação e pela disponibilidade para conversas. Obrigada aos técnicos Gustavo, Chico e Felipe pela ajuda com as linhagens, e aos funcionários das secretarias da Ecologia e da Pós-Graduação, principalmente à Vera e à Shirlene.

Aos amigos queridos que dividem casa e coração: Tama, Xano, Gustavo, Jolie e Jujubee — obrigada por aceitarem ser a minha família em SP. Aos amigos que não dividem casa, só coração: Orgi, Marx, Bala e Jorge — obrigada por pelegarem sempre!

Aos amigos "*PhD first years*", especialmente "*tall*-Tom", "*bee*-Thom", Rachael, Laura e Mel, agradeço pela companhia e apoio durante os seis meses em Penryn. Obrigada também aos amigos de Falmouth — Chris e Becky — que me ajudaram em momentos difíceis no início do intercâmbio.

Agradeço aos amigos da Toca, aos Bestas, à equipe do Alô Ciência? (obrigada Tom pela arte da capa), aos amigos do Pinheirinho e dos Cursos de Campo 2016 e 2017. Sobretudo, obrigada ao Pietro, Louise, Karina e Iluminado por recorrentes conversas sobre a vida e eventuais ajudas com R.

Muito obrigada aos amigos que, perto ou longe, estiveram presentes durante os anos do mestrado, particularmente à Aline e ao Phelps. Eu aprendi muito com vocês!

Por fim, agradeço à minha (grande) família de Santa Isabel e de Aparecida por todo o incentivo de sempre. Agradeço principalmente aos meus pais, Airtola e Cida, por serem grandes e fortes exemplos para mim e por continuamente me ajudar e me apoiar nas minhas escolhas. Obrigada também aos melhores irmãos que eu poderia ter: Vi, Loris, Mari e Ju — eu amo vocês.

CONTENTS

RESUMO

Trocas de hospedeiro — quando um patógeno passa de uma espécie de hospedeiro para outra — são descritas como um dos principais fatores causadores de doenças infecciosas emergentes (DIE). O dano que um patógeno causa a seu hospedeiro (virulência) varia quando trocas de hospedeiro ocorrem. Diferenças em susceptibilidade entre espécies de hospedeiros indicam que patógenos são mais propensos a realizar trocas entre determinados grupos de hospedeiros. Os fatores que determinam a variação na susceptibilidade do hospedeiro ainda são desconhecidos, porém um possível preditor é a história evolutiva do hospedeiro. Nesse estudo, nós examinamos como hospedeiros filogeneticamente relacionados variam em susceptibilidade ao lidar com duas infecções de vírus que variam em patogenicidade. Infectamos 39 espécies de Drosophilidae com o vírus A de *Drosophila* (DAV), inicialmente descrito como não virulento, e medimos mortalidade do hospedeiro (virulência) e replicação viral (carga). Em seguida, nós comparamos nossos resultados com informações do vírus C de *Drosophila* (DCV), um virulento, e analisamos os dados dos dois vírus conjuntamente. Encontramos uma grande variação nos dados de virulência e carga viral para DAV, com infecções benignas em alguns casos e alta mortalidade em outros. Encontramos correlação para carga viral, com espécies com cargas virais semelhantes aparecendo filogeneticamente próximas. Contudo, não há correlação filogenética para virulência, indicando que a virulência de DAV não pode ser predita com base na carga viral. Além disso, nós não encontramos correlação entre os resultados de DAV e DCV, indicando que a variação na susceptibilidade não pode ser predita por infecções de outros patógenos. É possível que fatores ecológicos e genéticos do hospedeiro e do parasita estejam influenciando a variação em susceptibilidade. Esses resultados sugerem que, apesar de alguns traços possam ser preditos pela filogenia,

determinar os fatores causadores da variação na susceptibilidade a diferentes patógenos após trocas de hospedeiros é um trabalho extremamente complexo.

ABSTRACT

Host shifts — where a pathogen jumps from one host species to another — have been described as one of the main factors leading to emerging infectious diseases (EID). The harm that a pathogen causes to a host (virulence) varies following a host shift. Differences in susceptibilities among host species means that pathogens may be more likely to switch between certain groups of hosts. Factors that determine the variation in host susceptibility are still unknown, but one possible predictor is the host evolutionary history. Here, we examine how phylogenetically related hosts vary in susceptibility when dealing with infections of two viruses differing in pathogenicity. We infected 39 species of Drosophilidae with *Drosophila* A virus (DAV), a virus initially described as avirulent, and we measured host mortality (virulence) and virus replication (viral load). Then, we compared our results to previously collected data from the virulent *Drosophila* C virus (DCV) and we analysed the data of both viruses together. We found large variation in DAV virulence and viral load, with benign infections in some cases and high mortality in others. There was phylogenetic correlation in viral load, with species presenting similar viral load clustering together in the phylogeny. However, we did not find correlation for virulence, indicating that DAV virulence was not predictable based on viral load. Also, we did not find correlation between DAV and DCV results, indicating that variation in host susceptibility is not predictable by other pathogens infections. It is possible that hosts and parasites ecology or genetic traits may be also influencing susceptibility variation. These results suggest that although some traits are predicted by phylogeny, to determine the factors driving host susceptibility variation to different pathogens following host shifts is a very complex task.

INTRODUCTION

Emerging infectious diseases (EID) — infections recognized in a host population for the first time (Morens & Fauci, 2013) — have significant negative impacts on the economy, human health, wildlife management and biodiversity conservation (Daszak et al., 2000; Morens et al., 2004; Jones et al., 2008). Factors that lead to the emergence of novel diseases are still not well understood, but socio-economic, environmental and ecological components have been pointed to as major drivers in the origin of EIDs (Jones et al., 2008). One potential cause of emerging diseases is host shifts, in which a pathogen jumps from one species into another (Woolhouse et al., 2005; Longdon et al., 2014). In humans, 60.3% of EID are result of pathogens changing from non-human animal reservoirs (Jones et al., 2008). For instance, the human immunodeficiency virus (HIV) jumped into humans roughly one hundred years ago from non-human primates in West-Central Africa (Sharp & Hahn, 2011; Faria et al., 2014), resulting in the human AIDS pandemic that has killed approximately 35 million people (World Health Organisation, 2018). Similarly, the Ebola virus shifted from fruit bats to humans in the 70's and caused an outbreak in Gabon and the Republic of Congo between 2001 and 2005 (Leroy et al., 2005).

Host shifts have been demonstrated to be frequent in systems such as phytophagous insects, plant fungi and plant and animal viruses (Nyman, 2010; de Vienne et al., 2013). Predicting the spatio-temporal occurrence of a host shift is still challenging, as it may be linked to a multitude of variables ranging from host and pathogen geographic dispersion to changes in host phenotype and genetics (Woolhouse et al., 2005). Understanding what are the factors favouring host shifts and identifying the potential susceptible taxa is crucial to novel emerging pathogen research and to mitigate their impacts in human and wildlife populations (Woolhouse et al., 2005; Burbrink et al., 2017). Although the host range of some pathogens

have been reported as ecologically and phylogenetically dispersed (Burbrink et al., 2017), there are also evidences for infections following some host features (Corey & Waite, 2008; de Vienne et al., 2009; Balaz et al., 2013; Zukal et al., 2014). For example, species of frogs from high-altitude, with a restricted range and with low fecundity are more susceptible to the fungus *Batrachochytrium dendrobatidis* than species that do not have these traits (Bielby et al., 2008; Smith et al., 2009).

An important factor underlying host susceptibility is the phylogenetic relatedness among the potential hosts (de Vienne et al., 2013; Longdon et al., 2014). Host phylogeny can influence pathogen replication in two ways: through phylogenetic distance effect and phylogenetic clade effect (Longdon et al., 2014). Phylogenetic distance effects suggest that a parasite's infection success decreases as the phylogenetic distance from the natural host increases (Figure 1A) (Longdon et al., 2014; Longdon et al., 2015a). This occurs when taxa phylogenetically closer to the natural host are more likely to be infected (Corey & Waite, 2008; de Vienne et al., 2009). For example, when a virus infects a novel host, adaptations to different cell receptors are necessary and, supposing that structural changes in cell receptors increase with phylogenetic distance from the original host, the virus will need more changes (Longdon et al., 2011). In turn, the phylogenetic clade effect predicts that the parasite infection success varies between different host clades, i.e. a particular clade of hosts may have a similar susceptibility to the parasite, independent of the distance from the natural host (Figure 1B) (Longdon et al., 2014; Longdon et al., 2015a). This occurs when particular clades share some features that made them particularly resistant or susceptible to the pathogen (Longdon et al., 2014).

During an infection, hosts use a combination of two different mechanisms to defend against parasites, resistance and tolerance (Ayres & Schneider, 2012). Resistance is when there is an activation of host's immune system to control pathogen's replication, and

tolerance, when the host is able to avoid a decrease in its own fitness without necessarily altering the parasite load (Medzhitov et al., 2012; Ayres & Schneider, 2012; Vale et al., 2016). Virulence is the cost in fitness a pathogen causes to its host due to infection (Read, 1994; Vale et al., 2016; Vale, 2018), and it may vary following a host shift, presenting high levels in particular species and leading to outbreaks and epidemics (Woolhouse, 2005; Jones et al., 2008). Initially, virulence was thought to be a direct consequence of parasite replication, being linked to the idea that the host-parasite interaction evolves towards avirulence, i.e. the pathogen does not cause a cost in fitness for the host anymore (Alizon et al., 2009). However, some host susceptibility features, e.g. resistance or tolerance, may affect how virulent a pathogen could be, decoupling virulence and parasite load measures (Gandon & Michalakis, 2000; Gandon et al., 2002).

Hosts have evolved diverse adaptations to avoid pathogen infection and replication, ranging from complex antiviral innate immune pathways in dipteran insects (Palmer et al., 2018) to behaviours that reduce the probability of encounter between host and parasite (Theimann & Wassersug, 2000). Despite some isolate cases where defence seems not to be costly (Heath et al., 2017), these strategies consume resources which the host could use to perform other activities (Sheldon & Verhulst, 1996). For instance, it has been suggested that the CRISPR-Cas system, used by bacterium to cut pathogen-derived nucleic acids, reduces *Streptococcus thermophilus* fitness when in the presence of a mutant not able to express Cas protein (Vale et al., 2015). In this way, as defence mechanisms against parasites may represent a cost in fitness for hosts (Sheldon & Verhulst, 1996) and as hosts have limited resources, hosts need to deal with trade-offs among investment in resistance or fitness, or among resistance traits. The trade-off among resistance traits means that an increase in resistance or tolerance to one pathogen must be balanced by an increase in susceptibility to another pathogen (Kariñho-Betancourt & Núñez-Farfán, 2015). For example, it has been

showed that plants express different resistant traits against herbivory depending on the place they are (Berenbaum & Zangerl, 2006) and on the time (Wäckers & Bonifay, 2004).

A powerful biological system to study variation in host susceptibility are drosophilid fruit flies. *Drosophila* species are a well-established genetic model, easily manipulated, with a huge diversity of lineages available as laboratory stocks and widely used in studies of host-parasite interaction (Lemaitre & Hoffman, 2007; Huszar & Imler, 2008; Merkling & Rij, 2015). Wild *Drosophila* populations are found to be infected by a range of natural pathogens, including numerous fungi, trypanosomatids, nematodes, parasitoid wasps, bacteria and viruses (Kraaijeveld & Godfray, 1997; Corby-Harris et al., 2007; Fleury et al., 2009; Jaenike et al., 2010; Wilfert et al., 2011; Magwire et al., 2012; Webster et al., 2015). RNA viruses, one of the most common pathogens types which become EIDs by host shifts (Woolhouse et al., 2005; Jones et al., 2008), are very abundant in fruit flies. More than 30% of wild *Drosophila melanogaster* carry a virus, and around 6% is infected with multiple viruses (Webster et al., 2015). Antiviral immunity in *Drosophila* may follow different pathways depending on the virus (Palmer et al., 2018), such as Toll pathway for the Nora virus (Cordes et al., 2013) and RNAi and JAK-STAT pathways when infected by *Drosophila* C virus (DCV) (Dostert et al., 2005; Kemp et al., 2013). In addition, fruit flies also vary in susceptibility among different species. A study performing virus cross infections in 48 *Drosophila* species showed large variation in virulence and viral load over all hosts analysed (Longdon et al., 2015).

Here, we evaluated the range of *Drosophila* susceptibility responses to viruses that naturally infect *Drosophila* and vary in pathogenicity. We infected 39 species of *Drosophila* with *Drosophila* A virus (DAV). DAV (Permutotetraviridae) is a positive-sense RNA virus isolated from *Drosophila melanogaster* (Ambrose et al., 2009) and very abundant in drosophilid populations (Webster et al., 2015). It has long been thought that DAV is a non-

virulent virus infecting *Drosophila* (Plus et al., 1975; Ambrose et al., 2009), although some new studies have demonstrated its potential effect reducing *Drosophila suzukii* survivorship (Carrau et al., 2018). We measured DAV's virulence and viral load to address the questions: (i) how do virulence and viral load vary among host species?; (ii) is there a phylogenetic effect (distance or clade) determining virulence and viral load variation?; (iii) are virulence and viral load correlated to each other? Then, we compared our DAV results to previous published DCV (Dicistroviridae) data (Longdon et al., 2015a) to answer: (iv) is there a correlation in host susceptibility (virulence and viral load) to different viruses? DCV is an RNA virus isolated from *D. melanogaster* (Christian, 1987), which has been recorded as the most virulent RNA *Drosophila* viruses (Brun & Plus, 1980; Ambrose et al., 2009; Arnold et al., 2013). Addressing the variation in host susceptibility to different pathogens in a broad phylogenetic scale is vital to understand why pathogens successfully jump between some host species but not to others.

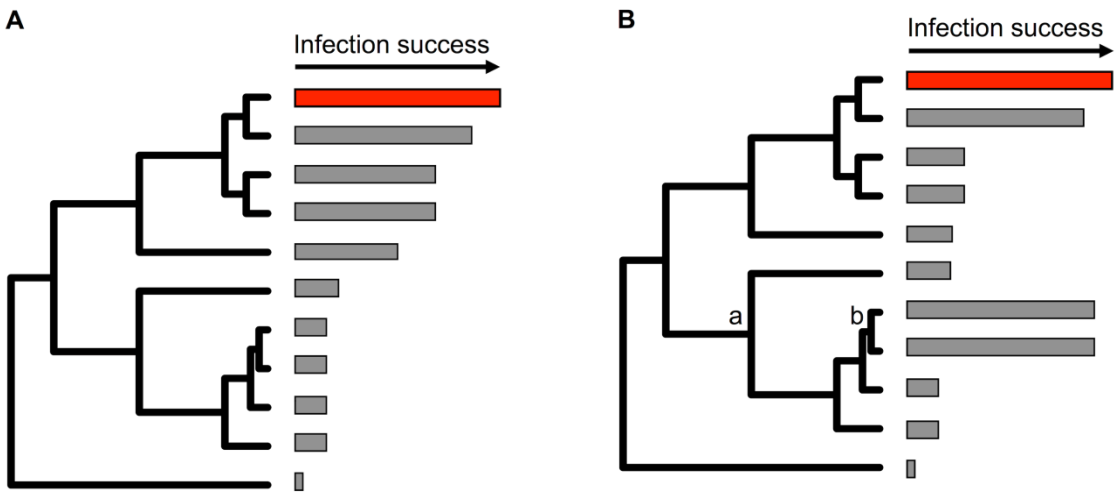


Figure 1. Phylogenetic distance and clade effects diagram. Bars represent a hypothetical measure of success in parasite infection and taxa with the red bar represents the parasite's original host. (A) Parasite's infection success declines as the phylogenetic distance from the natural host increases. (B) Phylogenetic distance from the original host has a minor effect, once the high tolerance or resistance is spread across the phylogeny. All the species from clade "a" are equally distant from the pathogen's original host. However, even having the same phylogenetic distance, species in clade "b" are more susceptible to the infection. Adapted from Longdon et al. (2014).

METHODS

Virus production

Drosophila A Virus (DAV) was kindly provided by Dr. Karyn Johnson (University of Queensland, Brisbane, Australia) (Ambrose et al., 2009). We injected DAV into flies of an isogenic virus-free lineage of *Drosophila melanogaster* (W1118) and flies were maintained on cornmeal medium (S1) at 25ºC, 70% humidity and 12h light-dark cycle for 10 days. Flies were then frozen in liquid nitrogen, stored at -80ºC and homogenised in Ringer's solution (4 µL/fly) as described in Ambrose et al. (2009). To remove any cellular components, we centrifuged the homogenate twice at 4ºC, each time retaining the supernatant. The supernatant was passed through Miller PVDF 0.45 µM and 0.22 µM syringe filters (Millipore, Billerica, MA, USA) to clear bacteria. We made aliquots from the DAV suspension, which were stored at -80ºC and used in all following experiments.

Flies inoculation

We used 39 species of Drosophilidae from the *Drosophila Species Stock Center*, University of California, San Diego, USA. These stocks were previously tested for the *Wolbachia* presence (Roberts et al. Unpublished), which is an endosymbiotic bacterium that affects resistance to RNA viruses (Hedges et al., 2008; Werren et al., 2008; Merkling & Rij, 2015), and so we only used *Wolbachia*-free species. All rearing and experiments were carried out at 22ºC, 70% relative humidity and 12h light-dark cycle. Flies were maintained in 250 mL bottles with ~35 mL of food medium (for stock rearing conditions see S2) and tipped into bottles with fresh medium once a week.

We inoculated 2-5 days old male flies using a 0.0125 mm diameter stainless steel needle (Austerlitz Insect Pins) bent ~0.3 mm from the end. We dipped the needle into a 10^{-5}

DAV solution or into a control Ringer's solution without DAV. The DAV solution concentration was defined based on a pilot test (S3). Flies were pricked in the right pleural suture on the thorax with the bent part of the needle (Merkling & Rij, 2015), then placed into vials of cornmeal medium and kept at 22ºC, 70% relative humidity and 12h light-dark cycle.

Assaying virulence

Host fitness was measured as flies' survival time after stabbing. We measured survival time for 20 days for two treatments: control and DAV inoculated flies. For each species we carried out three replicates of each treatment, each replicate being a vial of 15 flies. Inoculations were carried out over a period of two months, performing both control and experimental inoculations twice a week. A control and virus treatment were carried out for each species every day, and the order in which species were inoculated was randomised. We used the same needle in both inoculations to control for the size and depth of the needle, carrying out the virus infections after the controls. Mortality was recorded daily, and flies were placed into fresh medium every 3 days.

Virulence was measured as the hazard ratio (HR) calculated using the Cox proportional-hazards model (Cox, 1972), a regression model to examine the relationship between survival time of individuals and one or more predictor variables. HR indicates the effect size of a covariate, i.e. the hazard of death of an individual per time unit. We also calculated the proportion of flies dead on each day of observation, taking the mean across the 20 days and performing an arcsine square-root transformation.

Measuring RNA viral load

We quantified the change in *Drosophila* A virus load inside the host over three days, using qRT-PCR. The time for DAV inoculation was defined based on a pilot test (S4). In

total, we carried out 6 replicates, each one with 15 flies per species; 3 replicates were snap frozen on day 0 as a reference of the relative dose for that species, and 3 replicates were snap frozen 3 days post-infection. Samples were then homogenised in Trizol (Invitrogen) and then stored at -80ºC.

We extracted RNA from Trizol (Invitrogen) homogenised flies, reverse transcribed RNA using the Promega GoScript (Promega Corp, Madison, WI, USA) and random hexamer primers, and cDNA was diluted 1:10 with nuclease free water. DAV RNA load was measured relative to a housekeeping gene, *RpL32*. We used specific *RpL32* primers for each species as in Longdon et al. (2011) (S5) and, for the DAV gene amplification, we used the following primers: DAV-3-F (5' - AGAGTGGCTGTGAGGCAGAT - 3') and DAV-3-R (5' - TACTTTGGGTCTGGGATTCG - 3') (Cogni et al., Unpublished).

The qRT-PCR was performed on an Applied Biosystems StepOnePlus system using SYBR Green Master Mix reagent (Applied Biosystems) with the following PCR cycle: 95ºC for 2 minutes and 40 cycles of 95ºC for 5 seconds, 60ºC for 30 seconds. We carried out two qRT-PCR reactions (technical replicates), for both the DAV and *RpL32* primers per sample. Each qRT-PCR plate contained four standard samples, and all technical replicates were split into different plates in a randomised block design. A linear model was used to correct the cycle thresholds (*Ct*) values for differences between qRT-PCR plates. To estimate the change in viral load, we calculated ΔCt, i.e. the difference between the cycle thresholds of the DAV qRT-PCR and the endogenous control (*RpL32*). We then calculated the viral load of the day 3 relative to day 0 flies as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{day0} - \Delta Ct_{day3}$. ΔCt_{day0} and ΔCt_{day3} are a pair of ΔCt values a day 0 and a day 3 biological replicate for a given species.

Statistical analysis

1. Phylogenetic correlations

To examine the effects of host evolutionary history on virulence and viral load data, and to compare DAV and DCV results, we used a phylogenetic mixed model (Lynch, 1991; Housworth et al., 2004; Hadfield & Nakagawa, 2010) in the MCMCglmm R package (Hadfield, 2010; R Foundation for Statistical Computing, Vienna, Austria). We used a model with four variates, applying as response variables (traits) the hazard ratio and the viral load for each species of each virus (DAV and DCV). We used 31 species for hazard ratio and 39 species for viral load. The model structure was similar to those outlined in Longdon et al. (2011) and Longdon et al. (2015):

$$\eta_{hti} = \beta_{1:t} + distance\beta_{2:t} + wingsize\beta_{3:t} + \mu_{p:ht} + \mu_{s:ht} + e_{hti}$$

in which η_{hti} is the response for the i^{th} biological replicate for trait t of a host species h. β_1 is the intercept for each trait t, β_2 models the linear effect of genetic distance from *Drosophila melanogaster* (DAV and DCV's original host), and β_3 models the linear effect of wing size. We used wing size as a measure of the fly size (as in Longdon et al., 2011; Longdon et al., 2015a). μ are random phylogenetic species effects (p) and non-phylogenetic species effects (s), and e are residuals. We used the host phylogenetic tree from Longdon et al. (2015a) and excluded the species that we did not include in our experiments.

Random effects and residuals are assumed to be multivariate normal with zero mean and covariance matrices as described in Longdon et al. (2015a). We specified the prior with a list of three elements: priors for the fixed effects (B), the G-structure (G) for the (co)variance of phylogenetic and non-phylogenetic random effects, and the R-structure (R) for (co)variance for residuals. For the fixed effects B, we used a multivariate normal distribution,

with zero mean and a diagonal variance matrix with huge variances (10^{10}) (Hadfield, 2010). G-structure was a non-central F distribution (Gelman, 2006) and R-structure was an inverse-gamma distribution with shape and scale equal to 0.002. The MCMC chain was ran for 13000000 iterations with a burn-in of 3000000 and a thinning interval of 10000.

To calculate how much the host phylogeny could be related to traits variation, we used an inter-specific variance dependent on the host phylogeny (v_p) and a species-specific component independent of the host evolution (v_s) to calculate the proportion of between-species variance in an specific group that is explained by the phylogeny ($v_p/(v_p + v_s)$), which is related to Pagel's lambda (Pagel, 1999; Housworth et al., 2004). Correlations between traits were calculated pair wise. Ancestral state reconstructions were built using *phytools* R package (Revell, 2012).

2. Other correlations

For analysis that did not involve host phylogeny, we performed Spearman's correlation tests, as our response variables were not normally distributed even after transformations.

RESULTS

DAV virulence and viral load vary greatly among Drosophila host species

To investigate the effect of DAV virulence on *Drosophila* host species, we inoculated 3555 flies from 39 species of Drosophilidae — 1797 flies were stabbed with Ringer's control solution and 1758 flies were infected with DAV. Control samples were used to confirm that observed differences follow the virulence of DAV instead of intrinsic variation between species. We found large variation in DAV virulence when infecting different host species (Figure 2). In some species, such as *D. euronotus* and *Z. tuberculatus*, survival time is not affected by DAV infection, and the mortality does not increase over 20 days. In other cases, e.g. *D. tropicalis* and *S. lebanonensis*, DAV effect is more severe and ~50% of the population is killed by day 10 post-injection. Hazard ratios from the 39 species (Figure 3D) ranged from 0.142 in *D. virilis* to 26.012 in *S. lebanonensis*.

In about 1/3 of all the species, we observed mortality in control flies higher than expected. In most of these cases, DAV infected flies died in a higher rate than control stabbed flies (as seen in *D. melanogaster*, *D. santomea* and *D. simulans*). For some species, mortality in controls was higher than or similar to in infected flies (e.g. *D.ambigua* and *D. sechellia*). The mean proportional mortality between control and infected treatments were strongly correlated (correlation = 0.7445344, p-value < 0.001) (Figure 3B-C). Considering the high mortality in controls for some species, even using a metric which considers controls mortality in the analysis, such as the hazard ratio, our data interpretation could be biased. Thus, we kept 31 species in the following virulence results (see criteria for exclusion in S6). To measure viral load we inoculated 3250 flies from all 39 species and measured the change in viral RNA load by qRT-PCR (S7) — 1668 flies were frozen on day 0 and the rest was frozen three days after virus inoculation. We also found considerable variation in viral load between host

species, ranging from species who decreased the amount of virus since day 0, e.g. *D. baimaii*, to species where virus had very high replication in three days, such as *S. pattersoni* (Figure 3E).

Viral load, unlike virulence, is determined by the host phylogeny

We tested for a phylogenetic distance effect and a phylogenetic clade effect on our virulence and viral load results. For the phylogenetic distance effect, we tested if our response variables were affected by phylogenetic distance from *Drosophila melanogaster*, which is DAV's original host (Plus et al., 1975). In the hazard ratio analysis, we included the 31 species previously selected and we did not find a significant effect of phylogenetic distance from *D. melanogaster* on hazard ratio (2.958, 95% CI = -1.767, 8.541) (Figure 4). We then calculated the proportion of between-species variance that was explained by the host phylogeny and we found that only 27% of variation in hazard ratio was explained by the phylogeny. This estimate had a very broad confidence interval (95% CI = 0% - 86%), suggesting that we cannot infer that a clade effect does exist. Thus, changes in virulence are not affected by phylogenetic distance or clade effect. Additionally, we showed that virulence is not influenced by species size, as there was not a significant relationship between wing size and hazard ratio (-3.375, 95% CI = -11.258, 3.598).

In the viral load analysis, we used all the 39 *Drosophila* species. Similar to virulence, we found there was no significant change in viral load with distance from *D. melanogaster* (-3.854, 95% CI = -14.253, 5.137) (Figure 5). However, 89% of between-species variation in viral load was explained by host phylogeny (95% CI = 59% - 99%). Changes in viral load between host species are not influenced by phylogenetic distance effect, but they are strongly determined by host evolutionary history (phylogenetic clade effect), with species which have high viral titre clustering together in the phylogeny. We also showed that viral load is not

influenced by species size, as there was not a significant correlation between viral load and wing size (4.254, 95% CI = -5.055, 12.948).

Variation in DAV virulence is not coupled to viral load

To study whether the variation found in virulence was coupled to the viral load variation, we tested the phylogenetic and the non-phylogenetic correlation between hazard ratio and viral load traits. We did not find either a phylogenetic correlation (0.18, 95% CI = -0.70, 0.92) or a non-phylogenetic correlation (-0.04, 95% CI = -0.74, 0.75) between hazard ratio and change in viral load (Figure 6). Therefore, increasing levels of virulence is not a direct result of high viral titres.

DCV infection does not predict DAV virulence and viral load

To examine how the host susceptibility changes with different pathogens infection, we compared our data to DCV data from London et al. (2015). In general, DAV was less virulent than DCV. However, in some species, DAV was as virulent as DCV and, in three species — *Drosophila lummei, D. tropicalis* and *S. lebanonensis* —, DAV was more virulent than DCV. We did not find phylogenetic correlation between DAV and DCV hazard ratios (0.09, 95% CI = -0.76, 0.96), but there was a weak non-phylogenetic (0.45, 95% CI = 0.003, 0.92) correlation between virulence of these viruses (Figure 7). However, this correlation is weak and had a very wide confidence interval, suggesting that we cannot confidently infer that it is real. Also, there was no phylogenetic (0.33, 95% CI = -0.27, 0.85) or non-phylogenetic (0.46, 95% CI = -0.20, 0.95) correlation between DAV and DCV viral loads (Figure 8).

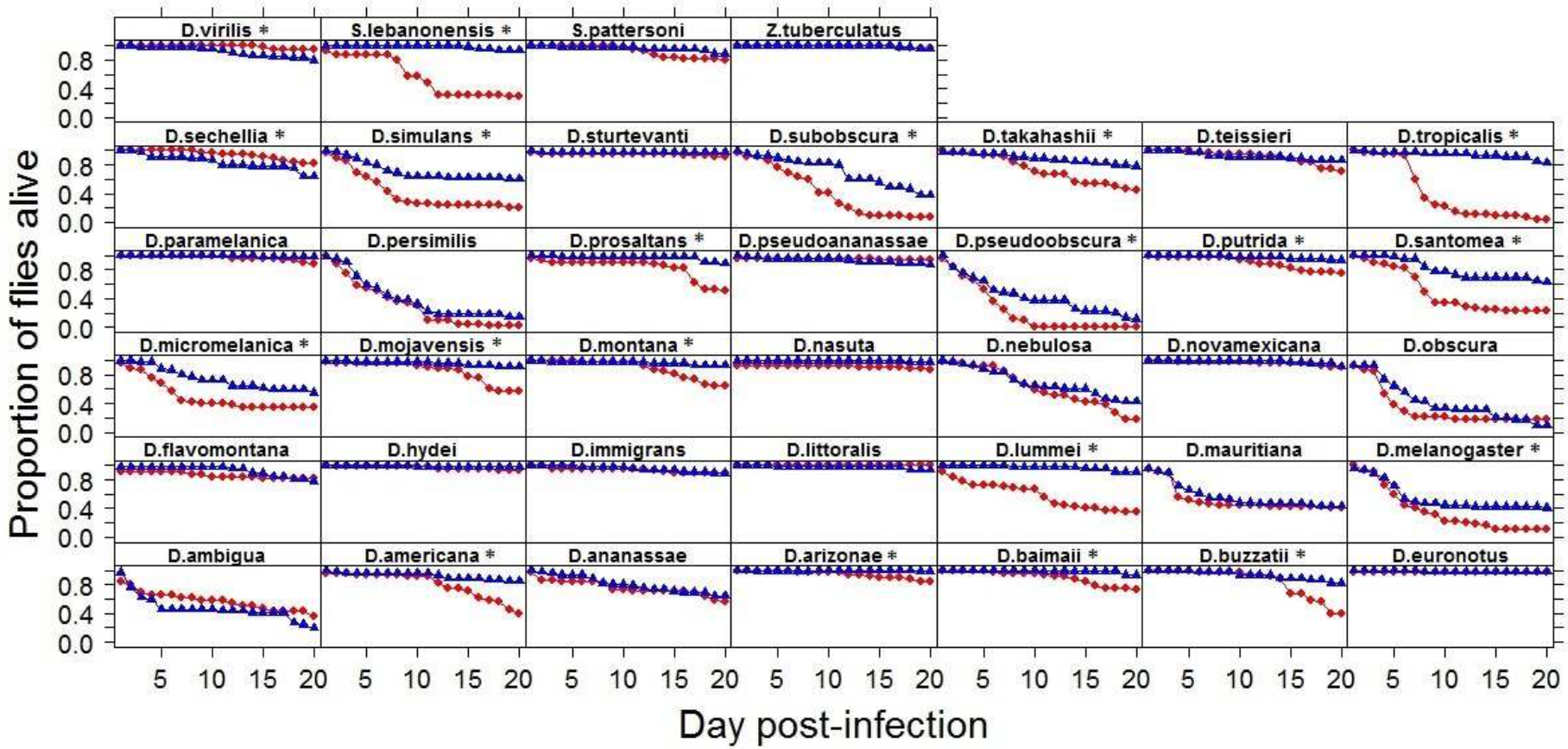


Figure 2. Mortality in 39 species of *Drosophila* after infection with DAV. Control flies are blue triangles and virus infected flies are red circles. Panels arranged in alphabetical order. Stared species have statistically different response curves (p-value < 0.05).

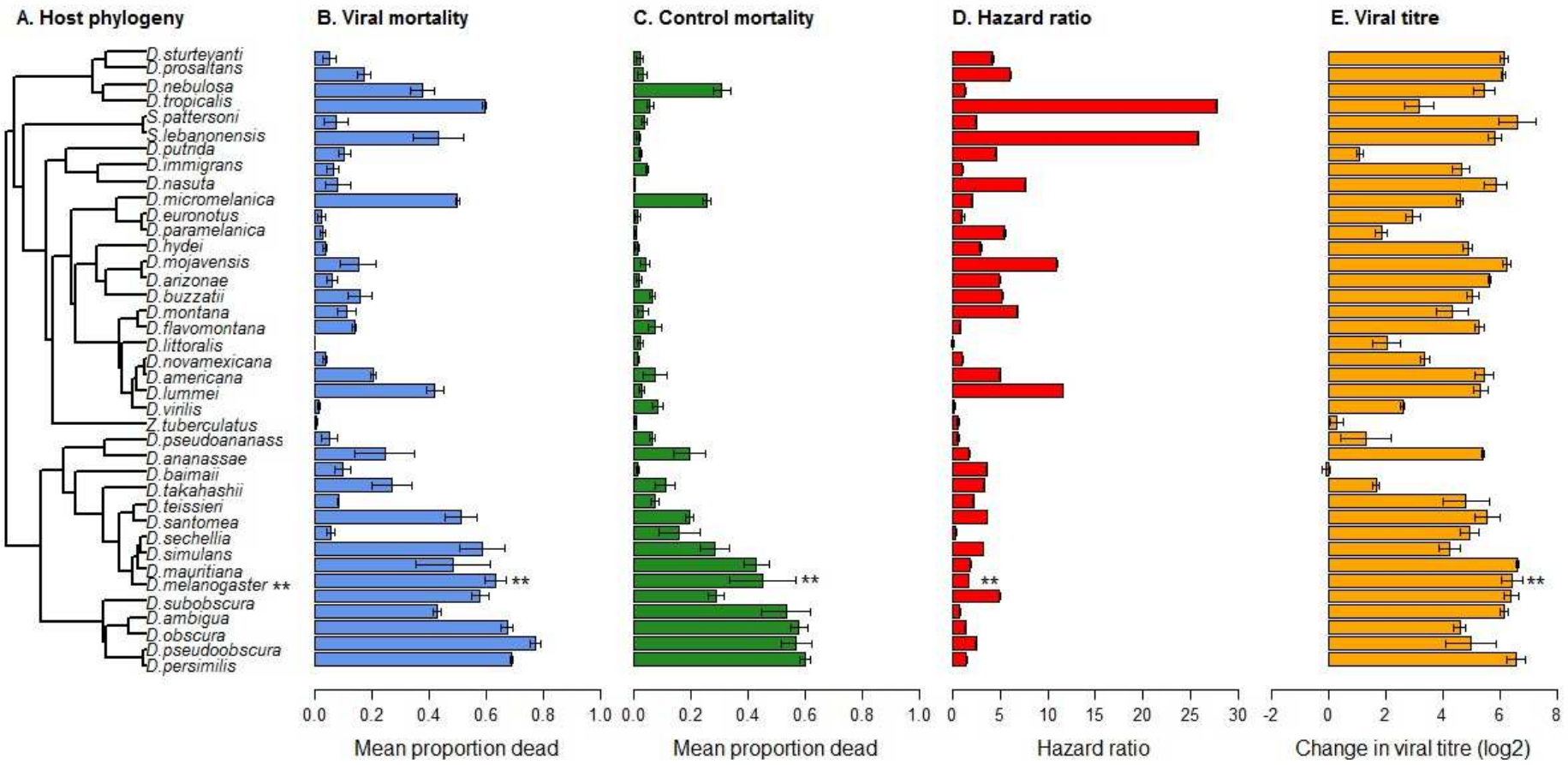


Figure 3. Phylogeny of host species (A), mean mortality of virus infected (B) and control flies (C) across all the days of observation, mortality hazard ratio (virulence) (D) and viral load (E). Hazard ratios indicate the hazard of death per unit time of infected flies compared to the control treatment. Viral load is the change in DAV load between day 0 and day 3 post infection. Species with (**) is *Drosophila melanogaster*, the DAV's original host. Graphs show mean and standard errors.

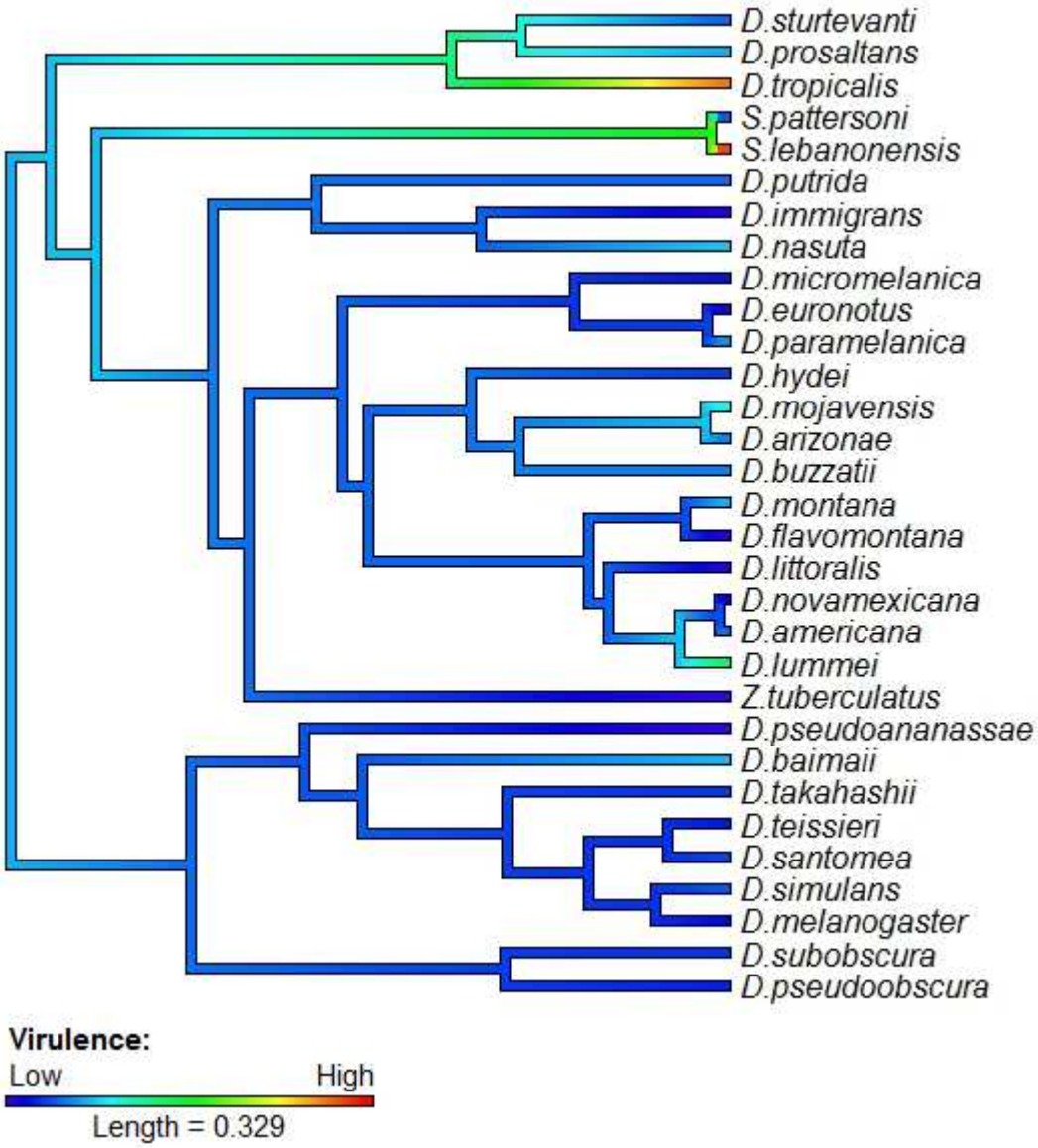


Figure 4. Ancestral state reconstruction of virulence. Ancestral states were plotted as colour gradient across the tree. Colours represent the virulence (hazard ratio), with red being the highest level of virulence and blue the lowest level of virulence at that time point. There was no phylogenetic distance (2.958, 95% CI = -1.767, 8.541) or clade effect on hazard ratio variation (host phylogeny explains 27% of variation between-species in hazard ratio data (95% CI = 0% - 86%). The colour spectrum length is the number of substitutions per site.

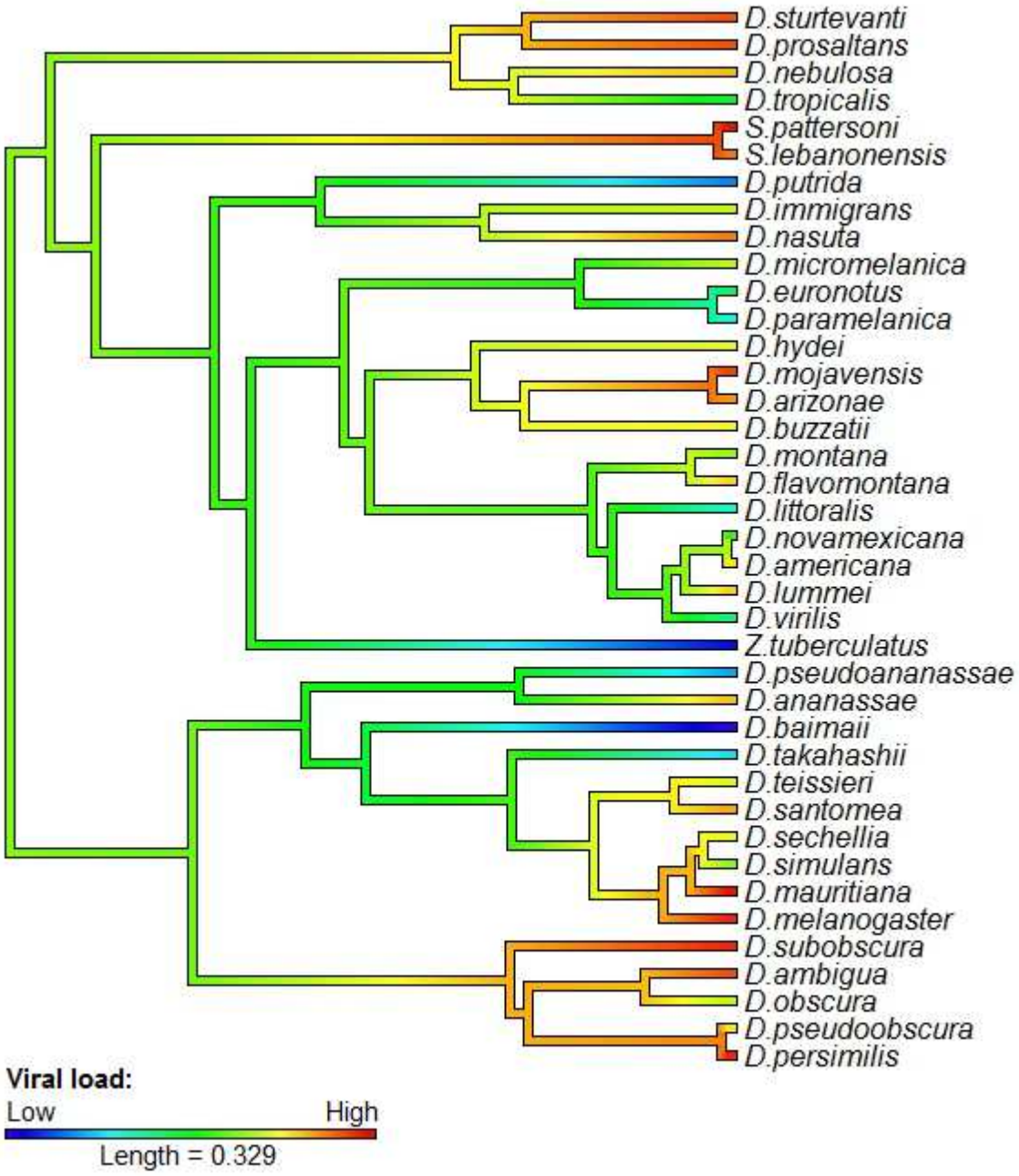


Figure 5. Ancestral state reconstruction of viral load. Ancestral states were plotted as colour gradient across the tree. Colours represent the mean viral load, with red being the highest level of viral load and blue the lowest level of viral load at that time point. Change in viral load was not correlated with distance from *Drosophila melanogaster* (-3.854, 95% CI = -14.253, 5.137), but 89% of the variation was explained by host phylogeny (95% CI = 59% - 99%). The colour spectrum length is the number of substitutions per site.

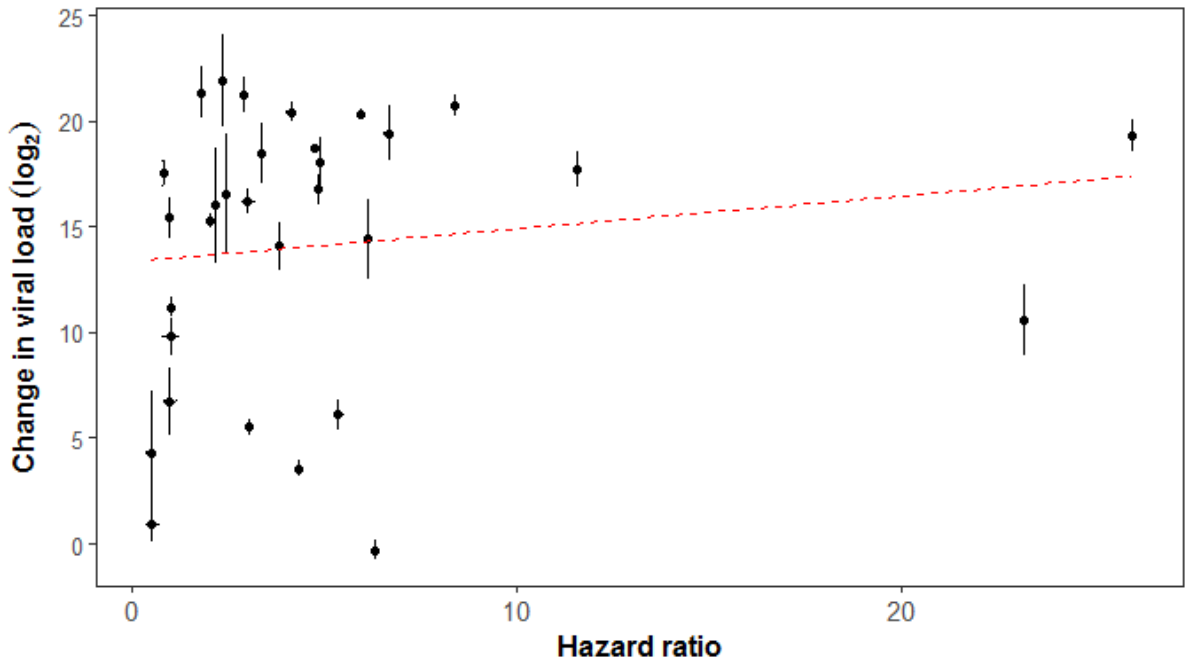


Figure 6. Correlation between DAV hazard ratio and change in viral load after three days of virus injection. 31 species were included. The trend line is estimated from a linear model. There was no phylogenetic correlation (0.18, 95% CI = -0.70, 0.92) or a non-phylogenetic correlation (-0.04, 95% CI = -0.74, 0.75). Error bars show standard errors.

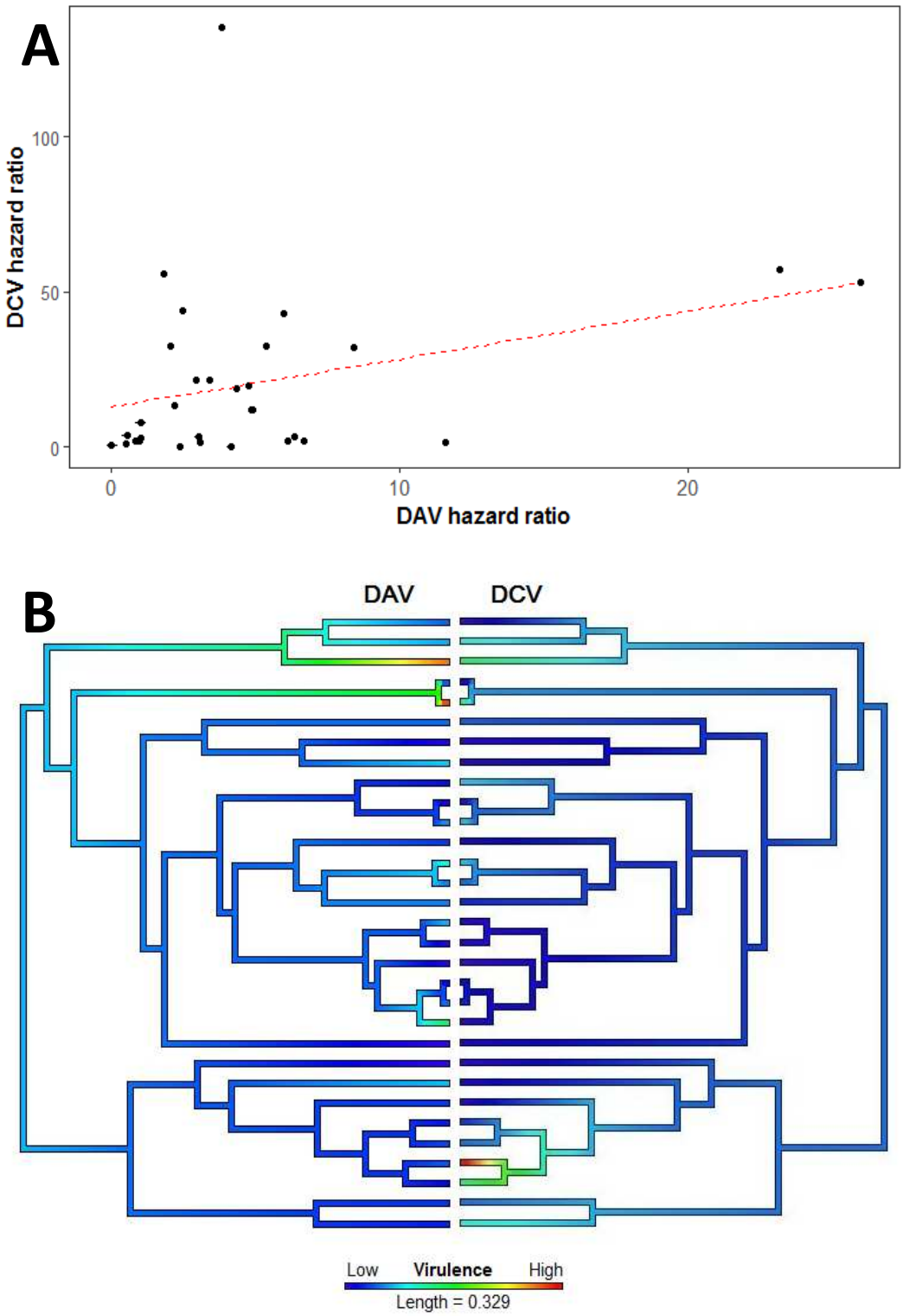


Figure 7. Correlation between DAV and DCV hazard ratios for 31 host species. (A) Hazard ratios for DAV and DCV, the trend line is estimated from a linear model, error bars show standard errors. DCV data is from Longdon et al. (2015). (B) Mirrored phylogenies showing ancestral state reconstructions for DAV (left) and DCV (right) hazard ratios. Colours represent the virulence (hazard ratio), with red being the highest level of virulence and blue the lowest level of virulence at that time point. There was no phylogenetic (0.09, 95% CI = -0.76, 0.96) or non-phylogenetic (0.45, 95% CI = 0.003, 0.92) correlation between DAV and DCV hazard ratios. The colour spectrum length is the number of substitutions per site.

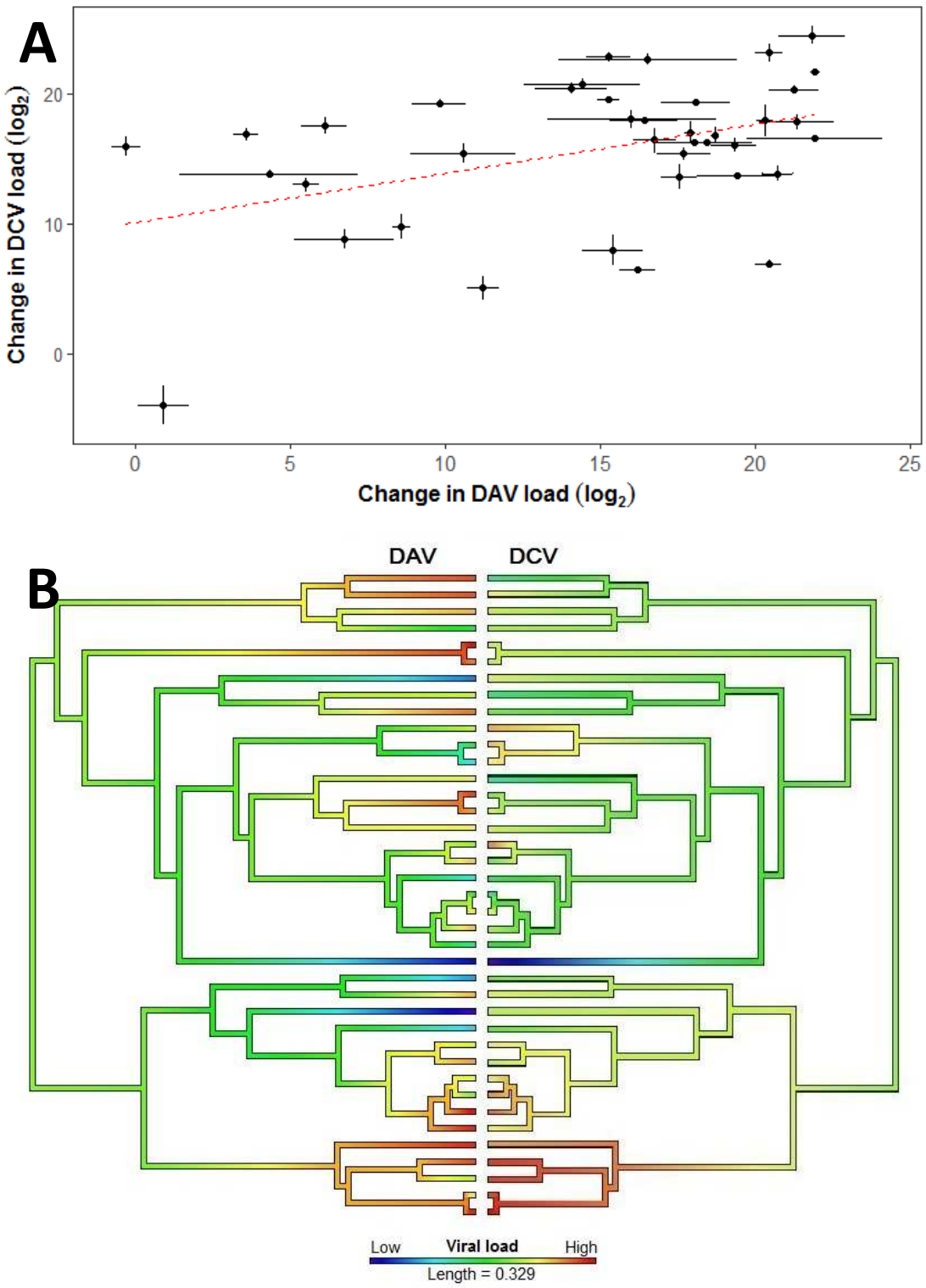


Figure 8. Correlation between DAV and DCV raw load data of 39 host species. (A) Change in viral load for DAV and DCV, the trend line is estimated from a linear model, error bars show standard errors. DCV data is from Longdon et al. (2015). (B) Mirrored phylogenies showing ancestral state reconstructions for DAV (left) and DCV (right) viral load. Colours represent the mean viral load, with red being the highest level of viral load and blue the lowest level of viral load at that time point. There was no phylogenetic (0.33, 95% CI = -0.27, 0.85) or non-phylogenetic (0.46, 95% CI = -0.20, 0.95) correlation between DAV and DCV viral load.

DISCUSSION

Pathogen host shifts are a major source of emerging infectious diseases (Woolhouse et al. 2005; Jones et al., 2008), with some of the major outbreaks in the last decades, such as Influenza, SARS and Ebola, each caused by pathogens that jumped into humans from other species (Webby & Webster, 2001; Leroy et al., 2005; Li et al., 2005). What determines the ability of a pathogen to infect some groups of host species but not others and how pathogens evolve in different hosts are questions that are just starting to be addressed. The research on how different viruses affect host fitness and how host susceptibility can vary at an evolutionary scale is crucial to understanding host switches and to predict future emerging infectious diseases. In this study, we have observed dramatic variation in DAV virulence and viral load between different *Drosophila* host species (Figures 2 and 3). Changes in virulence were not explained by the host phylogeny (Figures 4). Variation in viral load had a phylogenetic effect but it was not correlated to the distance from *Drosophila melanogaster*, indicating a phylogenetic clade effect. Surprisingly, virulence and viral load variables were not correlated to each other (Figure 6), indicating that the harm the pathogen causes to its host is not a consequence of the amount of virus inside the host. When comparing host susceptibilities to viruses differing in pathogenicity, DAV and DCV virulence and viral load were not correlated to each other (Figure 7 and 8), suggesting that host susceptibility to different pathogens vary depending on the species which are interacting.

DAV has been described since its identification (Plus et al., 1975) as the least virulent virus infecting *Drosophila* (Plus et al., 1975; Brun & Plus, 1980; Christian, 1987; Christian, 1992; Ambrose et al., 2009). However, most studies have only tested the virus' effect on survivorship of few species, most commonly in *D. melanogaster* and *D. simulans* (Christian, 1987). Despite DAV being less virulent than DCV for a large number of species, DAV

effects between *Drosophila* host species varied widely, both in virulence and in viral load. Recently, high levels of DAV virulence were registered for the first time in the pest *Drosophila suzukii*, with half of the population of infected flies dying three days after virus injection (Carrau et al., 2018). Because of this dramatic effect on *D. suzukii* survivorship, DAV has been proposed as a biological control agent to protect crops (Carrau et al., 2018). However, as DAV is vertically and horizontally transmitted, spreading easily by contact between adult flies (Christian, 1987), any control method involving the release of artificially inoculated flies into the wild will likely have serious impacts in other *Drosophila* species. Based on the results presented here the effect of this horizontal transfer can vary depending on the other species encountered and such a control strategy will therefore need further study before it could be put into effect.

We observed unexpected high control mortality in our experiments. Our main hypotheses for this result are based on Merkling & Rij (2015): (1) controls flies were dying because of tissue damage during injection with the needle or (2) stock lineages were already infected with an unknown virus or bacteria and, when the fly was pricked, the pathogen would spread and decrease survivorship. To test the first hypothesis, we obtained new lineages of each species that presented high control mortality and we performed tests investigating the survivorship of flies pricked and not pricked. We observed far less mortality in both controls treatments from this test when in comparison with control from the main experiment (S8). Thus, we discarded the first hypothesis, and our best explanation is that our fly stocks were infected with an unidentified pathogen. We are carrying further experiments to test the second hypothesis. Although we do not yet know exactly the causes of the mortality in control flies, the possibility of another pathogen infecting flies does not invalidate our results. Wild *Drosophila* species are infected with several natural enemies (Kraaijeveld & Godfray, 1997; Fleury et al., 2009; Jaenike et al., 2010; Wilfert et al., 2011;

Magwire et al., 2012; Webster et al., 2015) and even experiments in controlled conditions are not able to use flies free of all parasites. Also, to be sure that we were including in our analysis only species where we could measure a potential DAV effect, we excluded eight species whose results could bias data interpretation (see S6).

Host phylogeny has been indicated as a key factor to predict host shifts, determining the abundance and spread of parasites (Engelstadter & Fortuna, 2018). Most of empirical studies performing cross infection experiments describe the distance effect as one of the main effect's of host tree favouring host switch. For example, an empirical study examining 24 *Drosophila* species were artificially infected with five different nematode species and it was found that hosts closely related to the original host were more susceptible to infection, while distant ones were unsuitable for any of the parasites tested (Perlman & Jaenike, 2003). Similarly, three strains of Sigma viruses (Rhabdoviridae) inoculated in 51 species of *Drosophila* had lower replication ability as the host was more phylogenetic distantly from the donor host (Longdon et al., 2011). Despite this past evidence, DAV virulence and viral load were not correlated with the genetic distance from its original host *D. melanogaster*, but changes in viral load were affected by the host phylogeny, indicating it is influenced by clade effect. This conclusion is supported by a previous study examining *Drosophila* host species and DCV, where virulence and viral load responses were not correlated with phylogenetic distance from *D. melanogaster*, but viral load was affected by clade effect (Longdon et al., 2015a). This apparent pattern contradiction might be explained by host and parasite features not mapped in phylogenies. A study comparing phylogenetic specialism in mammal parasites found that both viruses and helminths exhibit intermediate generalism, being able to infect closely related host as well as hosts distantly related (Park et al., 2018). This suggests that multiple factors may be driving patterns of parasite sharing, such as parasite transmission mode (Antonovics et al., 2017), host geographical ranges (Davies & Pedersen, 2008), host

and parasite behaviours (Ezenwa et al., 2016; Hoverman & Searle, 2016) and host defences mechanisms (Becerra, 1997).

We did not find correlation between DAV virulence and viral load. For the most part of species we tested, our results show low virulence (exceptions are *D. tropicalis* and *S. lebanonensis*) and an intermediate to high virus replication rate (exceptions are *Z. tuberculatus* and *D baimaii*) (Figures 3-5). Virulence was initially thought to be a direct consequence of parasite replication inside the host (Alizon et al., 2009). However, it has been demonstrated that components of host susceptibility (resistance and tolerance) also affect virulence responses (Ayres & Schneider, 2012). Tolerance strategies are mechanisms used by hosts to keep healthy during an infection, without necessarily affecting pathogen replication (Vale et al., 2016). In this way, our results indicate that host species may be varying in tolerance during DAV infection. One potential way in which hosts may tolerate infections is via sickness behaviours, such as change in body temperature, reduced eating and drinking, anorexia, lethargy and hypothermia (Arnold et al., 2013; Vale, 2018). A study infecting *D. melanogaster* with DCV showed that this virus induces several physiological consequences in its host, such as reduction of metabolic and growth rates (Arnold et al., 2013). Thus, *Drosophila* species may vary in tolerance when facing other viruses infection (e.g. DAV), being possible that each species presents a specific type of sickness behaviour.

Host susceptibilities to DAV and DCV were not correlated to each other, but because too little is understood about DAV epidemiology, it is hard to understand the differences between these two pathogens that may be causing virulence and viral load variations. It is possible that DAV and DCV are varying in their mechanisms of infection. In insects, RNA interference is an important antiviral immune response (Palmer et al., 2018), and DCV is a suppressor of RNAi that binds dsRNA and inhibits siRNA production (van Rij et al., 2006). Changes in DCV virulence and viral load in 48 species of *Drosophila* were hypothesized to

be caused by variation in the ability of hosts to produce an antiviral RNAi response, or to a difference in the viral RNAi suppressor efficiency depending on the species (Longdon et al., 2015a). In turn, DAV may be targeted by the host via other immune response, which can also vary over host phylogeny. Another factor that may affect the variation in host susceptibility to different pathogens is the range of tissue tropism for each virus. Tissue tropism is defined by the group of cells and tissues of a host that can support growth of a given virus or bacteria (Levine, 1984). In *D. melanogaster* and *D. simulans,* DAV is known to infect intestinal cells and Malpighian Tubules, while DCV usually infects tracheal cells (Christian, 1987). However, in *D. suzukii,* DAV was found to be mainly infecting the head, suggesting infection in the brain, nerve ganglia and nerves (Carrau et al., 2018). Viruses tropism seems to vary not only between virus strains but also with the host species infected.

The variation in host susceptibilities to different pathogens found in this study can also be interpreted under an evolutionary approach. The species we used in this study shared a common ancestor about 40 million years ago (Obbard et al., 2012). When, in the host evolutionary history, DAV started to interact to each species of *Drosophila* (due to a host shift or not) and for how long was this interaction could have driven different evolutionary processes within each host species, such as co-evolution. Co-evolution implies in reciprocal genetic changes between individuals from populations of different species (Janzen, 1980). Co-evolutionary processes in host-parasite systems are constantly investigated. There are two different co-evolutionary dynamics generally described: arms race — where selection pressure leads to an increase in host resistance and/or tolerance and in pathogen's virulence (Buckling & Rainey, 2002) —, and Red Queen — when there is a fluctuation between traits features of host and parasite due to a negative frequency-dependent selection (Pirie et al., 2016). The occurrence of DAV in nature is known for a few species, such as *D. melanogaster, D. immigrans, D. simulans , D. sulfurigaster* (Christian, 1987) and *D. suzukii*

(Carrau et al., 2018) (these last two were not included in this study). Thus, depending on when DAV started the interaction with these species and whether there was interaction with other species, the (co)evolutionary dynamics may have varied for each host-parasite pair. The diversity of *Drosophila*-DAV evolutionary dynamics could have led to the variation in host susceptibility we observed.

The two viruses used in this study presented high variation in virulence and change in viral load over several species of drosophilid hosts. Although viral load responses for both pathogens were affected by phylogenetic clade effect, the replication of each virus differs within the same host. Therefore, it is not possible to predict host susceptibility to one pathogen based on the fitness response to infection of another pathogen. Even though both pathogens we used were RNA viruses, they differ in transmission mode, place of infection and, probably, in mechanism of infection, which is unknown for DAV. In order to develop a better estimate of potential host range for a pathogen, it is vital to include other traits (e.g. genetic or ecological) into the phylogenetic analysis. In conclusion, our study corroborates previous findings that pathogen replication is closely linked in related species. We add to this finding that it is not possible to infer host susceptibility to different pathogens based on host other infections, making predictions of EIDs an even more complex task.

REFERENCES

Alizon, S., A. Hurford, N. Mideo, and M. Van Baalen. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. Journal of Evolutionary Biology 22:245–259.

Ambrose, R. L., G. C. Lander, W. S. Maaty, B. Bothner, J. E. Johnson, and K. N. Johnson. 2009. Drosophila A virus is an unusual RNA virus with a T=3 icosahedral core and permuted RNA-dependent RNA polymerase. The Journal of general virology 90:2191–200.

Antonovics, J., A. J. Wilson, M. R. Forbes, H. C. Hauffe, E. R. Kallio, H. C. Leggett, B.Longdon, B. Okamura, S. it, and J. P. Webster. 2017. The evolution of transmission mode. Philosophical Transactions of the Royal Society. B 372:20160083.

Arnold, P. A., K. N. Johnson, and C. R. White. 2013. Physiological and metabolic consequences of viral infection in *Drosophila melanogaster*. Journal of Experimental Biology, doi: 10.1242/jeb.088138.

Ayres, J. S., and D. S. Schneider . 2012. Tolerance of infections. Annual Review of Immunology 30:271–294.

BALÁŽ, V., J. VÖRÖS, P. CIVIŠ, J. VOJAR, A. HETTYEY, E. SÓS, R. DANKOVICS, R. JEHLE, D. G. CHRISTIANSEN, F. CLARE, M. C. FISHER, T. W. GAER, and J. BIELBY. 2014. Assessing Risk and Guidance on Monitoring of *Batrachochytrium dendrobatidis* in Europe through Identification of Taxonomic Selectivity of Infection. Conservation Biology 28:213–223.

Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276, 253-256.

Berenbaum, M. R., and A. R. Zangerl. 2006. Parsnip webworms and host plants at home and abroad: trophic complexity in a geographic mosaic. Ecology 87(12): 3070–3081.

Betts, A., C. Gray, M. Zelek, R. MacLean, and K. King. 2018. High parasite diversity accelerates host adaptation and diversification. Science 360:907–911.

Bielby, J., N. Cooper, A. A. Cunningham, T. W. J. Garner, and A. Purvis. 2008. Predicting susceptibility to future declines in the world's frogs. Conservation Letters 1:82–90.

Bowers, R. G., M. Boots, and M. Begon. 1994. Life-History Trade-Offs and the Evolution of Pathogen Resistance: Competition between Host Strains. Proceedings of the Royal Society of London B: Biological Sciences 257:247–253.

Brun, G., and N. Plus. 1980. The viruses of *Drosophila*. In: *The Genetics and Biology of Drosophila*, pp. 625–702. Edited by M. Ashburner, and T. F. R. Wright. New York: Academic Press.

Buckling, A., and P. B. Rainey. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. Proceedings of the Royal Society of London B: Biological Sciences 269: 931–936.

Burbrink, F. T., J. M. Lorch, and K. R. Lips. 2017. Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. Science Advances 3:e1701387.

Carrau, T., N. Hiebert, A. Vilcinskas, and K.-Z. Lee. 2018. Identification and characterization of natural viruses associated with the invasive insect pest *Drosophila suzukii*. Journal of Invertebrate Pathology, doi: 10.1016/j.jip.2018.04.001.

Christian, P. D. 1987. Studies on Drosophila C and A viruses in Australian populations of *Drosophila melanogaster*. Australian National University. 305 p.

Christian, P. D. 1992. A simple vacuum dot-blot hybridisation assay for the detection of Drosophila A and C viruses in single Drosophila. Journal of Virological Methods 38:153–165.

Corby-Harris, V., A. Pontaroli, L. J. Shimkets, J. L. Bennetzen, K. E. Habel, and D. E. Promislow. 2007. Geographical Distribution and Diversity of Bacteria Associated with Natural Populations of *Drosophila melanogaster*. Applied and Environmental Microbiology 73:3470–3479.

Cordes, E. J., K. D. Licking-Murray, and K. A. Carlson. 2013. Differential gene expression related to Nora virus infection of *Drosophila melanogaster*. Virus Research 175:95–100.

Corey, S. J., and T. A. Waite. 2008. Phylogenetic autocorrelation of extinction threat in globally imperilled amphibians. Diversity and Distributions, 14: 614–629.

Cox, D. R. 1972. Regression models and life tables (with discussion). Journal of the Royal Statistical Society: Series B 34: 187–220.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science 287(5452): 443–449.

Davies, T. J., and A. B. Pedersen. 2008. Phylogeny and geography predict pathogen community similarity in wild primates and humans. Proceedings of the Royal Society of London B: Biological Sciences 275, 1695–1701.

de Vienne, D. M., G. Refrégier, M. López-Villavicencio, A. Tellier, M. E. Hood, and T. Giraud. 2013. Coespeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytologist 198: 347–385.

de Vienne, D. M., M. E. Hood, and T. Giraud. 2009. Phylogenetic determinants of potential host shifts in fungal pathogens. Journal of Evolutionary Biology 22:2532–2541.

Dostert, C, E. Jouanguy, P. Irving, L. Troxler, D. Galiana-Arnoux, C. Hetru, J. A. Hoffmann, and J.-L. Imler. 2005. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. Nature Immunology 6: 946–953. pmid:16086017.

Engelstaedter, J., and N. Z. Fortuna. 2018. The dynamics of preferential host switching: host phylogeny as a key predictor of parasite prevalence and distribution. *bioRxiv* 209254, https://doi.org/10.1101/209254.

Ezenwa, V. O., E. A. Archie, M. E. Craft, D. M. Hawley, L. B. Martin, J. Moore, and L. White. 2016. Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. Proceedings of the Royal Society of London B: Biological Sciences 283:20153078.

Faria, N. R., A. Rambaut, M. A. Suchard, G. Baele, T. Bedford, M. J. Ward, A. J. Tatem, J. D. Sousa, N. Arinaminpathy, J. Pépin, D. Posada, M. Peeters, O. G. Pybus, and P. Lemey. 2014. The early spread and epidemic ignition of HIV-1 in human populations. Science. 346(6205): 56–61.

Fleury, F., P. Gilbert, N. Ris, and R. Allemand R. 2009. Ecology and life history evolution of frugivorous *Drosophila* parasitoids. Advances in Parasitology 70:3–44.

Gandon, S. 2002. Local adaptation and the geometry of host–parasite coevolution. Ecology Letters 5: 246–256.

Gandon, S., and Y. Michalakis. 2000. Evolution of parasite virulence against qualitative or quantitative host resistance. Proceedings of the Royal Society of London B: Biological Sciences 267: 985–990.

Gelman, A. 2006. Prior Distributions for Variance Parameters in Hierarchical Models. Bayesian Analysis 1(3): 515–533.

Hadfield, J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software 33.

Hadfield, J. D., and S. Nakagawa. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. Journal of Evolutionary Biology 23:494–508. pmid: 20070460.

Heath, S. E., K. Knox, P. F. Vale, and S. Collins. 2017. Virus Resistance Is Not Costly in a Marine Alga Evolving under Multiple Environmental Stressors. Viruses 9:39.

Hedges, L. M., J. C. Brownlie, S. L. O'Neil, and K. N. Johnson. 2008. *Wolbachia* and virus protection in insects. Science 31(322):702.

Housworth, E. A., E. P. Martins, and M. Lynch. 2004. The phylogenetic mixed model. American Naturalist 163:84–96. pmid:14767838.

Hoverman, J. T., and C. L. Searle. 2016. Behavioural influences on disease risk: implications for conservation and management. Animal Behaviour 120:263–271.

Huszar, T., and J.-L. Imler. 2008. *Drosophila* viruses and the study of antiviral host-defence. Advances in Virus Research 72:227–265.

Jaenike, J., J. K. Stahlhut, L. M. Boelio, and R. L. Unckless. 2010. Association between Wolbachia and Spiroplasma within *Drosophila neotestacea*: an emerging symbiotic mutualism? Molecular Ecology 19(2): 414–425.

Janzen, D. H. 1980. When is it coevolution? Evolution 34(3): 611–612.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990.

Kariñho-Betancourt, E., and J. Núñez-Farfán. 2015. Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis. PeerJ 3:e789.

Kemp, C., S. Mueller, A. Goto, V. Barbier, S. Paro, F. Bonnay, C. Dostert, L. Troxler, C. Hetru, C. Meignin, S. Pfeffer, J. A. Hoffmann, and J.-L. Imler. 2013. Broad RNA interference-mediated antiviral immunity and virus-specific inducible responses in Drosophila. The Journal of Immunology 190: 650–658. pmid:23255357.

Kraaijeveld, A. R., and H. C. J. Godfray. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Nature 389(18):278–280.

Lemaitre, B., and J. Hoffmann. 2007. The host defence of *Drosophila melanogaster*. Annual Review of Immunology 25:697–743.

Leroy, E. M., B. Kumulungui, X. Pourrut, P. Rouquet, A. Hassanin, P. Yaba, A. Délicat, J. T. Paweska, J.-P. Gonzalez, and R. Swanepoel. 2005. Fruit bats as reservoirs of Ebola virus. Nature 438:575.

Levine, A. J. 1984. Viruses and differentiation: the molecular basis of viral tissue tropisms. In: Notkins, A. L., and M. B. A. Oldstone (eds) Concepts in viral pathogenesis. Springer, New York, NY.

Li, W., Z. Shi, M. Yu, W. Ren, C. Smith, J. H. Epstein, H. Wang, G. Crameri, Z. Hu, H. Zhang, J. Zhang, J. McEachern, H. Field, P. Daszak, B. T. Eaton, S. Zhang, and L. Wang. 2005. Bats are natural reservoirs of SARS-like Coronaviruses. Science 310(5748): 676–679.

Longdon, B., J. D. Hadfield, C. L. Webster, D. J. Obbard, and F. M. Jiggins. 2011. Host Phylogeny Determines Viral Persistence and Replication in Novel Hosts. PLoS Pathogens 7:e1002260.

Longdon, B., J. D. Hadfield, J. P. Day, S. C. Smith, J. E. McGonigle, R. Cogni, C. Cao, and F. M. Jiggins. 2015. The Causes and Consequences of Changes in Virulence following Pathogen Host Shifts. PLoS Pathogens 11:e1004728.

Longdon, B., M. A. Brockhurst, C. A. Russell, J. J. Welch, and F. M. Jiggins. 2014. The Evolution and Genetics of Virus Host Shifts. PLoS Pathogens 10:e1004395.

Lynch, M. 1991. Methods for the analysis of comparative data in evolutionary biology. Evolution 45:1065–1080.

Magwire, M. M., D. K. Fabian, H. Schweyen, C. Cao, B. Longdon, F. Bayer, and F. M. Jiggins. 2012. Genome-wide association studies reveal a simple genetic basis of resistance to naturally coevolving viruses in *Drosophila*. PLoS Genetics 8:e1003057.

Medzhitov, R., D. S. Schneider, and M. P. Soares. 2012. Disease tolerance as a defence strategy. Science 335:936-941.

Merkling, S. H., and R. P. van Rij. 2015. Analysis of resistance and tolerance to virus infection in Drosophila. Nature Protocols 10:1084–1097.

Morens, D. M., and A. S. Fauci. 2013. Emerging Infectious Diseases: Threats to Human Health and Global Stability. PLoS Pathogens 9:e1003467.

Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature 430(6996): 242–249.

Nyman, T. 2010. To speciate, or not to speciate? Resource heterogeneity, the subjectivity of similarity, and the macroevolutionary consequences of niche-width shifts in plant-feeding insects. Biological Reviews 85: 393–411.

Obbard, D. J., J. Maclennan, K.-W. Kim, A. Rambaut, P. M. O'Grady, and F. M. Jiggins. 2012. Estimating divergence dates and substitution rates in the Drosophila phylogeny. Molecular Biology and Evolution 29: 3459–3473. pmid:22683811.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401: 877–884. pmid:10553904.

Palmer, W. H., F. S. Varghese, and R. P. van Rij. 2018. Natural variation in resistance to virus infection in dipteran insects. Viruses, doi: 10.3390/v10030118.

Park, A., M. Farrell, J. Schmidt, S. Huang, T. Dallas, P. Pappalardo, Jake, P. Stephens, R. Poulin, C. Nunn, and T. Davies. 2018. Characterizing the phylogenetic specialism–generalism spectrum of mammal parasites. Proceedings of the Royal Society of London B: Biological Sciences 285: 20172613.

Perlman, S., and J. Jaenike. 2003. Infection success in novel hosts: an experimental and phylogenetic study of *Drosophila*-parasitic nematodes. Evolution 57(3): 544–557.

Pirie, M. D., E. G. H. Oliver, A. M. de Kuppler, B. Gehrke, N. C. Le Maitre, M. Kandziora, and D. U. Bellstedt. 2016. BMC Evolutionary Biology 16–190.

Plus, N., G. Croizier, F. X. Jousset, and J. David. 1975. Picornaviruses of laboratory and wild *Drosophila melanogaster*: geographical distribution and serotypic composition. Annals of Microbiology 126: 107–117.

R Core Team. 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. [http://www.R-project.org].

Read, A. F. 1994. The evolution of virulence. Trends Microbiology 2: 73–76. pmid: 8156274.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3: 217–223.

Sharp, P. M., and B. H. Hahn. 2011. Origins of HIV and the AIDS Pandemic. Cold Spring Harbor Perspectives in Medicine 1(1):a006841. doi:10.1101.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. TREE, 11(8): 317–321.

Smith, K. G., K. R. Lips, and J. M. Chase. 2009. Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. Ecology Letters 12:1069–1078.

Thiemann, G. W., and R. J. Wassersug. 2000. Patterns and consequences of behavioural responses to predators and parasites in Rana tadpoles. Biological Journal of the Linnean Society 71: 513–528.

Vale, P. F. 2018. Disease Tolerance: Linking Sickness Behaviours to Metabolism Helps Mitigate Malaria. Current Biology 28:R606–R607.

Vale, P. F., G. Lafforgue, F. Gatchitch, R. Gardan, S. Moineau, and S. Gandon. 2015. Costs of CRISPR-Cas-mediated resistance in Streptococcus thermophilus. Proceedings of the Royal Society of London B: Biological Sciences 282: 20151270.

Vale, P. F., L. McNally, A. Doeschl-Wilson, K. C. King, R. Popat, M. R. Domingo-Sananes, J. E. Allen, M. P. Soares, and R. Kümmerli. 2016. Beyond killing. Can we find new ways to manage infection? Evolution, Medicine, and Public Health 2016:148–157.

van Rij, R.P., M. C. Saleh, B. Berry, C. Foo, A. Houk, C. Antoniewski, and R. Andino. 2006. The RNA silencing endonuclease Argonaute 2 mediates specifc antiviral immunity in *Drosophila melanogaster*. Genes and Development 20: 2985–2995.

Wäckers, F. L., and C. Bonifay. 2004. How to be sweet? Extrafloral nectar allocation by Gossypium hirsutum fits optimal defense theory predictions. Ecology 85(6): 1512–1518.

Webby, R. J., and R. G. Webster. 2001. Emergence of influenza A viruses. Philosophical Transactions of the Royal Society of London 356(1416): 1817–1828.

Webster, C. L. , F. M. Waldron, S. Robertson, D. Crowson, G. Ferrari, J. F. Quintana, J.-M. Brouqui, E. H. Bayne, B. Longdon, A. H. Buck, B. P. Larazzo, J. Akorli, P. R. Haddrill, and D. J. Obbard. 2015. The Discovery, Distribution, and Evolution of Viruses Associated with *Drosophila melanogaster*. PLoS Biology 13(7): e1002210.

Werren, J. H., L. Baldo, and M. E. Clark. 2008. *Wolbachia*: master manipulators of invertebrate biology. Nature Reviews 6:741–751.

Wilfert, L., B. Longdon, A. G. A. Ferreira, F. Bayer, and F. M. Jiggins. 2011. Trypanosomatids are common and diverse parasites of *Drosophila*. Parasitology, 138:858–865.

Woolhouse, M. E. J., D. T. Haydon, and R. Antia. 2005. Emerging pathogens: the epidemiology and evolution of species jumps. Trends in Ecology and Evolution, 20(5): 238–244.

World Health Organisation. 2018. Global Health Observatory (GHO) data HIV/AIDS. [http://www.who.int/gho/hiv/en/].

Zukal, J., H. Bandouchova, T. Bartonicka, H. Berkova, V. Brack, J. Brichta, M. Dolinay, K. S. Jaron, V. Kovacova, M. Kovarik, N. Martínková, K. Ondracek, Z. Rehak, G. G. Turner, and J. Pikula. 2014. White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats. PLoS ONE 9:e97224.

SUPPORTING INFORMATION

S1 - Fly Food Recipes

Cornmeal

- 1400 ml distilled water
- 13 g agar
- 105 g dextrose
- 105 g cornmeal
- 23 g yeast
- 35 ml 10% Nipagin

  Combine, bring to boil for 5 minutes and whisk constantly. Cool to 80º - 70ºC and add Nipagin. This recipe must be sprinkled with dry yeast. Adapted from Longdon et al. (2015).

Banana

Mixture 1

- 1000 ml distilled water
- 30 g yeast
- 10 g agar

  Bring to the boil for 3-4 minutes and whisk constantly.

Mixture 2

- 150 g mashed banana
- 50 g cornmeal
- 30 g malt powder
- 2.5 g Opuntia powder
- 20 ml 10% Nipagin

  Add mixture 2 to mixture 1, whisk constantly and simmer for 5 minutes. Cool to 80º - 70ºC and add Nipagin. Adapted from Longdon et al. (2015).

Propionic

- 2000 ml water
- 160 g malt powder
- 16 g agar
- 36 g yeast
- 160 g cornmeal
- 20 g soya flour
- 44 g molasses
- 28 ml 10% Nipagin
- 12.4 ml propionic acid

  Combine and bring to a boil for 5 minutes. Cool to 80º - 70ºC and add Nipagin and propionic acid. Use gloves when handling propionic acid. This recipe must be sprinkled with dry yeast. Adapted from Longdon et al. (2015).

Malt

- 1000 ml distilled water
- 10 g agar

- 60 g semolina
- 20 g yeast
- 80 g malt powder
- 14 ml 10% Nipagin
- 5 ml propionic acid
  Mix all the ingredients, bring to the boil and simmer for 15 minutes. Cool to 80º - 70ºC and add Nipagin and propionic acid. Use gloves when handling propionic acid. This recipe must be sprinkled with dry yeast when required. Adapted from Longdon et al. (2011).

References:

Longdon B, Hadfield JD, Day JP, Smith SCL, McGonigle JE, Cogni R, Cao C, Jiggins FM (2015) The causes and consequences of changes in virulence following pathogen host shifts. PLoS Pathogens, 11(3):e1004728.

Longdon B, Hadfield JD, Webster CL, Obbard DJ, Jiggins FM (2011) Host phylogeny determines viral persistence and replication in novel hosts. PLoS Pathogens, 7: e1002260.

S2 - Rearing Conditions

Table S2.1. List of all species used. Each species was reared on its specific food (see recipes in S1), at 22ºC, 70% relative humidity and 12h light-dark cycle. The San Diego Stock Centre (SC) or the Cambridge (Cam) ID are identified.

Species	Food	Provenience	SC or Cam ID
D. ambigua	Malt + yeast	San Diego SC	14013-1011.00
D. americana	Malt	San Diego SC	15010-0951.20
D. ananassae	Cornmeal	Cambridge	JM wol free 3/6
D. arizonae	Banana	San Diego SC	15081-1271.26
D. baimaii	Cornmeal	San Diego SC	14028-0481.01
D. buzzatii	Malt	San Diego SC	15081-1291.01
D. euronotus	Cornmeal	San Diego SC	15030-1131.01
D. flavomontana	Malt + yeast	San Diego SC	15010-0981.05
D. hydei	Cornmeal	San Diego SC	15085-1641.71
D. immigrans	Malt + yeast	Cambridge	EEL-106 3/6
D. littoralis	Banana	San Diego SC	15010-1001.00
D. lummei	Malt + yeast	San Diego SC	15010-1011.07
D. mauritiana	Propionic	Cambridge	Cam 3/6
D. melanogaster	Cornmeal	Cambridge	Cam
D. micromelanica	Cornmeal	San Diego SC	15030-1151.01
D. mojavensis	Banana	San Diego SC	15081-1352.00
D. montana	Malt + yeast	Cambridge	Cam 3/6
D. nasuta	Cornmeal	San Diego SC	15112-1781.00
D. nebulosa	Cornmeal	San Diego SC	14030-0761.06
D. novamexicana	Banana	San Diego SC	15010-1031.04
D. obscura	Banana	Cambridge	Cam DA30 3/6
D. paramelanica	Cornmeal	San Diego SC	15030-1161.05
D. persimilis	Malt	San Diego SC	14011-0111.51
D. prosaltans	Propionic	San Diego SC	14045-0901.02
D. pseudoananassae	Cornmeal	San Diego SC	14024-0411.02
D. pseudoobscura	Banana	San Diego SC	14011-0121.94
D. putrida	Propionic	San Diego SC	15150-2101.00
D. santomea	Cornmeal	San Diego SC	14021-0271.00
D. sechellia	Propionic	Cambridge	Cam 48 3/6
D. simulans	Cornmeal	Cambridge	Cam
D. sturtevanti	Cornmeal	Cambridge	JM wol free 3/6
D. subobscura	Cornmeal	San Diego SC	14011-0131.15
D. takahashii	Cornmeal	San Diego SC	14022-0311.07
D. teissieri	Cornmeal	San Diego SC	14021-0257.01
D. tropicalis	Cornmeal	San Diego SC	14030-0801.01
D. virilis	Banana	Cambridge	Cam 3/6
S. lebanonensis	Propionic	Cambridge	Cam 3/6
S. pattersoni	Banana	San Diego SC	11010-0031.00
Z. tuberculatus	Banana	San Diego SC	50001-0001.05

S3 - Pilot test 1: DAV concentration for infection

To test what virus concentration of DAV we would use to infect the flies in the experiment, we performed a pilot test with three treatments using *Drosophila melanogaster*, *D. subobscura* and *D. tropicalis*. Each treatment received a different virus concentration — high (10^{-5}), medium (10^{-6}) or low (10^{-7}) — and had eight replicates, each one with 15 females flies which were 3 to 5 days old. In total, we infected 1080 flies. Flies from half of the biological replicates from each treatment were snap frozen in liquid nitrogen after virus injection, as a reference of day 0. The other half was left for virus incubation for three days. In the third day, these replicates were also snap frozen and we kept them at -80ºC. RNA extraction and change in DAV load calculation were performed as described in Methods section.

We observed a large variation in the change of viral load depending on the initial concentration of DAV (Figure S3.1). When we infected flies with a high virus concentration solution (10^{-5}), all the species showed similar change in viral load after three days. However, when flies were prickled with lower virus concentration solutions (10^{-6} or 10^{-7}), there was a dramatic change in viral load in *D. subobscura* and *D. melanogaster*.

As different species of flies seemed to respond distinctly to injections with lower virus concentration, and the higher virus replication in *D. melanogaster* (the original host) occurred with the high dose, we choose the higher DAV concentration solution (10^{-5}) to perform the experiments.

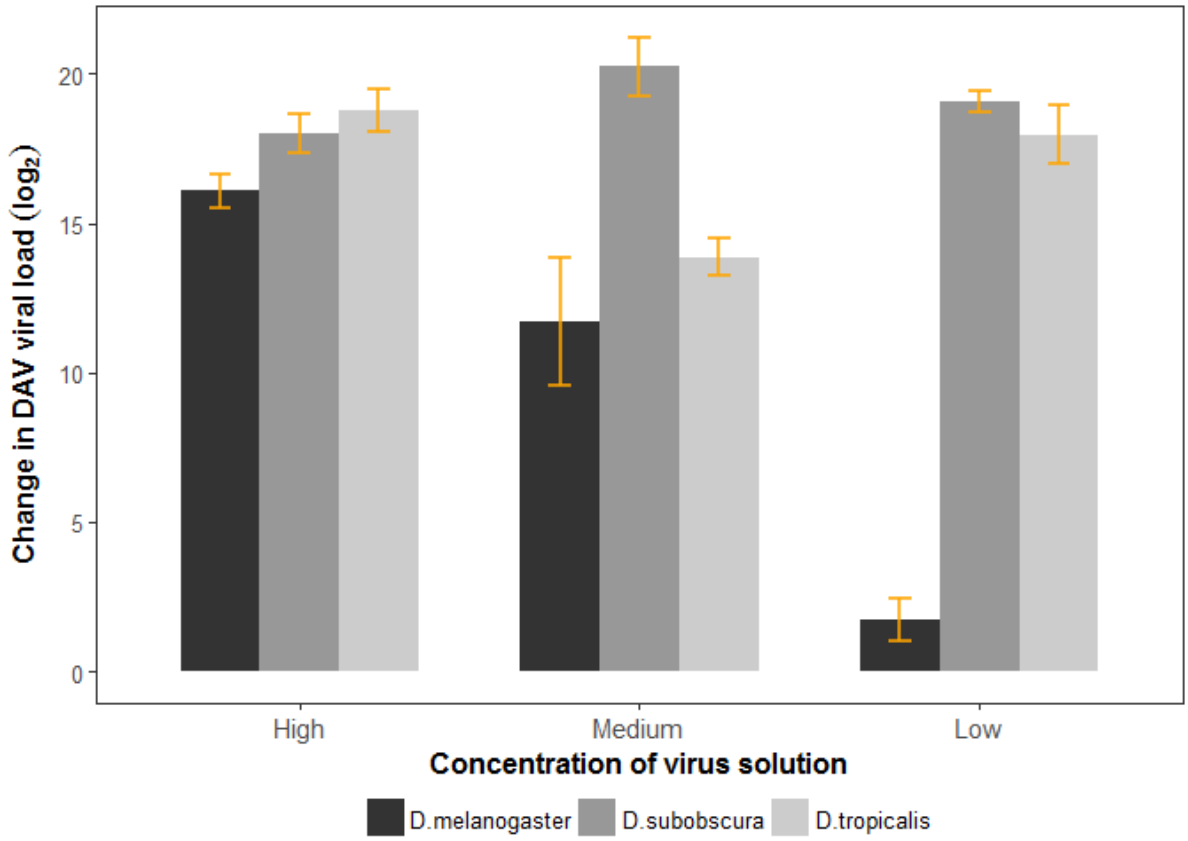


Figure S3.1. Change in DAV load after three days of injection in three species of *Drosophila*, with three different virus concentrations. Bars represent different species: *Drosophila melanogaster* in black, *Drosophila subobscura* in dark grey and *Drosophila tropicalis* in light grey. Each treatment received a solution with a different initial DAV concentration: high = 10^{-5}, medium = 10^{-6} and low = 10^{-7}). Error bars show standard errors.

S4 - Pilot test 2: Time of DAV incubation in host

To decide the number of days we would leave flies incubating DAV, we performed a pilot test with *D. baimaii, D. melanogaster, D. nasuta, D. subobscura, D. tropicalis* and *D. virilis*. We submitted each species to three different treatments — virus incubation for three, six and nine days — and for each one we settled up eight replicates with 15 females (3 to 5 days old). In total, we inoculated 2160 flies. Flies from half of the biological replicates from each treatment were snap frozen in liquid nitrogen after virus injection, as a reference of day 0. In the last day of incubation for each treatment, flies were also snap frozen and kept at -80ºC. RNA extraction and change in DAV load calculation were performed as described in Methods section.

We observed huge variation in the change in viral load in the first three days of incubation (Figure S4.1). After six and nine days of incubation, viral load did not seem to vary compared to viral load from day 3. Some exceptions were *D. baimaii* and *D. tropicalis*. In *D. baimaii*, viral load increased after three days of inoculation, but it had a dramatic decrease after six and nine days. *D. tropicalis* had a decrease in viral load in the first three days, lightly increased after six days and almost did not change anymore after nine days. As host species had a considerable change in viral load in the first three days, and as it did not appear to suffer many changes after six and nine days, we choose the day 3 to use in our experiments.

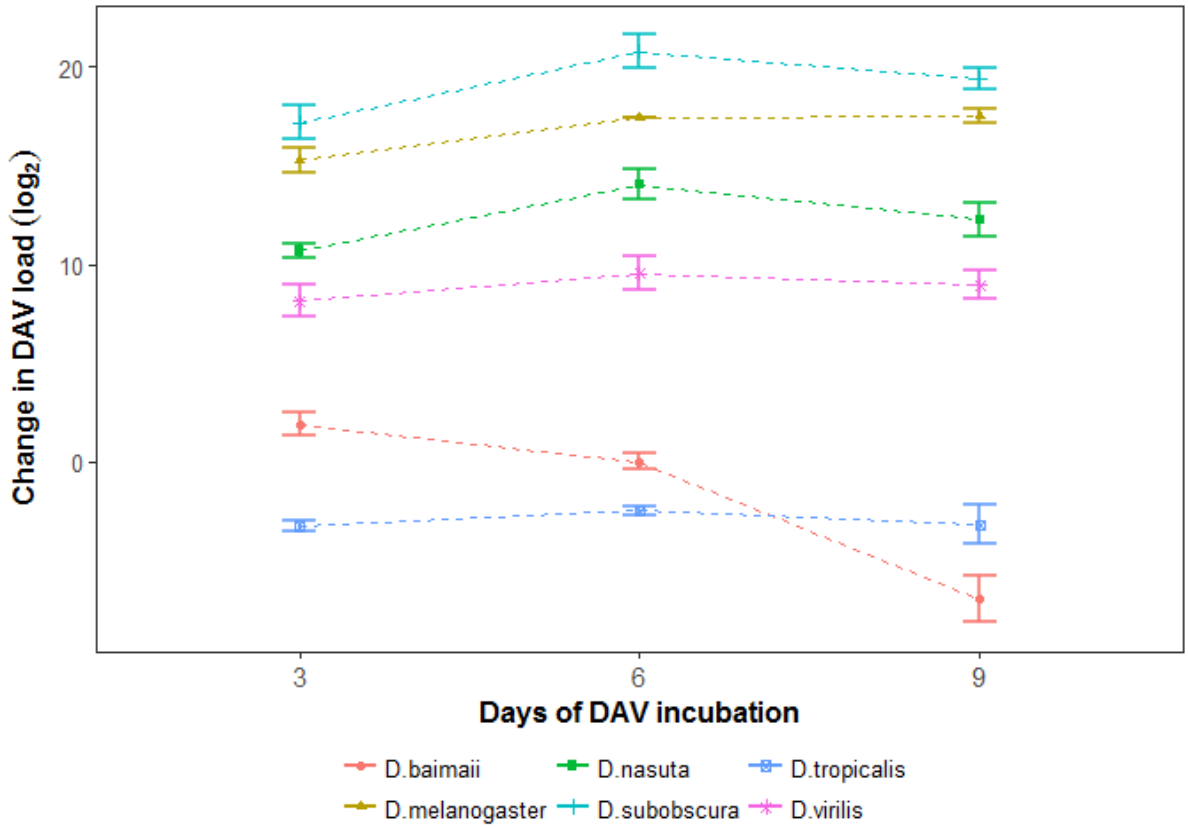


Figure S4.1. Change in DAV load after three, six and nine days of injection in six species of *Drosophila*. Symbols and colours represent different species. Error bars show standard errors.

S5 - Primers for RpL32 amplification

Table S5.1. Primers used in RpL32 gene RT-qPCR. F: forward, R: reverse

Primer	F/R	Code	Sequence 3'-5'
RpL32_qPCR_F-a	F	a	TGCCAAGTTGTCGCACAAATGG
RpL32_qPCR_F-b	F	b	TGCTAAGTTGTCGCACAAATGG
RpL32_qPCR_F-c	F	c	TGCCAAGCTGTCGCACAAATGG
RpL32_qPCR_F-d	F	d	TGCTAAGCTGTCGCACAAATGG
RpL32_qPCR_F-e	F	e	TGCGAAGTTGTCGCACAAATGG
RpL32_qPCR_F-f	F	f	TGCGAAGCTGTCGCACAAATGG
RpL32_qPCR_R-a	R	a	TGCGCTTGTTGGAACCGTAAC
RpL32_qPCR_R-b	R	b	TGCGCTTGTTGGATCCGTAAC
RpL32_qPCR_R-c	R	c	TGCGCTTGTTGGAACCATAAC
RpL32_qPCR_R-d	R	d	TGCGCTTGTTGGAGCCGTAAC
RpL32_qPCR_R-e	R	e	TGCGCTTGTTAGAACCGTAAC
RpL32_qPCR_R-f	R	f	TACGCTTGTTGGAACCGTAAC
RpL32_qPCR_R-g	R	g	TGCGCTTGTTGGAACCGTAGC
RpL32_qPCR_R-h	R	h	TGCGCTTGTTCGATCCGTAAC
RpL32_qPCR_R-i	R	i	TGCGCTTGTTGGAGCCATAAC
RpL32_qPCR_R-j	R	j	TGCGCTTGTTTGATCCGTAAC
RpL32_qPCR_R-k	R	k	TGCGCTTGTTTGAACCATAAC
RpL32_qPCR_R-l	R	l	TACGCTTGTTGGAACCATAAC
RpL32_qPCR_R-m	R	m	TACGCTTGTTGGAGCCGTAAC
RpL32_qPCR_R-n	R	n	TGCGCTGGTTGGAACCATAAC
RpL32_qPCR_R-o	R	o	TGAGCTTGTTCGATCCGTAAC
RpL32_qPCR_R-p	R	p	TACGCTTGTTGGAGCCATAAC
RpL32_qPCR_R-q	R	q	TGAGCTTGTTTGATCCGTAAC
RpL32_qPCR_R-r	R	r	TAAGCTTGTTGGATCCGTAGC
RpL32_qPCR_R-s	R	s	TCAGCTTGTTGGATCCATAGC

Table S5.2. Primers used in RT-qPCR for each species. The description of each forward and reverse primer is on S5.

Species	Forward	Reverse
D. ambigua	b	a
D. americana	c	a
D. ananassae	f	a
D. arizonae	a	a
D. baimaii	a	r
D. buzzati	a	e
D. euronotus	a	g
D. flavomontana	c	a
D. hydei	a	a
D. immigrans	b	p
D. littoralis	c	a
D. lummei	c	a
D. mauritiana	d	h
D. melanogaster	d	h
D. micromelanica	a	g
D. mojavensis	a	a
D. montana	c	a
D. nasuta	b	f
D. nebulosa	b	c
D. novamexicana	c	a
D. obscura	b	a
D. paramelanica	a	g
D. persimilis	a	b
D. prosaltans	a	n
D. pseudoananassae	e	a
D. pseudoobscura	a	m
D. putrida	d	q
D. santomea	a	n
D. sechellia	d	h
D. simulans	d	h
D. sturtevanti	a	l
D. subobscura	a	i
D. takahashii	d	o
D. teissieri	d	h
D. tropicalis	b	k
D. virilis	c	a
S. lebanonensis	d	h
S. pattersoni	a	m
Z. tuberculatus	a	c

S6- Criteria for exclusion of species from virulence tests

Considering the control mortality higher than expected, even the hazard ratio could influence data interpretation if all 39 species' virulence data were included in the analysis. Also, although there was not any correlation for the 39 species between hazard ratio and mean proportion mortality of virus infected flies (correlation = 0.22, p-value = 0.1745), there was correlation with control mean proportion dead (correlation = -0.37, p-value = 0.0210). Thus, we followed these criteria to exclude species from virulence tests:

1. We compared controls from this study to control data from Longdon et al. (2015) (Figure S6.1). Both control tests were implemented following the same method and with the same species. We calculated hazard ratios between those curves and kept for virulence analysis the species whose DAV controls were not statistically different from DCV controls (Cox model resulted in a p-value > 0.05) (Table S6.1). We kept 24 species.
2. For the 15 species left, we observed that there was a significant variation between control and infected flies in DAV experiment (see *D. subobscura* in Figure S6.2 in the manuscript). In order not to lose this information, we compared the hazard ratios from the curves of controls and DAV injected flies and we kept species whose infected flies curves were statistically different from control curves (Cox model resulted in a p-value < 0.05) (Table S6.2). We kept 33 species and excluded six species.
3. The control curve of *D. sechellia a*nd *D. virilis* were statistically different from DAV infected flies curve (Figures S6.2 and S6.3). However, the hazard ratio for these species (Table S6.2) indicates that there is a higher risk of death per time unit for controls when compared to DAV infected flies. As it is an unexpected pattern, and considering the high variation of proportion death for *D. sechellia*, we concluded that these species need deeper investigation. Thereby, we also discarded *D sechellia* and

D. virilis. In total, we excluded eight and kept 31 species for following mortality analysis.

References:

Longdon B, Hadfield JD, Day JP, Smith SCL, McGonigle JE, Cogni R, Cao C, Jiggins FM (2015) The causes and consequences of changes in virulence following pathogen host shifts. PLoS Pathogens, 11(3):e1004728.

Table S6.1. Hazard ratios from Cox model between DAV and DCV controls.

Species	**Hazard ratio**	**P-value**
D.ambigua	0.1282	<0.001
D.americana	0.078	0.035
D.ananassae	0.0238	0.00026
D.arizonae	3.543	0.13
D.baimaii	1.566	0.49
D.buzzatii	0.611	0.38
D.euronotus	5.1892	0.14
D.flavomontana	0.807	0.64
D.hydei	0.162	0.5
D.immigrans	0.83	0.78
D.littoralis	3.1609	0.076
D.lummei	2.062	0.24
D.mauritiana	0.074	<0.001
D.melanogaster	0.0297	<0.001
D.micromelanica	0.236	0.00072
D.mojavensis	2.745	0.258
D.montana	0	1
D.nasuta	10.847	0.04
D.nebulosa	0.236	0.00072
D.novamexicana	0.384	0.306
D.obscura	0.0732	<0.001
D.paramelanica	2.0982	0.55
D.persimilis	0.187	<0.001
D.prosaltans	0.591	0.665
D.pseudoananassae	1.415	0.58
D.pseudoobscura	0.0395	<0.001
D.putrida	2.356	0.28
D.santomea	0.1617	0.0011
D.sechellia	0.0535	0.00017
D.simulans	0.28066	0.019
D.sturtevanti	0	1
D.subobscura	0.0764	<0.001
D.takahashii	1.1236	0.79
D.teissieri	1.529	0.46
D.tropicalis	0.266	0.069
D.virilis	0.502	0.2266
S.lebanonensis	0.509	0.55
S.pattersoni	0	1
Z.tuberculatus	0.581	0.7

Table S6.2. Hazard ratios from Cox model between control and DAV injected flies.

Species	**Hazard ratio**	**P-value**
D.ambigua	0.609	0.11
D.americana	4.892	0.00019
D.ananassae	1.322	0.41
D.arizonae	4.776	0.046
D.baimaii	6.339	0.0031
D.buzzatii	4.853	0.00011
D.euronotus	1.0213	0.99
D.flavomontana	0.826	0.687
D.hydei	3.0357	0.34
D.immigrans	0.9715	0.96
D.littoralis	1	1
D.lummei	11.595	<0.001
D.mauritiana	1.762	0.0589
D.melanogaster	1.8098	0.018
D.micromelanica	2.041	0.015
D.mojavensis	8.404	<0.001
D.montana	6.129	0.004
D.nasuta	6.691291	0.078
D.nebulosa	1.504	0.12
D.novamexicana	1.0229	0.97
D.obscura	1.247	0.33
D.paramelanica	5.383	0.12
D.persimilis	1.444	0.1
D.prosaltans	5.957	0.00033
D.pseudoananassae	0.505	0.33
D.pseudoobscura	2.4516	0.00047
D.putrida	4.3339	0.025
D.santomea	3.389	<0.001
D.sechellia	0.316	0.0093
D.simulans	3.832	<0.001
D.sturtevanti	4.151	0.2
D.subobscura	2.933	<0.001
D.takahashii	3.0547	0.003
D.teissieri	2.206	0.11
D.tropicalis	23.207	<0.001
D.virilis	0.142	0.011
S.lebanonensis	26.012	<0.001
S.pattersoni	2.369	0.1241
Z.tuberculatus	0.536	0.61

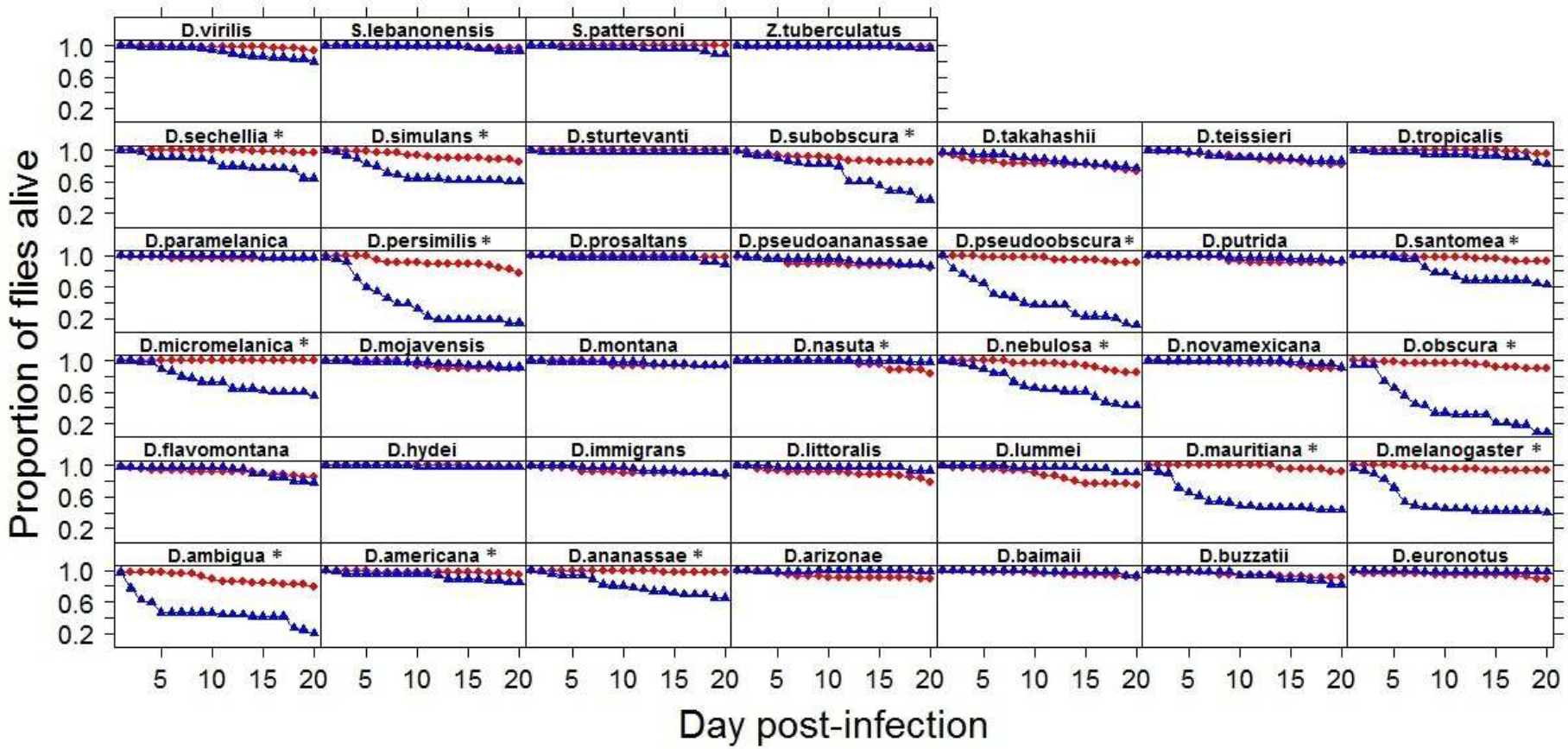


Figure S6.1. Mortality in 39 species of *Drosophila* stabbed with control solution. Control flies from DAV experiment are blue triangles and control flies from DCV experiment (Longdon et al., 2015a) are red circles. Panels arranged in alphabetical order. Stared species have statistically different treatment effects (p-value < 0.05).

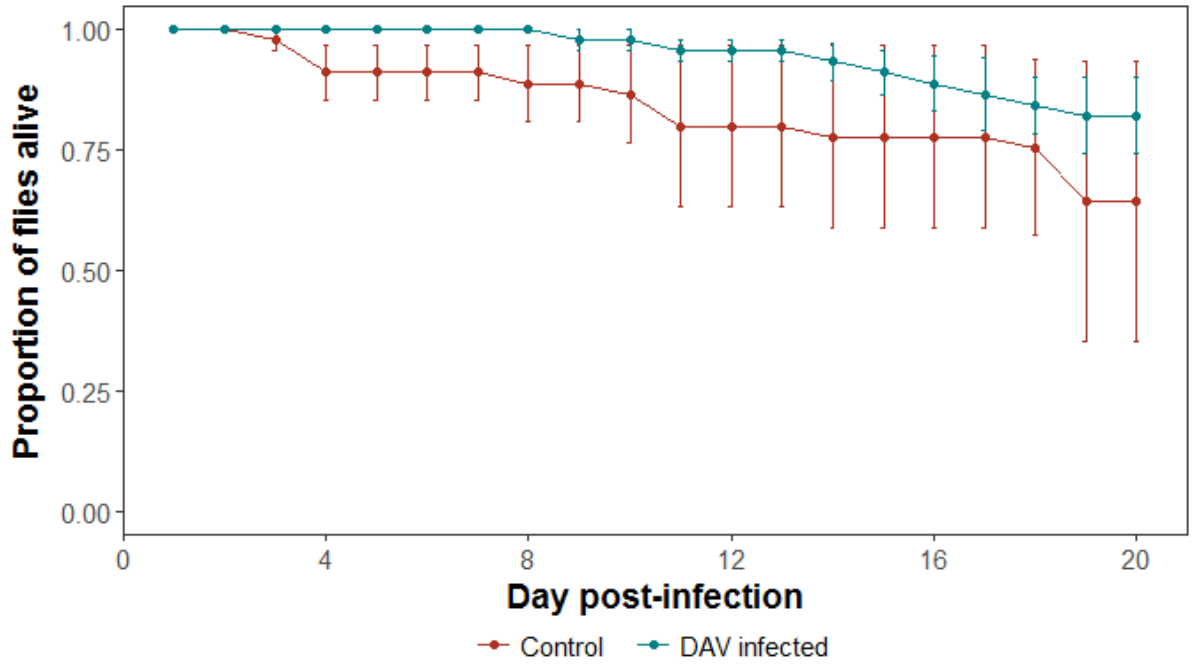


Figure S6.2. Mortality in *Drosophila sechellia* after infection with DAV. Control flies are in red and virus infected flies are in blue. Hazard ratio for this species is 0.316 (p-value = 0.0093).

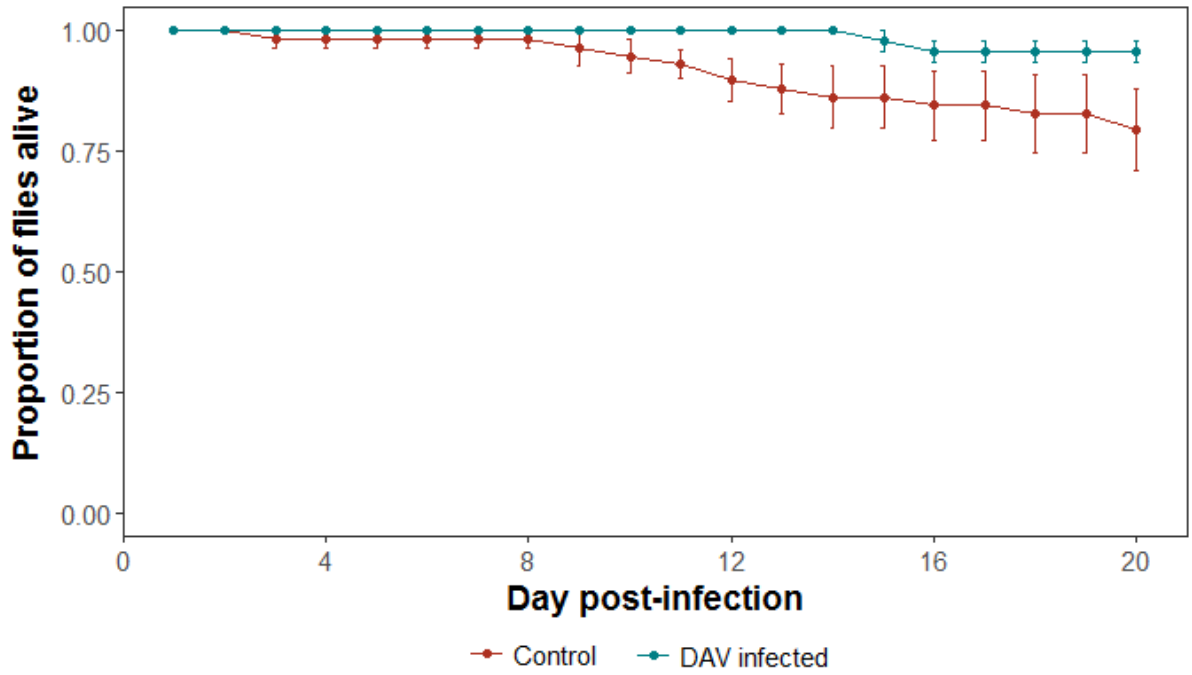


Figure S6.3. Mortality in *Drosophila virilis* after infection with DAV. Control flies are in red and virus infected flies are in blue. Hazard ratio for this species is 0.142 (p-value = 0.011).

S7 - Correlation between DAV viral load on day 0 and on day 3 post-infection

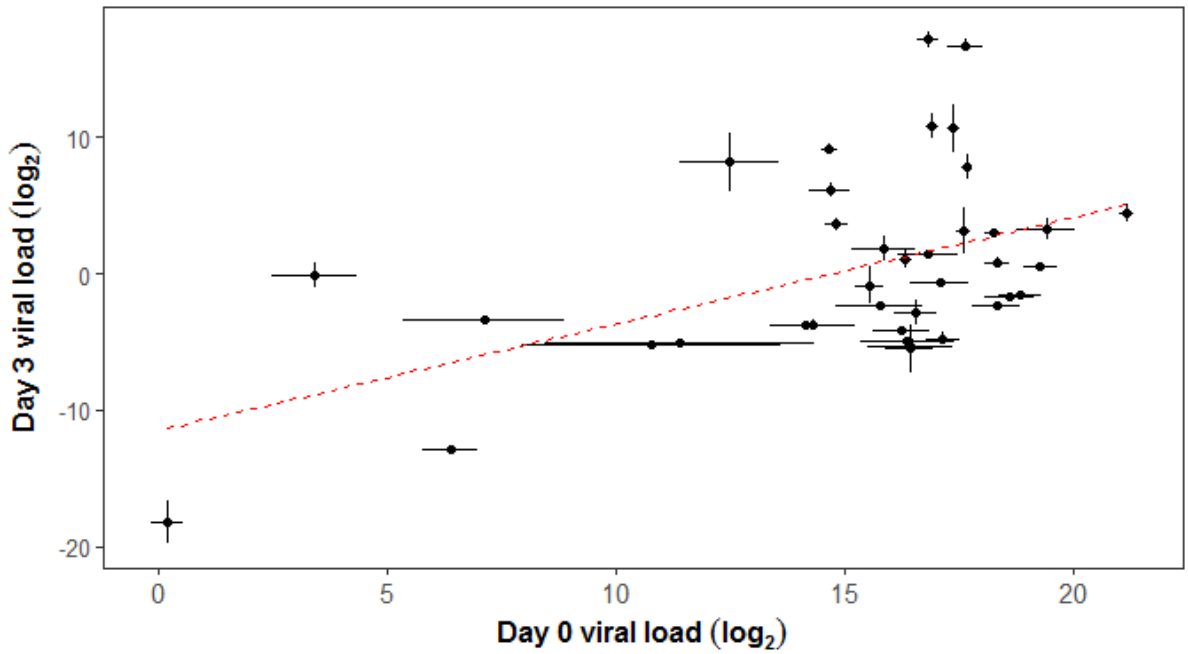


Figure S7.1. Correlation between DAV viral load in flies collected on the day and in flies collected three days after injection. 39 species were included. The trend line is estimated from a linear model. Correlation = 0.48, p-value = 0.0017. Error bars show standard errors.

S8 - Extra test: Effect of the needle during virus injection

To test whether control flies were dying due to harm caused by the needle during virus injection, we performed an extra experiment, only testing control flies, at the Insect Virus Ecology and Evolution Laboratory (University of Exeter, Penryn Campus). Flies at this laboratory were previously screened for DCV and Nora viruses and for *Wolbachia*, and all the results were negative. We selected 12 species that had high mortality in controls during virulence experiment (Table S8.1). For each species, we prepared 6 samples with 15 males. Half of the samples was anesthetized and stabbed with Ringer's solution. The other half was not stabbed — we only anesthetized flies in CO_2 for the same amount of time than stabbed samples. In total, we stabbed 540 flies. We recorded the number of dead flies per day during 10 days and then we calculated the proportion of flies alive in each day. Also, we compared these results to control flies from our virulence experiment.

We observed that for all the species, except for *Drosophila nebulosa*, mortality in control stabbed flies was lower than in virulence experiment (Figure S8.1). Thus, the injection with the needle probably is not harming the host and causing the death in control flies. We concluded that our stock flies might be contaminated with a virus or bacteria and need to be screened again.

Table S8.1. List of fly species used in the extra test. Each species was reared on its specific food (see recipes in S1), at 22ºC, 70% relative humidity and 12h light-dark cycle. The San Diego Stock Centre (SC) or the Cambridge (Cam) ID are identified. These species are maintained at the Insect Virus Ecology and Evolution Laboratory, supervised by Dr. Ben Longdon, in the College of Life and Environmental Sciences (University of Exeter, Penryn Campus).

Species	Food	Provenience	SC or Cam ID
D. ananassae	Cornmeal	Cambridge	JM wol free 3/6
D. mauritiana	Propionic	Cambridge	Cam
D. melanogaster	Cornmeal	Cambridge	Cam
D. micromelanica	Cornmeal	San Diego SC	15030-1151.01
D. nebulosa	Cornmeal	San Diego SC	14030-0761.05
D. obscura	Propionic	Cambridge	Cam DA30
D. persimilis	Malt	San Diego SC	14011-0111.51
D. pseudoobscura	Malt	Not available	1401-0121-103 (allo)
D. santomea	Cornmeal	San Diego SC	14021-0271.00
D. sechellia	Propionic	Cambridge	Cam
D. simulans	Cornmeal	Cambridge	Cam
D. subobscura	Cornmeal	San Diego SC	14011-0131.15

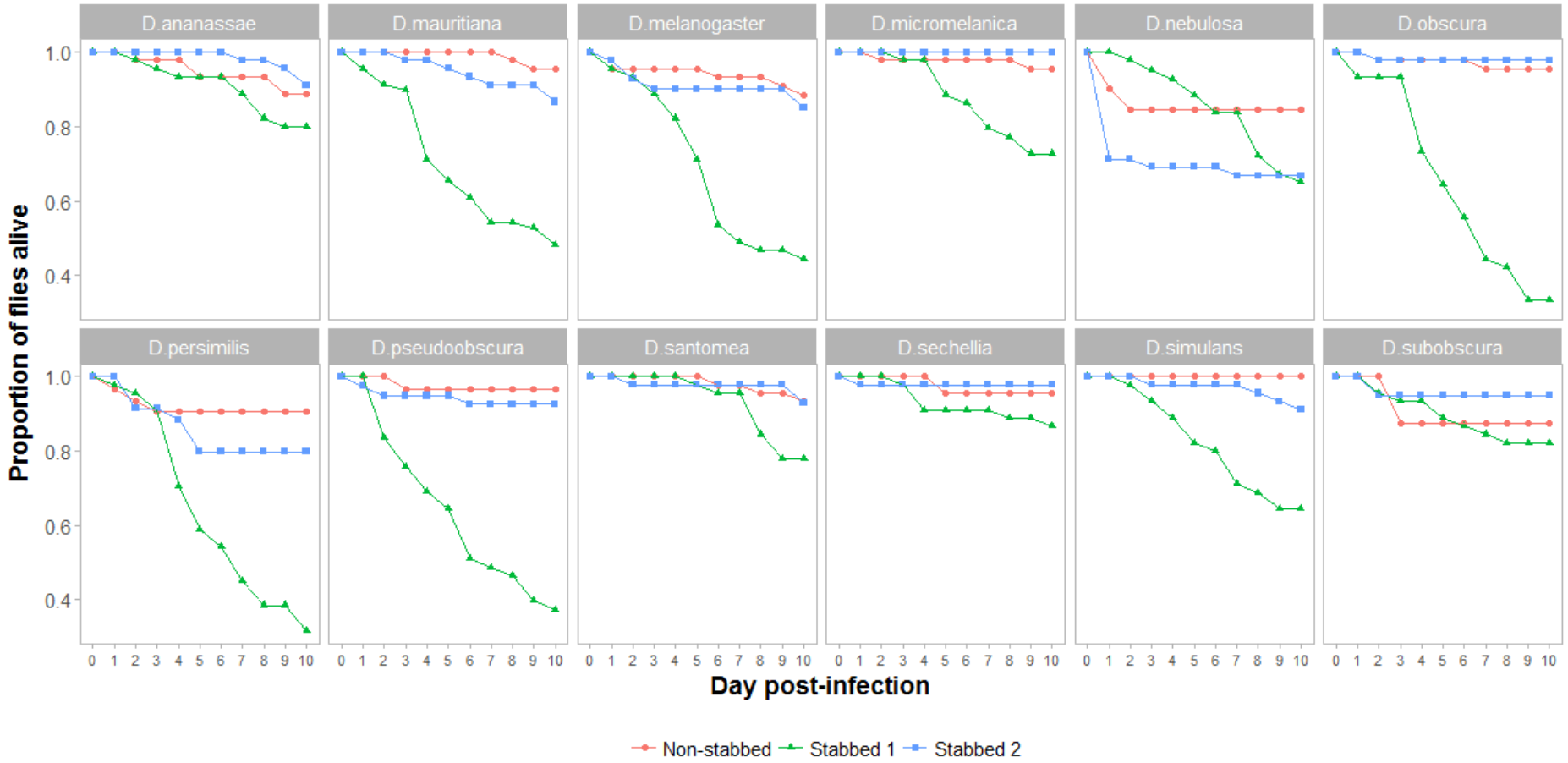


Figure S8.1. Mortality in 12 species of *Drosophila* stabbed with control solution. Control flies from DAV experiment are blue triangles and control flies from DCV experiment (Longdon et al., 2015a) are red circles. Panels arranged in alphabetical order. Stared species have statistically different treatment effects (p-value < 0.05).