Dear Ali,

Thanks very much to yourself, Greg and Anon for the thoughtful and constructive comments. In addition to uploading a revised manuscript to bioRxiv we have responded to the comments inline below and provide a version of the manuscript with tracked changes attached.

Many thanks,

Ben and Kat

Author's Reply:

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by Alison Duncan, 05 Jan 2023 11:27
Manuscript: **https://doi.org/10.1101/2022.11.01.514663** version 1

Minor revisions for preprint 'Sex and tissue differences in susceptibility across species'

I agree with both reviewers that this preprint is interesting, well written and will make a good contribution to PCI Evolutionary Biology. The preprint combines 2 experiments addressing sex differences in susceptibility to DCV across 31 different species and tissue tropism across males for 7 species. As it stands, the preprint is of high quality, however both reviewers make some useful comments. Reviewer 1 suggests that framing the preprint in terms of 'heterogeneity in infection outcomes' that might arise due to sex or tissue tropism may make the flow of the introduction a bit easier. Both reviewers also suggest that additional information about sex differences in infection may add to the preprint -notably Reviewer 1 suggests more information about STIs and how this differs to ordinary infectious diseases and Reviewer 2 some text explaining why sex differences in infections might arise due to a trade-off with reproduction. There are a few typos that I highlighted in the attached pdf.

Thanks – typos have been corrected.

Download recommender's annotations

Reviews

Reviewed by Greg Hurst, 24 Dec 2022 19:26

Roberts and Longdon

This is an interesting and thorough paper on sex differences in viral progression across drosophilids, and repeatability of tropism patterns. I found the methods and analysis well described (though note slightly above my expertise in analysis here) and the results clear.

I've attached a pdf with comments on for minor style issues.

Thanks – style issues have been addressed.

I'd ask the authors to consider the following:

a) There is a body of work on sex biased infection from the STI literature (Lockhart Biol Rev. is an entry point,also Knell and Webberley and some others), which does tend to find sex biased infection (through exposure differences e.g Ryder et al 2014 Am Nat) and sex biased virulence patterns (e.g. female limited sterility or female biased sterility). This literature also goes across to insects, so I would suggest mentioning this as a separate case and then examining what are sometimes (after Lockhart) known as Ordinary Infectious Diseases.

Thanks for this suggestion – we've mentioned this in the introduction (L76-78) and again in the discussion (L392-393).

"Sexually transmitted infections – which are primarily transmitted between the sexes – are particularly prone to sex biased infection through either exposure differences or sex biased virulence [20-22]."

"… sex biased prevalence and impacts are well known for sexually transmitted infections in insects [20, 21]."

b) The piece tries to do two things in one paper, which is always a tricky ask in terms of writing. It's not needed, but does make the introduction a little saltatory. I wonder if an overall framing in terms of 'heterogeneity in infection outcome' might be a way forward, saying this can be viewed both at the level of individuals and species.E.g sex of host wihtin species, pattern of infection between species .

Thanks – agree there is a little juggling of two different things here – the suggestion is a good one and we have adjusted the title, abstract, introduction and discussion to encompass this disease heterogeneity narrative.

c) The start of the discussion would benefit from a brief recapitulaiton of the main aims/ideas before reconciling to the literature.

Added L368-378

"Here, we examined whether heterogeneities in infection outcomes altered patterns of susceptibility across host species. We first examined whether males and females responded in consistent or different ways to infection with DCV. We found that viral susceptibility between females and males of 31 host species showed a strong positive correlation with a close to 1:1 relationship, suggesting that susceptibility across species is not sex specific. We next examined whether heterogeneities in the tissues infected across host species altered the outcome of infection. We found differences in viral load between tissues of seven host species, but no evidence of tissues showing different patterns of susceptibility in different host species."

d) Whilst experiments and analyses were strong to me, I think an important caveat for the discussion is that the experiments on sex bias were limited to i) young flies and ii) largely virgin flies. I would add 'exploration in a few systems should investigate if age and matedness status alter these results'. Further, the tropism were limited to a sex and age as well, so again a caution that tropism did not vary in young male flies is the formal conclusion, rather than there is no variation in tropism amongst species (which is true of generalised).

Sorry if the conclusions seemed a bit too broad - we've now added the following caveats to the discussion:

L405-407 "A caveat is that the flies used here were of a fixed age and largely virgins – future studies should explore if age and mating status can affect these results."

L418-419 "Further work should explore this further in a range of conditions including flies of both sexes and of varying ages."

L435-438 "Further work is needed to explore how sex differences can vary with factors such as host age, mating status, the environment and pathogen type, and the underlying mechanisms as to why species vary in their susceptibility."

Aside these, minor comments only. I enjoyed this paper and it clearly has a substantial body of work that can be published - just a matter of polish and the occasional tempering caveat.

Many thanks.

[Download the review]

Reviewed by anonymous reviewer, 27 Dec 2022 10:14

The preprint "Sex and tissue differences in virus susceptibility across species" reports the results of two experiments where Drosophila C Virus (DCV) genomes has been quantified by quantitative RT-PCR in males and females (experiment 1: effect of sex) and different tissues (experiment 2) of several species of fruit flies of the family Drosophilidae.

The first experiment was performed over 31 species, and showed no clear difference in viral loads between sexes. Although viral load varied greatly between species, males and females viral load were rather consistent between sexes of the same species, with a close to 1:1 overall ratio between sexes. Consistent with a previous paper of the same authors, host phylogeny explained most of the observed interspecific variation. Surprisingly, there was no effect of body size on viral, which I found particularly interesting, both within species and across species. Authors also found no effect of mating status, but I believe that the absence of effect is largely due to the experiment not designed to test it (only 4 species showed sign of uncontrolled mating).

The second experiment was performed over 7 species of flies, where males (only) were infected and dissected to measure viral load in different body parts/tissues. The main result here is again a strong effect of species on viral loads, and very little within species variation and across species tissue consistency. This is a very interesting study that investigated the possible effect of tissue tropism to explained variation in disease virulence across species, or interspecific variation of disease transmission.

Overall, I found this paper well written and very clear. The rational of the study is clear, the experiments are sound, without apparent flaw, and their analysis clearly described. I really enjoyed reading this preprint and I believe it provides highly valuable results and was very stimulating. I do not have comments to make on the experimental procedures, as I think experiments were done properly and reported clearly. I will only make comments on the report itself, with a few suggestions which I hope will be helpful to authors.

Many thanks.

Major suggestions:

In the second paragraph of the introduction, I found the background on sex differences in immunity in invertebrates a little light, with no mention of reproduction trade-off theory that may explain why males are generally more susceptible to diseases than females. One important paper to cite may be about the Bateman's principle in insect immunity (https://doi.org/10.1098/rspb.2002.1959).

In addition to the additional info requested by the other reviewer, we've added some additional information about the reproduction trade-off "Bateman's" theory L79-84

"Other hypotheses suggest that as longevity is a major determinant of female fitness investment in costly immune responses are more important than for males, who can maximise their fitness with shorter term mating success [24]. As such, investment in reproduction may trade-off with immunity in different ways between males and females. There is some support for this hypothesis in mammals but a lack of supporting data for insects [15]."

It is unclear to me why authors decided to report statistical significance from experiment 1 based on CI, and p-value for experiment 2. Is this because of different statistical models used? It may be better to announce the level of significance for each model in the method section.

Yes, the bivariate phylogenetic mixed models for the sex difference were implemented using a Bayesian approach (L243). For the tissue tropism data it seemed sensible to use a frequentist approach that more people are familiar with as we were not able to implement phylogenetic mixed models for just 7 species. We have added some additional information to the methods to try and make this clearer.

Why not illustrating the phylogenetic effects and the absence of effect of body size in experiment 1, even in a supplementary figure? These are valuable results, and I would have welcomed more plots.

We have illustrated the phylogenetic effects previously using ancestral state reconstructions (see refs [4-5]) but to allow us to quantify susceptibility in both males and females here we used fewer species of flies. This means that whilst the estimates for the phylogenetic effects are consistent with previous work, they have broader credible intervals. Given the absence of an effect of body size (consistent with previous among species data for males) this doesn't really warrant a figure. As such, we felt that keeping the paper streamlined and succinct was the way to go here.

Minor comments:

Line 23: I would add for clarity in the abstract "to DCV", in the sentence "suggesting that susceptibility [to DCV] is not sex specific". Otherwise, it sounds like a general statement.

Corrected

Line 55: missing letter, please correct "parasitism rates in mammals"

Corrected

When citing species names, please add a space between genus initial letter and species name, throughout the paper.

Corrected

Line 132, authors justified that pricking flies with DCV does not change the overall distribution of viruses across fly tissue compared to oral feeding. But authors may acknowledge this might be true for D. melanogaster only (as cited in ref. 28). I am not aware of other study testing this, and based on the rational of the present ms, one cannot rule out a transmission route by species interaction leading to a difference in tissue tropism.

We have explicitly stated that these studies were in D. mel in the methods, but agree it would be neat to test this in other species (its already on the to do list!)

In the section 'Inferring the host phylogeny', not all references are cited as number. Citation format should be homogenized.

Corrected

Line 233 and 251: It is unclear to me what is strain-specific effect and variance. Is this fly species? Please clarify.

Corrected, should have been "species"

Line 335-336: I think this sentence missed its reference (meta-analysis).

Corrected