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Host–pathogen immune feedbacks can explain widely divergent outcomes from similar infections

Stephen P. Ellner, Nicolas Buchon, Tobias Dörr and Brian P. Lazzaro

Article citation details
Proc. R. Soc. B **288**: 20210786.
http://dx.doi.org/10.1098/rspb.2021.0786

Review timeline

Original submission:	1 January 2021
1st revised submission:	2 April 2021
2nd revised submission:	29 April 2021
Final acceptance:	30 April 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2021-0004.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This manuscript presents a set of analytical models to explain a phenomenon – the bimodality of discrete infection outcomes - that has long puzzled scientists studying host-microbe interactions and that has received renewed attention thanks to recent empirical studies in insect-bacteria systems. The first model presents a simple scenario of bacterial growth, host antimicrobial peptide production, and the production of bacterial proteases to degrade AMPs. The results suggest that small differences in initial conditions (here, the rate of immune system induction) can produce bimodal infection outcomes. However, this simple model is not sufficient to explain the empirically observed chronic infection dynamics, so the second model allows the existence of multiple bacterial phenotypes (persisters and growth/immunosuppression specialists) that better reflect empirical growth and density dynamics.

I thoroughly enjoyed reading this manuscript, for multiple reasons. The main text does a good job of explaining the significance and motivation of the modeling work, and the first analytical model is simple and elegant while managing to capture the fundamental dynamic in question. The model and analyses are thoroughly described in the main text and supplement (such that I think a student could repeat the analyses as an exercise) while still being accessible to both theoreticians and experimentalists. In many places, the robustness of the initial conclusions are reinforced by additional modeling efforts to test the impact of potentially important factors like constitutive immunity. I also appreciate the thoughtful nature of the paper, from the careful motivation of each of the model components to the conclusions and predictions that are suitably restrained by the limitations of the modeling framework.

I do have some comments and concerns, although they do not raise any fundamental issues about the quality and suitability of the work for this journal.

Moderate Concerns
1. The chronic infection model is interesting, and does recapitulate the empirical dynamics nicely, but as the manuscript admits, there may be many routes to similar dynamics and fit alone isn't strong evidence for mechanistic inference. In this case, there is a layering of several assumptions: that chronic infection is down to persistence phenotypes, that there is a trade-off between persistence and growth/immunosuppression, and that there is some kind of stochastic phenotype switching to produce daughter bacteria with the N phenotype. All of these are possible, but I think (given the lack of strong empirical evidence, or at least lack of evidence cited here), that this is only one of several possible scenarios that could lead to chronic infection. Alternative hypotheses (which to my mind are equally or perhaps even more likely) are not

developed here. For example, what if chronic infection is immune-mediated rather than pathogen-mediated, such that host recovery involves stronger negative regulation than an unperturbed host and it therefore takes more bacteria to initiate a response? In that case I would imagine variation acting through S (or K in the reduced model), but in a time- structured way. Or, what if the bacteria are undergoing within-host evolution to evade particular AMPs (I'm thinking about Haine et al 2008 Science), which drives some kind of arms race? I suppose in that case we would see recrudescence or other type of cycling behavior, but given sufficiently crude time sampling it could resemble a stable equilibrium? At any rate, it would be interesting to see how these alternative hypotheses might fit into the system, and whether they would also be sufficient to generate bimodal outcomes.

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4. One of the really interesting aspects of the Duneau et al paper, which motivates this one, is that different bacteria produced different distributions of outcomes. Do we know anything about the natural history or genetics of those bacteria to support variation in protease production (for example) or other microbe-mediated drivers of variation in infection outcomes that can connect the model to those empirical results?
5. Figure 5: The dynamics here are a bit hard to compare. It might be helpful to also represent these in phase space, to see (for example) how protease production maps onto the total bacterial population; to my eye it seems to recover, but aren't the protected bacteria supposed to be producing fewer proteases?
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11. While it does no actual harm, ESM S.3 seems pretty unnecessary. We already have an abundance of data and models about variation in innate immune induction rates, such that I don't think you need to justify adding this variation into the main models with a new, really complex kinetic model. This section seems a bit like reinventing the wheel, given its purpose in the manuscript (to motivate the assumption of variation in AMP induction), and could more naturally be spun off as a separate manuscript. I didn't review this section thoroughly so I can't provide detailed comments on its relative merits.

Review form: Reviewer 2 (Clayton Cressler)

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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Comments to the Author
In this mansuscript, Ellner and co-authors demonstrate that simple, but general, mathematical models for host-pathogen interaction can generate bimodal infection outcomes, and develop model extensions that they use to help understand previously observed infection dynamics in a Drosophila-bacteria infection system. As someone who appreciates the insights that can come from using simple analytical techniques (such as nullclines) to understand the dynamics of mathematical models, I really enjoyed reading this paper. The paper was well-written and easy to read, with a tight and punchy message. As one of the authors of van Leeuwen et al. 2019, I am also personally gratified to see such an elegant extension and generalization of the ideas presented in that paper. I have some comments and questions that might help to clarify some points about the dynamics of the minimal model, in particular. Otherwise, I feel that this paper is well worth publication in Proc B.

The one source of confusion for me in the paper was in lines 219-234. There, in discussing the limitations of the minimal model in you say, “that the alternative to host death may be a chronic,

low-level infection where bacteria remain present at substantial but non-lethal levels, and the host immune response is never fully down-regulated." That seems to imply that, in the minimal model, the alternative to host death is an infection with very, very low bacterial abundance and a down-regulated immune response. This is echoed later in the paragraph, when you say, "$B << 1$ implies that the immune system is almost completely downregulated, which is also out of line with the experimental observations." Although it is obvious from Fig. 2 and eq. (3) that the A nullcline approaches 0 as B approaches 0, intersections of the A and B nullclines intersect will be at low B and very high A. It's hard to see how this could be otherwise, seemingly indicating that the alternative to host death is not a down-regulated immune response.

Although I appreciate the brevity of the paper, I did feel like the Discussion was quite short, and perhaps missed some opportunities to discuss the generality of the model and the connections with existing literature. For example, in the Introduction, there are a number of experimental systems that are referenced as also demonstrating bimodality in infection outcomes. How do the models apply (or not) to those systems? For example, Fig. 1C shows that immune priming can drive bimodality, suggesting that differences in the initial immune state can matter – this is not something you mention in the paper because the natural initial state for the immune response on these models is $A = 0$, but it is implicit in your drawing some of the purple curves in Fig. 2B that start at non-zero immune states.

There is some nuance amongst the two models known to produce bimodality that could be discussed. In the model of van Leeuwen et al., the bimodality is between an equilibrium representing a chronic infection with sustained immune activation or an acute infection where the parasite is cleared and the immune system eventually downregulates. In the minimal model presented here, the bimodality is technically between two chronic infections (you are just assuming that the host dies before one of these two equilibria can be reached), one with a very low burden and a high immune response and one with a high burden and a low immune response. It seems plausible that each set of model assumptions might be better suited to particular host-pathogen systems – for example, vertebrate versus invertebrates?

When the extended model is mentioned on lines 89-100, it becomes a bit unclear what were the two bimodal infection outcomes that you had been talking about. When bimodality is first introduced, it is in the context of the experimental system, when the bimodality is between high replication and death and a chronic infection. On lines 67-69, you say, "depending on the balance between host immune response and pathogen counter-response, the outcome can be bistable dynamics, in which similar initial states lead to widely divergent outcomes." But you don't actually mention what those two outcomes are at that point; it might be worth mentioning that there.

In the discussion of the results of the minimal model (e.g., lines 166-169), I think it would be worthwhile to mention the fact that, in this model, the initial state of the immune system is always $A = 0$, meaning that what determines the outcome of infection in this model is always pathogen dose. Unless there are circumstances where it is reasonable to assume that A is non-zero (e.g., due to the presence of some other immune-inducing stimulus)? Is AMP production in Drosophila zero prior to the start of an infection experiment? Another implication of the model is that, at a high enough dose, there will be no possibility of seeing bistability. Is that also confirmed experimentally? That is, if you give Drosophila a high enough dose of Providentia, do you only see rapid proliferation and host death?

The discussion on lines 170-186 are so elegant. It was lovely to see how you could use the approximation to gain biological insight into the conditions required for bistability.

I found Fig. 2 to be a bit busy. I was wondering whether it was necessary to plot the unstable manifold. It's not discussed, and its inclusion kind of clutters the figure.

The goal of Fig. 3 was to try to approximate the dynamics observed experimentally, and while I liked the phenomenological way that you incorporated variability in immune induction, it's worth noting that it's not quite the same as model (2). That is, to what extent is another way to think about what you're doing in creating Fig. 3 is actually generating a 100 different parameter sets? In other words, rather than simulating 100 trajectories with a fixed phase-plane diagram, what this analysis basically does is simulate 100 different trajectories across 100 slightly different phase-plane diagrams. How would the results differ if you had instead drawn 100 different initial parasite doses and simulating the dynamics? Presumably, even in a tightly controlled experiment, it's hard to give individuals the *exact* same dose of parasites, so this would be a potentially important source of variation in infection outcome.

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Minor grammatical stuff:
Line 30: should be "sustain" rather than "sustaining"

Decision letter (RSPB-2021-0004.R0)

16-Feb-2021

Dear Dr Ellner:

I am writing to inform you that your manuscript RSPB-2021-0004 entitled "Host-pathogen Immune Feedbacks Can Explain Widely Divergent Outcomes from Similar Infections" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that revisions are necessary. Both reviewers and the Associate Editor are very positive about the work though, as am I. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript, as is customary at Proceedings B. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.
4) Data - please see our policies on data sharing to ensure that you are complying (https://royalsociety.org/journals/authors/author-guidelines/#data).

To upload a resubmitted manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
This manuscript was very well received by both reviewers, my own assessment is that it is high quality and of general interest. The reviewers highlighted a number of ways that the manuscript can be strengthened, particularly by fleshing out the discussion to further contextualize the findings and to discuss potential alternative hypotheses that may be relevant. Please address these and other comments in a revised version of the manuscript.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
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Author's Response to Decision Letter for (RSPB-2021-0004.R0)

See Appendix A.

RSPB-2021-0786.R0

Review form: Reviewer 1

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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Is it clear?
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Is it adequate?
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Do you have any ethical concerns with this paper?
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Comments to the Author
This revised manuscript presents a set of analytical models to explain a phenomenon – the bimodality of discrete infection outcomes - that has long puzzled scientists studying host-microbe interactions and that has received renewed attention thanks to recent empirical studies in insect-bacteria systems. The first model presents a simple scenario of bacterial growth, host antimicrobial peptide production, and the production of bacterial proteases to degrade AMPs. The results suggest that small differences in initial conditions (here, the rate of immune system induction) can produce bimodal infection outcomes. However, this simple model is not sufficient to explain the empirically observed chronic infection dynamics, so the second model allows the existence of multiple bacterial phenotypes (persisters and growth/immunosuppression specialists) that better reflect empirical growth and density dynamics. These models set up new, testable predictions for futher empirical work, and are likely to be broadly generalizable to many host-microbe systems.

I appreciate the thoughtful responses to my previous concerns about alternative hypotheses for chronic infections among other more minor issues, all of which I think have been adequately addressed in the current version of the manuscript. Overall, I think this an interesting, well-written, and timely paper that is likely to generate substantial interest among evolutionary biologists and infectious disease researchers, and I do not have any lingering or new concerns that require further revision.

Decision letter (RSPB-2021-0786.R0)

26-Apr-2021

Dear Dr Ellner

I am pleased to inform you that your Review manuscript RSPB-2021-0786 entitled "Host-pathogen Immune Feedbacks Can Explain Widely Divergent Outcomes from Similar Infections" has been accepted for publication in Proceedings B.

The referee does not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

To upload your manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor Hans Heesterbeek
mailto:proceedingsb@royalsociety.org

Associate Editor,
Board Member
Comments to Author:
Thank you for thoroughly addressing the reviewers' comments.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s).
This revised manuscript presents a set of analytical models to explain a phenomenon – the bimodality of discrete infection outcomes - that has long puzzled scientists studying host-microbe interactions and that has received renewed attention thanks to recent empirical studies in insect-bacteria systems. The first model presents a simple scenario of bacterial growth, host antimicrobial peptide production, and the production of bacterial proteases to degrade AMPs. The results suggest that small differences in initial conditions (here, the rate of immune system induction) can produce bimodal infection outcomes. However, this simple model is not sufficient to explain the empirically observed chronic infection dynamics, so the second model allows the existence of multiple bacterial phenotypes (persisters and growth/immunosuppression specialists) that better reflect empirical growth and density dynamics. These models set up new, testable predictions for futher empirical work, and are likely to be broadly generalizable to many host-microbe systems.

I appreciate the thoughtful responses to my previous concerns about alternative hypotheses for chronic infections among other more minor issues, all of which I think have been adequately addressed in the current version of the manuscript. Overall, I think this an interesting, well-written, and timely paper that is likely to generate substantial interest among evolutionary biologists and infectious disease researchers, and I do not have any lingering or new concerns that require further revision.

Decision letter (RSPB-2021-0786.R1)

30-Apr-2021

Dear Dr Ellner

I am pleased to inform you that your manuscript entitled "Host-pathogen Immune Feedbacks Can Explain Widely Divergent Outcomes from Similar Infections" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Dear Editors and reviewers,

Thank you for your positive evaluation of our paper, and for the very helpful comments. “Thanks for the helpful comments” is formulaic, but in this case we really mean it. We believe that the revised manuscript is substantially clearer about the biological basis for our models, about our specific conclusions, and about the broader implications of this work. We hope that this resubmission is now acceptable for publication, and we look forward to hearing from you.

Please note: with the resubmission we have re-uploaded all R scripts that have been changed in the process of revising the manuscript, but not scripts or data files that are the same as the original submission.

Steve Ellner, on behalf of all authors.

Associate Editor

This manuscript was very well received by both reviewers, my own assessment is that it is high quality and of general interest. The reviewers highlighted a number of ways that the manuscript can be strengthened, particularly by fleshing out the discussion to further contextualize the findings and to discuss potential alternative hypotheses that may be relevant. Please address these and other comments in a revised version of the manuscript.

Thank you for this positive assessment. The reviewer comments are addressed individually below.

Referee: 1

This manuscript presents a set of analytical models to explain a phenomenon – the bimodality of discrete infection outcomes - that has long puzzled scientists studying host-microbe interactions and that has received renewed attention thanks to recent empirical studies in insect-bacteria systems. The first model presents a simple scenario of bacterial growth, host antimicrobial peptide production, and the production of bacterial proteases to degrade AMPs. The results suggest that small differences in initial conditions (here, the rate of immune system induction) can produce bimodal infection outcomes. However, this simple model is not sufficient to explain the empirically observed chronic infection dynamics, so the second model allows the existence of multiple bacterial phenotypes (persisters and growth/immunosuppression specialists) that better reflect empirical growth and density dynamics.

I thoroughly enjoyed reading this manuscript, for multiple reasons. The main text does a good job of explaining the significance and motivation of the modeling work, and the first analytical model is simple and elegant while managing to capture the fundamental dynamic in question. The model and analyses are thoroughly described in the main text and supplement (such that I think a student could repeat the analyses as an exercise) while still being accessible to both theoreticians and experimentalists. In many places, the robustness of the initial conclusions are reinforced by additional modeling efforts to test the impact of potentially important factors like constitutive immunity. I also appreciate the thoughtful nature of the paper, from the careful motivation of each of the model components to the conclusions and predictions that are suitably restrained by the limitations of the modeling framework.

I do have some comments and concerns, although they do not raise any fundamental issues about the quality and suitability of the work for this journal.

Thank you for this positive assessment.

Moderate Concerns

1. The chronic infection model is interesting, and does recapitulate the empirical dynamics nicely, but as the manuscript admits, there may be many routes to similar dynamics and fit alone isn't strong evidence for mechanistic inference. In this case, there is a layering of several assumptions: that chronic infection is down to persistence phenotypes, that there is a trade-off between persistence and growth/immunosuppression, and that there is some kind of stochastic phenotype switching to produce daughter bacteria with the N phenotype. All of these are possible, but I think (given the lack of strong empirical evidence, or at least lack of evidence cited here), that this is only one of several possible scenarios that could lead to chronic infection. Alternative hypotheses (which to my mind are equally or perhaps even more likely) are not developed here. For example, what if chronic infection is immune-mediated rather than pathogen-mediated, such that host recovery involves stronger negative regulation than an unperturbed host and it therefore takes more bacteria to initiate a response? In that case I would imagine variation acting through S (or K in the reduced model), but in a time- structured way.

Or, what if the bacteria are undergoing within-host evolution to evade particular AMPs (I'm thinking about Haine et al 2008 Science), which drives some kind of arms race? I suppose in that case we would see recrudescence or other type of cycling behavior, but given sufficiently crude time sampling it could resemble a stable equilibrium? At any rate, it would be interesting to see how these alternative hypotheses might fit into the system, and whether they would also be sufficient to generate bimodal outcomes.

The reviewer is correct that we do not yet have any direct evidence for Protected bacteria phenotypes in our system. We chose to model the Protected bacteria hypothesis because of the evidence, cited in the manuscript, for defended phenotypes that arise in response to antibiotics and to immune defenses in other host-pathogen interactions. Testing the protected phenotype hypothesis for chronic infections in *Drosophila* is one component of the R01 proposal we recently submitted to NIH. If this reviewer happens to review our NIH proposal, we hope that you will highlight the importance of this question.

The first hypothesis suggested by the reviewer is that after fighting off the initial infection, the host has a different immune response, leading to a stable equilibrium with a low but not minuscule bacterial population. However, we are not aware of any empirical evidence, in any study system, to support or motivate this hypothesis. We also show, in a new ESM section (S.3) that the hypothesized change cannot occur just through changing the parameters in our model – it would require a qualitative change in how AMP production is modulated in response to pathogen abundance.

The pathogen evolution hypothesis is also possible in principle. Indeed, if a Protected phenotype can generate chronic infections, it must be possible for that to happen (in principle) through bacteria evolving to express the Protected phenotype. However, this hypothesis has been tested experimentally in our system, and rejected. Bacteria taken from flies with either outcome (lethal infection, chronic infection) and then re-injected into uninfected flies, again produce exactly the same split outcomes (unpublished experiments by N. Buchon and B.P. Lazzaro).

These same ideas might occur to other readers, so we have added a paragraph about them in the main text, as well as the new ESM section to provide the technical details about the first hypothesis.

Minor Concerns

1. The term –cAB occurs identically in both the bacterial and AMP equations of system 1. Does this imply that there is a 1:1 stoichiometry in the interaction of AMPs, bacteria, and the rate that they are degraded? I would think that the term in the AMP equation would have a (potentially) different rate parameter or scaling coefficient (e.g. if it takes more than one AMP to kill a bacterium). Or maybe the rescaling solves this problem?

Good catch! That was an omission. We are letting one unit of AMP be amount that it takes to kill one bacterium, which is consumed in the process, so each bacterial death is matched by loss of one unit of AMP. In the revision we state this explicitly in the paragraph after eqn. (1).

2. Hosts do produce protease inhibitors that could degrade bacterial proteases – is it assumed that the response would be too little too late and thus be linearized out of the dynamics?

We are not aware of any experimental evidence for this in *Drosophila*, which our model is based on, although it may happen in other systems. Moreover, unless it were a markedly delayed response to bacterial protease production, this host response would be equivalent to a reduction in protease production rate by the bacteria. So it would not change our model, it would just change the value of a parameter, and it would not affect our general analysis of the model.

3. In some places I think both motivations and conclusions could be fortified with the literature a bit more. For example, in the discussion of the constitutive defense scenario (213-217), there are several models that have investigated the evolution of constitutive vs. inducible defenses. Are these relevant here or have they focused too much on evolutionary bistability rather than individual outcomes?

Some work by Shudo and Iwasa (discussed below) has considered constitutive vs. inducible defenses, but those models are indeed operating on the time scale of host evolution, over multiple host generations. Our model is operating on the short time scale of a single infection, so evolution of host defense strategies would not interact with the dynamics we are studying. And since the bimodal outcomes occur even among genetically identical flies, evolutionary bistability in the hosts could not be the explanation. We take the host strategy as a "given", and uniform or nearly so across hosts, which is appropriate for the time scale of the observations that we are trying to explain.

4. One of the really interesting aspects of the Duneau et al paper, which motivates this one, is that different bacteria produced different distributions of outcomes. Do we know anything about the natural history or genetics of those bacteria to support variation in protease production (for example) or other microbe-mediated drivers of variation in infection outcomes that can connect the model to those empirical results?

We agree that this is an excellent question. Exploration of possible microbe-mediated drivers of variation in infection outcomes is one component of the R01 proposal we recently submitted to NIH. If this reviewer happens to review our NIH proposal, we hope that you will highlight the importance of this question. But at the moment, unfortunately, we can't say anything about this.

5. Figure 5: The dynamics here are a bit hard to compare. It might be helpful to also represent these in phase space, to see (for example) how protease production maps onto the total bacterial population; to my eye it seems to recover, but aren't the protected bacteria supposed to be producing fewer proteases?

A new panel in the Figure (bottom right panel) has been added to show the relationship between total bacteria and protease in a phase plane. It confirms that the ratio of protease to bacteria is lower at the later times, when Protected bacteria are dominant, exactly as it ought to be.

6. Lines 310-317: I do think the discussion is fair about the contributions and limitations of the model, and the stated 'new predictions' are similar to ones I thought of while contemplating the model (this is a compliment, not a concern)

Thank you!

7. Lines 324-327: I think there is probably an intermediate option here between a linear ramp and a detailed kinetic systems biology approach. There is a fair amount of recent empirical data on these dynamics (with regard to both time post infection and AMP production vs bacterial density) as well as a bunch of older models with simpler dynamics (e.g. some of the Shudo and Iwasa models from the mid-2000s) that could adequately capture variation in immunological dynamics with 3-4 parameters rather than dozens.

We agree that simpler would be good, but (assuming that Web of Science found all the papers this reviewer was thinking of) none of the Shudo and Iwasa models seems like it would be appropriate for our purposes. The mechanistic models are specifically for vertebrates, whose immune system is very different from what we're modeling. The others are optimal control models, in which the immune up-regulation trajectory is derived as the solution to an optimization problem, without specifying how the optimum is achieved mechanistically. Moreover, the optimization problems are not appropriate for our system, because the Shudo-Iwasa models do not include the counter-attack by the pathogens (protease production).

That being the case, we still see no better alternative to developing the detailed kinetic model. The mechanisms are all known, the rate equations are built from the standard toolkit, and using numerical optimization to find parameters generating desired outcomes is a few minutes of work for a cheap desktop PC. Eventually we are hoping to generate the data necessary to parameterize the "systems biology" model we develop here, and at that stage we might benefit from stripping it down to essential features and fewer parameters, but the full ugly beast does fine for our present purposes.

8. Lines 451-453: I don't know off the top of my head either Too bad, thanks!

9. Line 531: indicate that the parameter is "a" after "low-level constitutive defense" Done, thanks.

10. Lines 531-539: I had a hard time following this section, particularly in terms of how the first term should be interpreted. Also, bacterial density reduces AMP production in two places in eq. S10: the first term and the last? Is it necessary to have both terms to get the model behavior?

Some additional text has been added at this point to try to clarify what processes each part of S10 represent. As the reviewer notes, the two AMP-inhibitory effects of bacteria in the first and last terms of S10 are not both necessary to get the key model behavior, bimodality of outcomes. Indeed, the point of this model and its analysis is that adding some known host-pathogen interactions that were left out of the basic model in the main text results in quantitative changes in model solutions but has no qualitative effect on model behaviors.

11. While it does no actual harm, ESM S.3 seems pretty unnecessary. We already have an abundance of data and models about variation in innate immune induction rates, such that I don't think you need to justify adding this variation into the main models with a new, really complex kinetic model. This section seems a bit like reinventing the wheel, given its purpose in the manuscript (to motivate the assumption of variation in AMP induction), and could more naturally be spun off as a separate manuscript. I didn't review this section thoroughly so I can't provide detailed comments on its relative merits.

As we noted above, there doesn't seem to be a pre-existing simpler alternative model that could play the same role in this manuscript, so we have decided to leave the harmless ESM S.3 (now S.4) as it is. While the complexity of the model isn't a benefit, it also isn't a problem for our purposes in this paper. We don't think we are reinventing the wheel - we are not aware of any existing mechanistic model for the IMD signaling pathway. While a separate manuscript sounds appealing, at this point we don't have (or know of) any data on the kinetics of immune induction in *Drosophila* that we could use to parameterize or test the model and we think it would be important to have those data for the extended manuscript. Obtaining these data and building out the model is another objective of our pending NIH proposal.

Referee: 2

In this manuscript, Ellner and co-authors demonstrate that simple, but general, mathematical models for host-pathogen interaction can generate bimodal infection outcomes, and develop model extensions that they use to help understand previously observed infection dynamics in a Drosophila-bacteria infection system. As someone who appreciates the insights that can come from using simple analytical techniques (such as nullclines) to understand the dynamics of mathematical models, I really enjoyed reading this paper. The paper was well-written and easy to read, with a tight and punchy message. As one of the authors of van Leeuwen et al. 2019, I am also personally gratified to see such an elegant extension and generalization of the ideas presented in that paper. I have some comments and questions that might help to clarify some points about the dynamics of the minimal model, in particular. Otherwise, I feel that this paper is well worth publication in Proc B.

The one source of confusion for me in the paper was in lines 219-234. There, in discussing the limitations of the minimal model in you say, "that the alternative to host death may be a chronic, low-level infection where bacteria remain present at substantial but non-lethal levels, and the host immune response is never fully down-regulated." That seems to imply that, in the minimal model, the alternative to host death is an infection with very, very low bacterial abundance and a down-regulated immune response. This is echoed later in the paragraph, when you say, "B << 1 implies that the immune system is almost completely downregulated, which is also out of line with the experimental observations." Although it is obvious from Fig. 2 and eq. (3) that the A nullcline approaches 0 as B approaches 0,

intersections of the A and B nullclines intersect will be at low B and very high A. It's hard to see how this could be otherwise, seemingly indicating that the alternative to host death is not a down-regulated immune response.

The "B << 1 implies that…" that you quote above is an instance of forgetting that readers can't read our minds, so thank you for pointing this out. The statement is correct but we omitted the explanation, which has been added at that point in the revised manuscript. Namely: because of how the model is scaled, the rate of AMP production at the equilibrium is lower than the maximally up-regulated rate by a factor $B/(1+B)$, which for B<<1 is approximately equal to **B**. So just from the fact that $B<<1$ at the low-B equilibrium, we can conclude that the host is producing AMPs at a rate far below the completely up-regulated rate.

The axis limits in that figure make it look like the equilibrium is at a high A value. But for comparison, the equilibrium A at full up-regulation, with production balanced only by natural decay of AMPs, would be $A=80$ for the parameter values used in drawing that figure.

Although I appreciate the brevity of the paper, I did feel like the Discussion was quite short, and perhaps missed some opportunities to discuss the generality of the model and the connections with existing literature. For example, in the Introduction, there are a number of experimental systems that are referenced as also demonstrating bimodality in infection outcomes. How do the models apply (or not) to those systems? For example, Fig. 1C shows that immune priming can drive bimodality, suggesting that differences in the initial immune state can matter – this is not something you mention in the paper because the natural initial state for the immune response on these models is A = 0, but it is implicit in your drawing some of the purple curves in Fig. 2B that start at non-zero immune states.

Relative to the Proc. B length limits, the paper is not brief but long - estimated to be 9 pages in print, where the hard maximum is 10 pages. So a short Discussion is necessary to avoid cutting content.

Figure 1C comes from Tate et al. (2017), and they don't actually show that "immune priming can drive bimodality". Bimodality happens with or without immune priming, but (as we say in the paper) immune priming increases the change of the infection being contained.

However, you're right that differences in initial host immune state can also matter in the model, if it includes a mechanism where the system can start be at A>0 already when the pathogen invades. This is discussed in the analysis of the extended model in the ESM, which includes constitutive AMP production.

We decided that the new paragraph doing the compare-and-contrast between our model and the van Leeuwen model (see the next comment and response below) was the best place to talk about generality and how the model relates to other experiments. The Proc. B space limits mean that we had to make it very brief. Our initial submission was estimated to be 9 pages "in print", and the hard and fast limit is 10 pages.

There is some nuance amongst the two models known to produce bimodality that could be discussed. In the model of van Leeuwen et al., the bimodality is between an equilibrium representing a chronic infection with sustained immune activation or an acute infection where the parasite is cleared and the immune system eventually downregulates. In the minimal model presented here, the bimodality is

technically between two chronic infections (you are just assuming that the host dies before one of these two equilibria can be reached), one with a very low burden and a high immune response and one with a high burden and a low immune response. It seems plausible that each set of model assumptions might be better suited to particular host-pathogen systems – for example, vertebrate versus invertebrates?

We agree – there is value in putting the two models side-by-side and talking about their differences and similarities. We have added a paragraph to the Discussion in which we try to do that. The key difference, we think, is not vertebrate vs. invertebrate, but the two different mechanisms whereby the pathogen fights back against suppression by the immune system. In van Leeuwen et al., it's by diverting resources from the host and thereby diminishing its capacity for immune response. In our model, it's by chemical warfare, producing substances that degrade the host's chemical weapons or block their production. These differences result in the model having two different forms of bimodality. But the important take-home, we think, is that the common feature of a double-negative feedback loop leads to bimodality in both cases.

To complete(?) the suite of models, somebody should next do a model that includes the multiple interacting components of vertebrate immunity (B cells, T cells, etc.), and any known mechanisms that pathogens can use to fight back against those. But it won't be us – three invertebrate biologists, and an applied mathematician who knows even less than they do about vertebrate immunity.

When the extended model is mentioned on lines 89-100, it becomes a bit unclear what were the two bimodal infection outcomes that you had been talking about. When bimodality is first introduced, it is in the context of the experimental system, when the bimodality is between high replication and death and a chronic infection. On lines 67-69, you say, "depending on the balance between host immune response and pathogen counter-response, the outcome can be bistable dynamics, in which similar initial states lead to widely divergent outcomes." But you don't actually mention what those two outcomes are at that point; it might be worth mentioning that there.

We have added text at various places in the Introduction to try to be clearer about these points. The additions are scattered, but they should be evident in the "track changes" version of the revised manuscript.

In the discussion of the results of the minimal model (e.g., lines 166-169), I think it would be worthwhile to mention the fact that, in this model, the initial state of the immune system is always A = 0, meaning that what determines the outcome of infection in this model is always pathogen dose.

Done – good idea, thanks!

Unless there are circumstances where it is reasonable to assume that A is non-zero (e.g., due to the presence of some other immune-inducing stimulus)? Is AMP production in *Drosophila* zero prior to the start of an infection experiment?

Probably it is not exactly zero, but it is very low relative to the amount required to control an infection, so it is harmless to assume that it is exactly zero. In the analysis of the extended model in the ESM, we discuss what can happen if AMP production is possibly non-negligible prior to infection. We know experimentally that immune priming, producing high AMP production before infection, will result in hosts being completely successful in fighting off infection.

Another implication of the model is that, at a high enough dose, there will be no possibility of seeing bistability. Is that also confirmed experimentally? That is, if you give Drosophila a high enough dose of Providentia, do you only see rapid proliferation and host death?

Thank you for this suggestion. This is indeed a model prediction for which we already have experimental confirmation: host mortality risk exhibits a positive dose-response relationship with initial pathogen load, as the model predicts (Duneau et al. 2017, Figure 2 figure supplement 1).

The discussion on lines 170-186 are so elegant. It was lovely to see how you could use the approximation to gain biological insight into the conditions required for bistability. Thank you!

I found Fig. 2 to be a bit busy. I was wondering whether it was necessary to plot the unstable manifold. It's not discussed, and its inclusion kind of clutters the figure. Done - excellent suggestion!

The goal of Fig. 3 was to try to approximate the dynamics observed experimentally, and while I liked the phenomenological way that you incorporated variability in immune induction, it's worth noting that it's not quite the same as model (2). That is, to what extent is another way to think about what you're doing in creating Fig. 3 is actually generating a 100 different parameter sets? In other words, rather than simulating 100 trajectories with a fixed phase-plane diagram, what this analysis basically does is simulate 100 different trajectories across 100 slightly different phase-plane diagrams. How would the results differ if you had instead drawn 100 different initial parasite doses and simulating the dynamics?

There would be no difference in the results. Those simulation experiments are equivalent to drawing 100 different pathogen levels at the moment when the immune response ramps up, because the effect of varying the time delay for up-regulation is to vary how much time the pathogen has for growth before the immune system kicks in. We have added an explanation of this equivalence to the text.

Presumably, even in a tightly controlled experiment, it's hard to give individuals the *exact* same dose of parasites, so this would be a potentially important source of variation in infection outcome.

It not exactly the same dose, but it's pretty close. In measurements of bacterial load right after the initial dosing, the coefficient of variation is about 20%, and that includes variation due to measurement error so the variation in actual initial dose is probably smaller. A typical doubling time for the bacteria in the initial growth phase of those experiments is one to two hours, so a 20% change in abundance happens in around 15 to 30 minutes. So yes, there is some variation in initial dose. But the big difference in our experiments is that 4 hours after the initial dose some flies have their immune response "up and running" and others lag behind, so our simulation experiments focus on variation in the time delay for immune up-regulation.

In Fig. 4, I was curious what the relative abundance of N versus P bacteria is in the two bimodal outcomes. Later (line 313), you say that the chronic, low bacterial abundance infections are dominated by P, but it would be interesting to see this in Fig. 4 to foreshadow that point. Perhaps by shading the points somehow?

Excellent idea, done!

Minor grammatical stuff:

Line 30: should be “sustain” rather than “sustaining” Done, thanks!