Comments from *Ecology Letters* referees

Referee: 1  
  
Comments for the Authors  
The study investigates the role of host immune diversity on disease emergence using a Pseudomonas-phage system. The authors find that host diversity limits disease emergence. This work is a valuable addition to the existing literature on evolutionary experiments investigating the impact of host diversity on disease emergence using bacteriophage. I can intuitively agree with the authors speculation that both ecological and evolutionary forces are at play in this experiment. However, without explicit knowledge of ecological dynamics, genetic architecture and mutational schedule of the escape mutant, it is difficult to directly disentangle the ecological impact of host diversity on disease emergence from an evolutionary one. While acknowledging that this might be beyond the intended scope of this work, I believe adapting a previously developed evolutionary epidemiology model (e.g. Ohtsuki & Sasaki 2006) to fit to the data presented in this work might allow for causal inference. In its current form, it is difficult to see conceptual novelty of this work over previous studies (e.g. van Houte et al., 2016, Chabas et al. 2018), as the authors main conclusion that host diversity limits disease emergence has been echoed elsewhere. I also recommend that more attention should be paid to writing as there are numerous hidden assumptions in the Introduction that make the section a tough read. I list minor comments below.  
  
Minor comments:  
In places, the authors talk about the concept of evolution before ecology (i.e., epidemiology): e.g. L4 & L45. I find this ordering a little confusing as it is in the wrong chronological order and ecology is a shorter timescale process (i.e., made explicit in the adaptive dynamics framework of evolution) and the authors themselves write “We therefore set out to explicitly test the epidemiological role of host diversity and its knock-on evolutionary effects” : L97).   
  
Genetic diversity in this experiment is extremely one-sided (i.e., no diversity among pathogens initially), in the opposite direction from how we might expect most natural infections to be (e.g., parasites typically have larger population size and hence more diverse). Therefore, the relevance of this one-sided assumption needs to be more carefully motivated in the Introduction (e.g. by framing the topic in the context of novel pathogen emergence).   
  
Many theoretical works on the topic of host diversity and parasite emergence make explicit assumptions about the genetic architecture of infection – e.g. gene-for-gene (Sasaki 2000, Ohtsuki & Sasaki 2006) and matching allele (Lively 2010) – and the impact of host diversity depends on infection genetics (e.g. compare Lively 2010 with Springbett et al. 2003 Genetics). Can the authors speak to the genetic architecture of the system studied?   
  
Line-specific comments:  
L45-49: The authors write “…host diversity may limit the evolutionary emergence of novel pathogen genotypes, potentially because the increased prevalence of susceptible hosts in less diverse populations can increase the supply of novel pathogen mutations”. I struggle to follow the logic of this sentence. Can the authors explain this more gently?  
  
L50-51: The authors write “Further theory and experimental work have suggested that this evolutionary effect of diversity may peak at intermediate levels of host diversity”. As far as I can work out, there is no explanation for this intermediate level effect.   
  
L87-88: The authors write “CRISPR diversity can provide increased resistance by limiting the ability of phage to acquire the mutations needed to overcome CRISPR”. It is not clear what the authors mean by “ ability of phage to acquire the mutations”. I’m not able to work out if this “acquisition” is an individual-level process or a population level one? If the former is true, I’m not familiar with the mechanism involve. If the latter is true, I would suggest rephrasing.  
  
L95-96: The authors write “Moreover, this in turn is predicted to reduce the ability of the phage to evolve to overcome other CRISPR resistance alleles in the population.” I’m guessing this is because of the reduced population size, but the readers would benefit from the reason stated explicitly.   
  
  
Referee: 2  
  
Comments for the Authors  
In this manuscript the authors investigated how host genetic diversity impacts host and pathogen fitness.  They did using a bacteria phage system in which bacteria genetic diversity was manipulated at the CRISRP locus and infectious phage strains were selected through experimental evolution.  In general the manuscript is well written and addresses a pertinent research topic.  The investigators have clearly taken advantage of the malleable nature of their experimental system.  However, there were many instances where the conclusions reached did not appear to be adequately supported by the results presented.  This could be partly remedied by the investigators providing more detail of their results, which they clearly worked hard to generate.  Specific comments are as follows.  
1)      The introduction was well written and provides a nice overview of research demonstrating the impact of host diversity on pathogen and host fitness.  However, there seems to be an omission of studies demonstrating no relationship between host diversity and resistance, or the opposite effect of diversity actually increasing susceptibility.  There are numerous such studies, and it would be worth acknowledging them to adequately represent the breadth of findings on this topic.  
2)      The authors have done an excellent job describing the molecular methods used to generate the bacterial and virus clones used in this study.  However, the description of the experiment conducted seemed to lack many crucial elements, which made them difficult to interpret.  A suggestion might be to provide statements of what question or hypothesis each experiment was directed towards testing.  
3)      I have some concerns about the use of bacterial and viral strains that had previously undergone experimental evolution using those same bacteria and virus strains.  One might expect that would introduce certain biases on the data.  For example, by adapting a virus to a specific host, you might already be limiting its potential for future evolution.  The authors may want to consider discussing these limitation or biases.  
4)      The statistics section would benefit from additional details.  For example, what were the response and predictor variables in the models?  Somewhere it would also be useful to provide the minimal models that were ultimately selected and their respective coefficients, perhaps as supplemental information.  
5)      In many cases fitness was calculated using a ratio.  The limitation to this method is that one does not know if differences are due to changes in the denominator or numerator.  For example it could be because the amount of strain A went up or strain B went down.  The authors might consider just comparing raw quantities instead.  
6)      Many of the figure legends lacked sufficient detail to allow for interpretation. For example, the various treatments in the figures were not identified.  This was particularly problematic for figure 2.  
7)      Line 303:  It is unclear what data the authors are using to reach this conclusion  
8)      Line 308-310: Again, it is unclear what data the authors are using to reach this conclusion  
9)      Much of the material on lines 331-348 seem more property presented in the discussion.  
10)     The authors have used the term selection when referring to the performance of various bacterial clones.  Would the term fitness be better?  Selection seems to imply some sort of evolutionary response, which was not examined in the bacteria.  
11)     Line 367: It is surprising that bacteria selection did not differ between ancestral and pre-evolved phage.  Does this imply that bacteria were resistant to pre-evolved phage?  
12)      Line 392:  The conclusion seems over generalized given that the effect was only true when comparing 3 vs. 1 clone treatment.  
13)     Line 350 and 409:  This conclusion also seems over generalized given that evolution selection peaked at a diversity of 6 bacterial clones then decreased thereafter.  
  
  
  
Referee: 3  
  
This work presents a meticulous empirical study that manipulated host and pathogen genetic diversity in a phage-bacteria system. The manuscript is clearly written and the complete factorial design manipulating diversity is quite impressive. However, the scope is somewhat narrower than what I would expect for this journal. While I found the evolutionary context and results to be quite convincing and grounded in theory, I am concerned about the treatment of the ecological (referred to as ‘epidemiological’) results and framework. I have two main concerns.

First, the context for the study is set up as the importance of within-species variation, or genetic diversity, in the evolutionary and ecological dynamics of infectious disease. Specifically, the authors seek to elucidate the ecological and evolutionary mechanisms by which more genetically diverse populations have lower parasite prevalence and slower spread of disease. The prior evidence for effects of genetic variation on disease prevalence is quite strong, from both theoretical and empirical studies. However, the introduction text currently mixes species-level diversity and population-level (or within-species) diversity almost interchangeably, in both the general statements and the use of references. For example, the dilution effect (line 60)is an effect of host species diversity, and all of the studies cited on the dilution effect (e.g. Ostfeld & Keesing, Civitello et al) exclusively discuss this mechanism in terms of species diversity. While in some cases the same models can be used and similar conclusions drawn for different host types as for different host species (e.g. Gandon 2004), in other cases the underlying assumptions and mechanisms really do depend on which of those contexts are being considered. For example, in much of the dilution effect literature, the primary underlying mechanism is differences in transmission rates between hosts of different species compared to within-species transmission. It is possible that the phage-bacteria system used in this study is truly more of an equivalent to a multi-species system, given that bacteria are reproducing clonally and thus might be treated as separate populations. If this is the assumption being made here, this would need to be discussed explicitly, and justified. In addition, the differences between these two types of diversity should be clarified in the mention of prior work both in the introduction and the discussion.

Second, the population dynamics shown in Figure 1 suggest that in most cases except the lowest diversity treatment, the pathogen fails to invade the population. In other words, the phage population decreases linearly from its starting population of 106, without ever spreading in the host population. This concerns me with regards to the conclusions that are being drawn about effects of genetic diversity on the ecological or epidemiological dynamics. This is also a place where additional analyses, perhaps fitting a simple mechanistic SIR-type model to the data, could greatly add to the generalizability of the results.

Minor comments:

Materials and Methods: I suggest moving the first paragraph of results (lines 280-297) to the beginning of the methods section, and greatly condensing the rest of this section. Many of these details can be moved to the supplement, including most/all of the following sections: Library of BIMs and escape phages (lines 120-146), Generating labelled BIMs (lines 148-193; lines 195-204 I would suggest keeping in the main text), Co-culture experiment (can be condensed and some details moved).Perhaps a modified version of Figure S1 that includes number of replicates for the various diversity treatments would be helpful in clarifying the complex experimental design structure.

Lines 264-266:Can take out sentence on packages used for data cleaning.

Line 301 (and other lines reporting model fitting for GLMMs): I am confused by the treatment of time in these models. The text states that “Phage densities decreased more rapidly as CRISPR diversity increased” but based on Figure S2 it appears that Time and Diversity were separate fixed effects in the model, with no interaction effect. Thus the model shows an effect of time and an effect of diversity on phage populations, but no effect of diversity on the rate at which phage density changes over time. It also seems that Time in treated as a categorical variable, which further confuses the analysis. I would suggest a different analysis here, either including Time as a continuous variable and testing for an interaction with diversity, or fitting slopes to each replicate phage density over the three days post-infection and using those slopes as the response variable to look for an effect of diversity. This would be a clearer and more appropriate analysis, and I have no doubt that the clear patterns shown in Figure S2 would hold up (I found Figure S2 very helpful and perhaps it should be moved to the main text).

Line 301 (and other lines reporting model fitting for GLMMs): Also, although Likelihood Ratio values are compared to a X2distributionto produce p-values, I have typically seen these statistics reported as LR=15.7 rather than X2=15.7, to avoid confusion with a X2 test.

Figure 1 and 2: More informative labels and caption would help to convey results to a general ecological audience. For example, “Phage density (Pfu mL-1)”rather than just Pfu, specifying “Days post infection” instead of dpi, etc

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Editor  
Editors Comments for the Author(s):  
In common with the reviewers, I very much enjoyed this work; which is also clearly and crisply presented, but I think the conceptual scope is too narrow for Ecology Letters.