Peer Community In Ecology

Are all the roads leading to Rome?

Simon Blanchet *based on peer reviews by* ***Nadia Aubin-Horth*** *and 1 anonymous reviewer*

Nathalie Charbonnel, Maxime Galan, Caroline Tatard, Anne Loiseau, Christophe Diagne, Ambroise Dalecky, Hugues Parrinello, Stephanie Rialle, Dany Severac and Carine Brouat (2019) Differential immune gene expression associated with contemporary range expansion of two invasive rodents in Senegal. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Ecology. 10.1101/442160

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Identifying the factors which favour the establishment and spread of non-native species in novel environments is one of the keys to predict - and hence prevent or control - biological invasions. This includes biological factors (i.e. factors associated with the invasive species themselves), and one of the prevailing hypotheses is that some species traits may explain their impressive success to establish and spread in novel environments [1]. In animals, most research studies have focused on traits associated with fecundity, age at maturity, level of affiliation to humans or dispersal ability for instance. The “composite picture” of the perfect (i.e. successful) invader that has gradually emerged is a small-bodied animal strongly affiliated to human activities with high fecundity, high dispersal ability and a super high level of plasticity. Of course, the story is not that simple, and actually a perfect invader sometimes – if not often- takes another form... Carrying on to identify what makes a species a successful invader or not is hence still an important research axis with major implications. In this manuscript, Charbonnel and collaborators [2] provide an interesting opportunity to gain novel insights into our understanding of (the) traits underlying invasion success. They nicely combine the power of Next-Generation Sequencing (NGS) with a clever comparative approach of two closely-related invasive rodents (the house mouse *Mus musculus* and the black rat *Rattus rattus*) in a common environment. They use this experimental design to test the appealing hypothesis that pathogens may be actors of the story, and may indirectly explain why some non-native species are so successful in invading novel habitats. It is generally assumed that the community of pathogens encountered by non-native species in novel environments is different from that of their native area. On the one hand (the enemy-release hypothesis), it can be hypothesized that non-native species, when they arrive into a novel environment, will be relaxed from the pressure imposed by their native pathogens because local pathogens are not adapted (and hence do not infect) to this novel host. Because immune defence against pathogens is highly costly, non-native species establishing into a novel environment could hence reallocate these costs to other functions such as fecundity or dispersal apparatus. This scenario

has been termed the "evolution of increased competitive ability" (EICA) hypothesis [3]. On the other hand (the EICA-refined hypothesis [4]), one can assume that invaders will encounter new pathogens in newly established areas, and will allocate energy toward cost-effective immune pathways to permit allocating a non-negligible amount of energy toward other functions. Finally, a last hypothesis (the "immune protection" hypothesis) assumes major changes in pathogen composition between native and invaded areas, which should lead to an overall increase in immune investment by the native species to successfully invade novel environments [4]. This last hypothesis suggests that only non-native species being able to take up the associated costs of immunity will be successful invaders. The role of immunity in invasion success has yet been poorly investigated, mainly because of the difficulty to simultaneously analyse multiple immune pathways [4]. Charbonnel and collaborators [2] overpass this difficulty by screening all genes expressed (using a whole RNA sequencing approach) in an immune tissue: the spleen. They do so along the invasion routes of two sympatric invasive rodents in Africa and compare anciently and newly invaded areas (respectively). For one of the two species (the house mouse), they found a high number of immune-related genes to be up-regulated in newly invaded areas compared to anciently invaded areas. All categories of immune pathways (costly and cost-effective) were up-regulated, suggesting an overall increase in immune investment in the mouse, which corroborates the "immune protection" hypothesis. For the black rat, patterns of gene expression were somewhat different, with much less pronounced differentiation in gene expression between newly and anciently invaded areas. Among the few differentiated genes, a few were associated to immune responses and some of theses genes were even down-regulated in the newly invaded areas. This pattern may actually corroborate the EICA hypothesis, although it could alternatively suggest that stochastic processes (drift) associated to recent decrease in population size (which is expected during a colonisation event) are more important than selection imposed by pathogens in shaping patterns of immune gene expression. Overall, this study [2] suggests (i) that immune-related traits are important in predicting invasion success and (ii) that two successful species with a similar invasion history and living in similar environments can use different life-history strategies to reach the same success. This later finding is particularly relevant and intriguing as it suggests that the traits and strategies deployed by species to colonise new habitats might actually be idiosyncratic, and that, if general trends actually emerge in regards of traits predicting the success of invaders, the devil might actually be into the details. Comparative studies are extremely important to identify the general rules and the specificities sustaining actual patterns, but these approaches are yet poorly used in biological invasions (at least empirically). The work presented by Charbonnel and colleagues [2] calls for future comparative studies performed at multiple spatial scales (native vs. non-native areas, anciently vs. recently invaded areas), multiple taxonomic resolutions and across multiple traits (to search for trade-offs), so that the success of invasive species can be properly understood and predicted.

References:

[1] Jeschke, J. M., & Strayer, D. L. (2006). Determinants of vertebrate invasion success in Europe and North America. Global Change Biology, 12(9), 1608-1619. doi: [10.1111/j.1365-2486.2006.01213.x](https://dx.doi.org/ 10.1111/j.1365-2486.2006.01213.x)

[2] Blossey, B., & Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology, 83(5), 887-889. doi: [10.2307/2261425](https://dx.doi.org/ 10.2307/2261425)

[3] Charbonnel, N., Galan, M., Tatard, C., Loiseau, A., Diagne, C. A., Dalecky, A., Parrinello, H., Rialle, S., Severac, D., & Brouat, C. (2019). Differential immune gene expression associated with contemporary range expansion of two invasive rodents in Senegal. bioRxiv, 442160, ver. 5 peer-reviewed and recommended by PCI Ecology. doi: [10.1101/442160](https://dx.doi.org/ 10.1101/442160)

[4] Lee, K. A., & Klasing, K. C. (2004). A role for immunology in invasion biology. Trends in Ecology & Evolution, 19(10), 523-529. doi: [10.1016/j.tree.2004.07.012](https://dx.doi.org/ 10.1016/j.tree.2004.07.012)

Reviews

Evaluation round #2

DOI or URL of the preprint: **10.1101/442160**
Version of the preprint: 3

Authors' reply, 13 February 2019

We thank Simon Blanchet for his last recommendations. We have considered and clarified the main point addressed concerning the description of the patterns expected under the EICA hypothesis. In this study, enemy release (helminths) occurs between anciently and recently invaded sites, so that we can apply the EICA predictions (decrease of immune responses) to our study.

Download tracked changes file

Decision by Simon Blanchet, posted 12 February 2019

Revisions required for preprint "Differential immune gene expression associated with contemporary range expansion of two invasive rodents in Senegal"

Dear Authors,

I have now read the new version of the MS and your replies to the referees' comments. You've done a very good job and all comments were very well accounted for. My opinion is that there is no need to send the MS back to the referees. Nonetheless, I have attached an edited copy of the MS in which I have provided some change from place to place; these changes are suggestions, so feel free to accept them or not. Moreover, I still have a series of comments (comments 3 to 5) that are all related to the hypotheses being tested and their expectations. More specifically, I still have a problem with expectations regarding the EICA hypothesis. You mention in the Introduction that the EICA hypothesis is related to the enemy release hypothesis and that -because of that- the energy allocated to immunity should be released (an I agree). According to this statement, I would expect that the expression of immune genes is not different between newly and anciently invaded sites as both types of sites are in the non-native area and should be both "enemy free". On the contrary you mention p 5-6 that according to this hypothesis you expect an overall higher immune gene expression in recently invaded sites than in anciently invaded sites. I think that I disagree with this expectation, and I also have the feeling that is is in contradiction with what you said in the previous pages and in pages 12 (see comment 5). If I'm correct I think you should clarify this issue.

Once done, I'll be happy to recommend this very nice piece of work.

Best regards

Simon Blanchet **Download recommender's annotations**

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/442160
Version of the preprint: 2

Authors' reply, 05 February 2019

Download author's reply
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Decision by Simon Blanchet, posted 27 November 2018

revision needed

Dear authors,

Two reviewers have now read you contribution to PCI and have raised some comments. The two reviewers are specialists in the fields of invasion biology and genomics, and they both appreciated your manuscript. They especially like the way genome-wide patterns of gene expression were used to test clear and specific hypotheses related to invasion success in animals. They also highlight the facts that the studies was replicated both spatially and taxonomically, which is highly original and strong.

As you will see, they raised several comments, and my feeling is that all these comments should improve the general value of this manuscript. I therefore encourage you to update a new version of your manuscript that would take into account the comments both reviewers have made.

As you will see I have also uploaded an annotated pdf file with some comments I had.

Best regards

Simon Blanchet **Download recommender's annotations**

Reviewed by Nadia Aubin-Horth, 12 November 2018

Review

"Differential immune gene expression associated with contemporary range expansion of two invasive rodents in Senegal"

Peer Community in Ecology

November 2018

This ms by Charbonnel and colleagues aims to test predictions associated with the EICA hypothesis (« evolution of increased competittive ability ») in two invasive species. They use transcriptomes from spleen of individuals for populations with different history of invasion (recent versus ancient) and four replicate populations within each invasion history type to find if certain biological functions are differentially activated between recent and ancient populations.

I think that this ms is interesting as the RNA-seq data is used to test an evolutionary model. It is also interesting that the dataset seems to support the opposite of the predicted pattern. The ms is also well written and most analyses are useful and appropriate. However, I have comments on the manuscript that woul dneed to be adressed to streamline it and make it clearer to more generalist readers.

1-The EICA hypothesis is central to the ms but its predictions are not spelled out clearly for non specialists.

The authors should explain in greater details what is proposed by the EICA hypothesis. This could be done on page 3 in the introduction. Specifically, it is not clear what increased competitive ability means, since "success" (or fitness) is not measured in the individuals studied. On page 5, the authors state: "We investigated two alternative hypotheses. On one hand, we expected an overall higher immune gene expression of rodent populations in recently invaded sites, as a response to novel parasite pressures encountered 31 »

This is clear « On the other hand, we expected trade-offs between immune pathways in recently invaded sites under the EICA-refined hypothesis. «

Trade-offs between immune pathways and …what? With other traits (which ones?) . If it is a trade off with other traits such as life history traits that is expected, shouldn't these life history traits be measured also? Or were the authors expecting to find that trade offs are visible at the gene expression levels, as in Aubin-Horth et al. 2005 (Proc Roy Soc B) who did this in an unrelated study and another tissue in fish? This is especially important for the EICA-refined hypothesis

Or is it « between immune pathways » as in « different immune strategies? « if yes, explain what those different immune pathways are for non specialists. If you expect that different immune strategies have different costs, make a table describing each one with predictions of what is expected

This topic comes back on page 12 in the discussion.

"we did not find any evidence of immunity or particular immune pathways being dampened at the expense of other life history traits or of less energetically costly immune defences. «

Which other life history traits did the authors measure? Not clear.

I understand that the authors found the opposite, with higher immunity expression instead of lower, but still, how could they validate their original prediction with the data they had collected?

On page 10, the authors talk about their predictions "Contrary to what was expected, along the mouse invasion route, all immune-related genes detected were over-expressed in recently invaded sites, and among them, inflammation and complement pathways were over-represented. «

This should be clearer in the introduction

Minor comments INTRODUCTION p.3 "From an eco-evolutionary perspective, invasion success may rely on pre-adaptation within the original range 5,6 or on the rapid evolution of phenotypic traits that would be advantageous in newly colonized areas 5,7. Some supports for this latter process come from the identification of phenotypic variation along invasion gradients, «

I think that phenotypic gradients could be found even if it is based on standing genetic variation, if the alleles are at low frequency in the original population, such that it is difficult to sample the resulting phenotype and / or the alleles that result in the new phenotype are rarely found in the same individuals, but the smaller effective population size of the invading front could result in the « encounter » of these alleles by chance.

RESULTS Even if this information is given in table 1, it would be best to orient the reader by starting the paragraph with 1-tissue studied (spleen) 2-that there are two invasion history (it is really well explained on page 15, maybe pu tat beginning of results?) 3-that there are 4 sites within each invasion history 4-that POOLS of individuals are used. This is important when we try to understand the analysis strategy presented later with the 4vs4 and 8 vs8

The analysis is very complicated with the different ways and different levels of stringency. Could it be possible to only present one?

p.9 the authors use the DE genes in mouse to study protein protein interactions. They focus on the genes related to immunity (using GO terms) and find that they interact within a cluster. Is that a trivial finding / unsurprising? Are there examples of proteins related to the same biological function that have no interaction? What does it tell us more than what we already knew?

TABLE

Table 1 Add a colum on the left with « invasion time » instead of using superscript for each population name

FIGURES FIGURE 1 can be supplementary

Figure 2 should be removed, it is not of enough quality for a public document

Figure 3 I really liked how this figure showed us that even tough there are trends at the average level between invasion histories, there are specific populations that have their own expression profiles for these genes. That is very interesting and will probably warrant more attention in the future.

DISCUSSION p.10 « our results suggested that variations of immune phenotypes were a less important strategy for R. rattus invasion success «

I don't see where in the ms is invasion success quantified? Could it be that rats are actually not as successful than if they had modulated their immune system? Or that they would be even less successful if they did, since most of the immune activity is up-regulation anyway (which suggest that the invasive mouse is fighting new pathogens)?

p.11 « Although mouse and rat populations experienced reduced genetic diversity due to founder events, they may have developed adaptive responses to novel selection pressures through high levels of plasticity «

Are the authors suggesting that there is genetic accomodation? Please explain in more detail and propose what it means for invasive species in general. Also, this study is a correlation study, and we do not have relationship between the gene expression phenotype and fitness, such that wording should be modulated accordingly.

p.12 « The up-regulation of all immune genes found to be differentially expressed in sites recently invaded by

the house mouse strongly supported the assumption of an increased overall infection risk in recently invaded sites. « How do we know this? Please add relevant references

Reviewed by anonymous reviewer 1, 22 November 2018

In the manuscript entitled: « Differential immune gene expression associated with contemporary range expansion of two invasive rodents in Senegal » the authors assess the gene expression patterns between anciently and recently established populations of two rodent species in Senegal. They hypothesized that invasion success may relies on immune phenotypic traits that would be advantageous in recently invaded sites. The authors indeed showed that the species Mus musculus domesticus showed an over-expression of immune related genes (notably the complement activation pathway), in recently invaded sites compared to anciently invaded sites and likely related to novel parasite pressures encountered in recently invaded sites. Regarding the species Rattus rattus the authors suggest that some stochastic events may be associated with colonization history since no particular pattern of differential gene expression related to immunity were found.

First, I would like to congratulate the authors for this very interesting work. I found the objectives clear and concise notably with a well written introduction. The analyses are various with a good use of the replicates and seem robust with a deep investigation regarding the biological processes involved. I nevertheless think that the manuscript could be improved notably regarding the structure (see comments below).

Major comments:

1) Even if I found the objectives very clear, I think that the manuscript could still explicit some information earlier in order to gain in clarity. Notably, the whole study focuses on one tissue (the spleen) to analyze gene expression patterns and this information is given a bit late (in the discussion section). However, since the spleen is an immune related tissue I think that this could be explicitly mentioned in the introduction and related to the mains hypothesis investigated, since studying other tissue would test other hypothesis (such as using brain to study behavioral related genes as mentioned page 14).

2) Two approaches are used to test for differentially expressed genes (4x4 and 8x8), however, the subsequent functional analysis mainly focus on the genes identified with the 8x8 approach. I think that the 4x4 approach could be better justified regarding the main questions addressed or better linked to the others results. For instance, are some of the differentially expressed genes of the 4x4 approach related to immune processes?

3) Discussion is a bit redundant (the mention of stochastics events involved in the rat invasion history for instance are discussed at the end of the first paragraph, in the second paragraph and in the fifth paragraph) and could beneficiate from a better structure, notably by addressing the question mentioned in the end of the introduction more directly. For instance in the introduction addressing whether the EICA or the EICA refined hypothesis are supported comes as the last question but is discussed quite early in the discussion (before the functional categories involved that is the second question presented in introduction).

Minor comments

4) Page 6, the number of transcriptome libraries is given, but I think that giving at this stage the number of studied sites and replicates would also ease the comprehension of the subsequent analysis.

5) Page 18, it is mentioned that genes with less than 20 reads or 40 reads were discarded. I suppose that it echoes the “10 occurrences” filter mentioned page 7. If so, I suggest to homogenize and explicit this in order to avoid confusion.

6) Page 6, the authors mentioned that they used a PCA on standardized read counts. I may be wrong, but I think that a multidimensional scaling analysis (MDS) would fit better over dispersed count data (see Bankers et al. 2017, fig. 3 for some example on transcriptomic data).

Bankers, L. et al. 2017. Genomic evidence for population-specific responses to co-evolving parasites in a New Zealand freshwater snail. - Mol. Ecol. 26: 3663–3675.

7) Fig. S1, the PCA is difficult to read, take into consideration to increase front size for instance.

8) Fig. S3 and S4, check carefully the supplementary there is some mismatch between the figure caption for Fig. S3 and S4 and the actual figures displayed.

Typography comments:

- A coma just be added just after “e.g.” and “i.e.”.
- Page 13, remove the double “x” in exhibit.