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Fragmentation mediates thermal habitat choice in ciliate microcosms

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Review timeline

Original submission:	9 January 2019
1st revised submission:	24 July 2019
2nd revised submission:	3 December 2019
3rd revised submission:	7 January 2020
Final acceptance:	7 January 2020

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

Review History

RSPB-2019-0057.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?
Poor

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.
Yes

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?
No

Is it clear?
N/A

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this study the authors test whether an experimental elongation of the corridors linking a central release patch with two peripheral patches differing in temperature, affect the total proportion of individuals (a unicellular ciliate) arriving in any of the peripheral sites, and the relative proportions between peripheral sites.
I am very sympathetic to the argument that fragmentation not only should hamper dispersal, as is well-known, but also the ability to select patches. This is an aspect of fragmentation that has been little explored, so an experimental test of this is very relevant. However, with the design as presented and the information available to the reader, I do not think that the experiments and analyses as performed can actually reliably address these topics.
As I see it, there are several problems:
- The dispersal and immigration rates are inadequately defined, and this creates problems with analysis and interpretation (see further below)
- The corridors do not only differ in length, but likely also in temperature and maybe even nutrients gradients
- We don´t know how fast these organisms move, and how much informaton they might be able to acquire and handle. Do they really make a decision on whether to disperse or not given the distance they need to transverse through unsuitable matrix habitat? How do they know how great this is, before initiating dispersal? Or can they decide to return when in transit? Which temperature gradients could they detect? Etc.
- All genotypes seem to prefer the warmer temperatures, which is surprising but might instead be an artifact of other temperature-related processes, like movement or reproduction
Hence, it is not clear to what extent this experiment really sets out to do what it is said to do. It is possible that the data is actually useable, but more background information and re-analyses are necessary for this.
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L 144: so dispersal rate is defined as the proportion that has arrived at another patch, not the proportion that has left the central patch (which you can also calculate if you measured central density as well, or is there much reproduction during your trials?). So, even if the same (or even a greater) proportion of individuals left the central patch, more individuals will be in transit at the end of a trial if the connecting tube is longer, and that reduces the value of dispersal rate the way you define it. You predicted: "... that increased inter-patch distance should reduce the tendency of individuals to engage in costly dispersal movements". This feels like a plastic decision of the individuals, but the way you test it, it is more the result of a physical process. Brownian diffusion of inert particles would likely yield the same result.

L 159: this isn´t correct. Random factors are used to take into account multiple measures of the same entity (like repeated measures, which are positively correlated), but the way you defined immigration rates, they always sum to 1 so they are actually negatively correlated. As a consequence, there cannot possibly be an effect of fragmentation on average immigration rate, since the average is always 0.5 (this is nicely shown in Fig. 2.). I think you should just analyse a single immigration rate, and test for the interactions involving fragmentation treatment, and then interpret in which of the treatments the genotypes made the better decisions (based on prior knowledge about their preference of performance, which you presumably have).

L 161: why not just run the full model, and test all factors separately for their significance, so we have all results? For example, excluded and unreported variables cannot be used for meta-analyses. I am also not a fan of binary significance testing according to an arbitrary significance criterion, and therefore would prefer to see all p-values (a p-value of 0.051 tells me that something is going on with a probability only slightly lower than p=0.049 – p values were designed to allow continuous interpretation of probability!).

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L 192: based on Fig. 2, it seems like the proportions are more different between temperatures when the tubes are shorter. This might look like a greater ability to choose habitat, but all genotypes arrived in a greater proportion in the warmer habitat. To what extent isn´t this also an artifact of greater movement at higher temperatures (in the matrix) by an ectothermic organism? Or greater reproduction rate? Did you expect all genotypes to prefer warm temperatures, based on their performance or preference in previous experiments? If so, why didn´t you select some genotypes which would have preferred 27 degrees to see if they could choose better with less fragmentation? If not, then how do you explain these results?

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L 407: they way you measured dispersal, you don´t have a binary classification between stay or disperse, but three categories: stay, transit, arrival. Moreover, it is not clear why this is a decision, if individuals have no way of knowing how long a tube is? Unless they decide to go back while in transit, but is that realistic?

Review form: Reviewer 2

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?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
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Comments to the Author

This paper reports the results of an experiment in the lab, using simple metapopulation microcosms and the ciliate Tetrahymena, to test the idea that increasing distance between patches in a fragmented landscape constrains both dispersal and habitat choice. The introduction does a very good job in giving motivations for this study and placing it in the broader context of study of the effects of habitat fragmentation on dispersal and its consequences. Artificial systems with very similar design have been previously used by the authors and others to reveal interesting complex variation in dispersal and habitat choice. Four types of 3-patches metapopulations are tested, varying the distance between microcosms and the temperature of the central patch (the temperature differs between the marginal patches but is kept constant across metapopulation). This design is replicated across 6 different genotypes, with 6 repetitions per genotype. The authors ask (i) whether emigration from the central patch decreases depending on distance between patches, (ii) for those having dispersed whether choice between warmer and colder patches is affected by distance between patches. There is no clear expectation about how the central patch temperature should affect this. Given past results on this system (and other ciliates) showing a lot of variation between genotypes in their dispersal behavior and plasticity, replicating the experiment for different genotypes seems a relatively standard test of generality and robustness of conclusions.

The analysis of the data shows that emigration indeed decreases with distance between patches as expected, but only in two genotypes out of 6, the rest showing no response to distance. As found before, emigration also varies across genotypes and temperature with complex interactions between the two, but no significant interaction between the effect of temperature and fragmentation. The effect of fragmentation on habitat choice at immigration is more consistent across genotypes: warmer environment are preferred, but with less intensity when distance between patches are larger.

The paper is well written, easy to follow and straightforward, the topic is interesting and addressing a very relevant question in ecology and evolution, the conclusions are well discussed. Even if the system is very artificial, the experiment helps to disentangle the effect of evolution of reduced dispersal and plastic response of dispersal to habitat fragmentation, which can be difficult to tease apart in a natural field system. The most novel exciting aspect however is the evidence that fragmentation constrains habitat choice, which would be very difficult to show in most system, and also seems to be a stronger more general pattern, less contingent on specific genotypes. Despite all these positive aspects, I still have concerns, which should be addressed or clarified by the authors. Most of them concern the statistical analysis and its interpretation.

1) My first comment however is more about content. Even though the experiment is solid and well replicated, I find that the paper lacks a bit of depth. This probably reflects my taste for richer contents and longer papers exploring various aspects of the question, mechanisms and further exploration in a more complete picture. I am aware that the present journal aims for short papers and that simple message are more easily communicated, but I wondered whether this was the right amount of results for this format.

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explanation is needed here.
3) I fail to understand the structure of the statistical model for immigration rates. Given the definition of the immigration rates as a proportion of all migrants immigrating at cold or warm temperature, those proportions must sum to one within a dispersal assay (as figure 2 actually shows) and yet they seem to be treated as independent observations in the statistical model. So basically all the information is in the proportion immigrating in cold patches, temperature of the patch receiving the migrants should not be in the model (but temperature of the central patch should be). I am quite worried by the structure and degrees of freedom of this model unless I am very confused by the explanations of the model structure given by the authors; maybe the definition of local and neighbor temperature should be clarified and this is the source of my confusion.
4) Given that all the explained variables are proportions I was a bit surprised that linear or mixed linear models were used everywhere and not generalized models with a logit link for instance as is typical for proportions; could the authors explain why they did not need to do this?
5) It is a bit confusing and somehow leading to contradictory statements that authors discuss patterns per genotype even though they find no evidence for an interaction when testing globally for it. For instance for immigration rates, there is no evidence for any genotype effect or interaction with genotype: then what is the point of showing and discussing the variation of patterns among genotypes as in figure 2 if the global analysis says that none of this variation is statistically supported and something else than noise; similarly, the analysis for emigration rates concludes that the triple interaction is not significant, yet the discussion of joint effects of temperature and fragmentation as in line 174 differing across genotypes suggests an interaction worth looking at. It would make it much simpler to understand the significant interaction and ignore the non significant ones, if not only the raw data was plotted but the predictions of the best model or the model with only the significant interaction terms; this would help to see the patterns that are statistically supported and separate it from noise.
6) The title, abstract and discussion put emphasis on the fact that emigration rate decreases with fragmentation; yet it is found only in two genotypes out of 6. Therefore for the majority of genotypes fragmentation does not affect emigration. I therefore think that this conclusion is not so well supported by the present data and the emphasis could have been on the absence of an effect rather than on the minority of cases. Much more nuanced conclusion is required here.
7) Concerning the interpretation of the reduced (but not abolished) habitat discrimination with increasing distance between patches, the authors seem to favor the interpretation that exploration and prospection is made more difficult by distance (with the idea that individuals would go and prospect different sites and return if they do not like it?). Yet, alternatively, is it possible that a temperature gradient establishes in the corridor and that with long corridors this gradient is necessarily shallower and therefore information about difference in temperature between patches more difficult to get; if this is the case, is this mechanism a bit artificial meaning that the reduced habitat choice may not be seen in more natural systems? In other words is the issue here the limits to movement of individuals or the limits to diffusion of cues about habitat quality through space?
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Decision letter (RSPB-2019-0057.R0)

08-Feb-2019

Dear Miss Laurent:

I am writing to inform you that your manuscript RSPB-2019-0057 entitled "Fragmentation reduces dispersal rate and hampers habitat choice in ciliate microcosms" 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 substantial revisions are necessary. 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. 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.

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,
Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Both reviewers and I think that the MS addresses an important topic, presents an interesting experiment that produces results of wider relevance. However, all three of us expressed some doubts and criticisms that make that the MS cannot be further considered at this stage. Both reviewers comment on aspects of the statistical analysis that are unclear and possiby overinterpreted. But both referees also have questions about the justification of the setup of the experiment. My own doubts resemble very much the ones expressed by reviewer 1; in particular, I aim not so convinced that longer corridors between patches adequately reflects the notion of increased fragmentation. The reviewer remarks that at the level of the experimental organisms all sorts of gradients can result from this. To this I would add that the experiment doesn't seem to allow a distinction between individuals that turned halfway and those that made it to a neighbouring patch but decided they didn't like it there. From this perspective, Reviewer 2's

problems with the potential effect of the temperature of the central patch is very relevant in this respect too.
So in sum, I think the study is potentially interesting for the Proceedings but the combined criticisms necessitate a very thorough revision. Given that statistical analyses have to be modified it is not even certain that the main conclusions will make it through unscathed.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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- All genotypes seem to prefer the warmer temperatures, which is surprising but might instead be an artifact of other temperature-related processes, like movement or reproduction
Hence, it is not clear to what extent this experiment really sets out to do what it is said to do. It is possible that the data is actually useable, but more background information and re-analyses are necessary for this.
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Referee: 2

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Author's Response to Decision Letter for (RSPB-2019-0057.R0)

See Appendix A.

RSPB-2019-1733.R0

Review form: Reviewer 1

Recommendation
Reject – article is not of sufficient interest (we will consider a transfer to another journal)

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

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

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

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.
Yes

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?
No

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
I was also a reviewer of a previous version. Compared to that one, the ms has much improved, especially regarding the statistical analyses. The topic remains interesting, especially regarding the second of the two objectives (does fragmentation affect the ability to choose habitats)? While the authors do find this affect, it is not quite how we expect it to play out: with greater fragmentation the ciliates seem to prefer more strongly the habitats to which they are less adapted, i.e. habitat choice becomes not weaker but stronger (counter to prediction), and in a maladaptive way. While the authors provide some discussion (speculation) on why this might be expected, perhaps I did not understand it but I was not so convinced. The authors also find some genetic variation in these effects, which is interesting. This leaves me a bit conflicted about the paper. While the title is corrent, it does not reflect this unexpected result. The same is true for the abstract. This suggests to me that the authors also do not really know what to make of their results, and are unwilling to stress or even mention the full results. Hence, much remains to be understood, and so perhaps this paper isn´t for the Proceedings which often publishes papers with more final conclusions.
Even though the authors have cleared up many questions and issues in the ms and/or their cover letter, a few doubts remain here and there, and I suggest that these are also addressed in any next version that the authors may prepare.
L 44: patches, and thereby inflate
L 72: from your description, with patch selection depending on the match with phenotype, it seems you are talking about matching habitat choice? I would be good if you make clear here and in the discussion which type(s) of habitat choice your ciliates employ using the classification in Akcali & Porter 2017 Evolution, since the dynamics and predictions can be quite different.
L 76:... , in general habitat choice
L 100+129, and maybe elsewhere: corridors that were twice as long.
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L 152: dispersed out of the
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L 167: most optimal of the two thermal
L 173: is that the expected fitness of both patches? And how does habitat choice enter into a habitat choice parameter? Basically, you cannot just cite another paper here, you need to explain how you obtain this critical parameter, and why it varies between -1 and 1.
L 175: explain what the nls function is
L 180: the decision of where to settle depends on leaving the local (do you mean central?) patch. This means that settlement is a function of both donor and recipient patch. How is that incorporated in your metric?
L 181: I´m surprised you don´t fit or investigate any potential nuisance/error/confounding terms, like date, block, batch, spatial location, observer, etc. Perhaps you are sure that all replicates are completely comparable, but it would be good to see that that is indeed the case, especially since some results are hard to understand and might reflect spurious results.
L 185+195: genotype by fragmentation
L 196: I think you don´t report any effect for central temperature?
L 202: given that you talk about emigration, maybe say avoidance of the least optimal temperature?
L 208: instead of choice ability, maybe say choosiness? The ability to choose might be high even when organisms don´t choose.
L 209: omit Interestingly.
L 226: I suggest ... a behaviour that can mediate the ... consequences of fragmentation.
L 228: dispersal rate in 2 out of 6 genotypes, ...
L 231: you say indeed, but I´m not sure if you ever made this prediction explicit in the introduction. If not, do so.

I agree with that prediction. At the same time, I´m not sure if there is any a priori prediction for what would happen at emigration (given that they don´t know what the length of the tube and the quality of the patch at the other side will be?). If so, make this lack of a prediction also clear in the introduction. You also allude to this in L 254-256, suggesting it is not clear what would happen or why it happened.
L 248: I think it is three?
L 268: remove Interestingly
L 269: was this speed measured in your experiment, or inferred from previous studies?
L 301: if that is an explanation, then you could correlate your results to the movement patterns of the 6 genotypes, to test if that is indeed so.

Review form: Reviewer 3

Recommendation
Major revision is needed (please make suggestions 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?
Excellent

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

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.
Yes

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Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
See attached file for detailed comments. (See Appendix B)

Decision letter (RSPB-2019-1733.R0)

07-Oct-2019

I am writing to inform you that this version of your manuscript RSPB-2019-1733 entitled "Fragmentation mediates thermal habitat choice in ciliate microcosms" 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 substantial revisions are necessary. 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. 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.
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.

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,

Dr Sasha Dall
mailto: proceedingsb@royalsociety.org

Associate Editor Board Member
Comments to Author:
The revised MS has been seen by two reviewers, one who commented on the original MS and a new one. The first reviewer concluded that the MS has definitely improved, but not up to the point where it should be accepted by the Proceedings. This reviewer's main issue is that the MS is confused about how the findings relate to the original hypotheses. This reinforces to some extent the views expressed by the new reviewer. This reviewer likes the problem and experimental methodology, but got confused by the analysis. There is not only some confusing sloppiness in the terminology used, but also some deep issue about what are explained and explanatory variables in the study.
My personal feeling is that if the authors paid more attention to what kind of information might be gained by the experimental animals (and where!) it might help them to resolve some of these issues. But whatever the case, the MS definitely needs more work.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
I was also a reviewer of a previous version. Compared to that one, the ms has much improved, especially regarding the statistical analyses. The topic remains interesting, especially regarding the second of the two objectives (does fragmentation affect the ability to choose habitats)? While the authors do find this affect, it is not quite how we expect it to play out: with greater fragmentation the ciliates seem to prefer more strongly the habitats to which they are less adapted, i.e. habitat choice becomes not weaker but stronger (counter to prediction), and in a maladaptive way. While the authors provide some discussion (speculation) on why this might be expected, perhaps I did not understand it but I was not so convinced. The authors also find some genetic variation in these effects, which is interesting. This leaves me a bit conflicted about the paper. While the title is corrent, it does not reflect this unexpected result. The same is true for the abstract. This suggests to me that the authors also do not really know what to make of their results, and are unwilling to stress or even mention the full results. Hence, much remains to be understood, and so perhaps this paper isn´t for the Proceedings which often publishes papers with more final conclusions.
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L 301: if that is an explanation, then you could correlate your results to the movement patterns of the 6 genotypes, to test if that is indeed so.

Referee: 3

Comments to the Author(s).
See attached file for detailed comments.

Author's Response to Decision Letter for (RSPB-2019-1733.R0)

See Appendix C.

RSPB-2019-2818.R0

Review form: Reviewer 1 (Pim Edelaar)

Recommendation
Accept with minor revision (please list 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?
Good

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?
No

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
I was also a reviewer of previous versions. The ms has again much improved, this time with respect to providing better context and more moderate interpretation. The topic remains interesting, especially regarding the second of the two objectives (does fragmentation affect the ability to choose habitats)? One of the surprising observations is that habitat choice at emigration becomes not weaker but stronger (counter to prediction), and with more genotypes preferring suboptimal habitats. Unfortunately this is still not mentioned in the abstract. The discussion surrounding this observation is clearer now, and the authors correctly highlight that more research is needed. The authors also find some genetic variation in these effects, which is interesting. Even though the authors have cleared up many questions and issues in the ms and/or their cover letter, a few doubts remain here and there, and I suggest that these are also addressed in any next version that the authors may prepare.

L 99: the first prediction is about dispersal rate, so replace habitat choice at emigration for rate of emigration. If you want, the part on habitat choice at emigration could be mentioned under the second prediction. Or even better, mention that you split this prediction into two: with respect to departure, and with respect to arrival.
L 31: corridors connecting patches differing in quality, ...
L 111: chose
L 113: why was that, why not take one from the known set of 12 of previous studies?
L 188: was computed
L 209: left ... fitness f at the central patch, habitat choice ability ... Also say here in words that if the habitat choice ability is positive, it means that a smaller proportion of cells leave the central patch as fitness at the central patch is greater.
L 222: say here that this is a similar equation as for emigration, but here a positive habitat choice ability h_i means that a greater proportion of cells arrive at the arrival patch as fitness at the arrival patch is greater.
L 240: from previous review rounds it is clear that the term habitat choice as used here (for the estimated parameter h) is problematic. Habitat choice is a process, whose outcome depends on conditions (the options that can be chosen). What you describe here is a feature of the organisms, presumably or potentially a fixed genetic trait which is also present in homogeneous environments, when habitat choice is absent. You have used habitat choice ability for this parameter before, which is an option. Or maybe habitat choosiness? Or habitat selectiveness?
L 250: what do you mean with in each condition? Maybe just omit?
L 251: what are your habitat choice estimates – the same as habitat choice metrics in L 249? Use unified terminology!
L 252: not clear what tests you will do, it reads as if you do a t-test for each genotype but that is not possible (no degrees of freedom)? Omit "for each genotype"?

L 255: you do not say whether there was an overall treatment effect – also provide (and discuss) that result, in addition to the results per genotype.
L 264: this addition only confuses – omit?
L 267: don´t refer to fig. 2d here, because you only talk about emigration.
L 272: replace by "while one genotype with a preference for more optimal habitat showed increased choosiness in the fragmented treatment"?
L 278: maybe say "mostly vanished" as, D11 still shows a nearly significant habitat choice ability (p=0.06).
Results: You wrote before in Methods: "Five of these six genotypes were previously characterized for habitat choice [13,24]: all performed habitat choice at emigration, and we chose two genotypes that also performed habitat choice at immigration (D3 and D11), and 3 that did not (D4, D13 and D17)." Comparing in more detail the specific previously reported h-values of these strains from Table S2 from ref. 24, I find that this pattern is largely repeated in this study for emigration (all five again significant, and the same four strains with positive values as before and the same one negative as before). For immigration, it is also roughly repeated: the two previously significant ones are again or nearly so, and with the same sign. (One that was not now almost is). I would mention this repeatability between different experiments somewhere, because it strongly suggests that you are measuring something meaningful and robust (various previous reviews had expressed doubts about this, so this is a corroboration of your methodology). Perhaps even plot the values from the separate experiments against each other, and provide this as a supplementary figure. For me, this repeatability really increases the value of the results.
L 295: remove also, and add (L 296): two even switched from preferring the better habitat to preferring the worse habitat.
L 307: Figure 2a? and also mention table 1b. Also, I would not say random dispersers (h=0); one genotype has an h = 0.16 with p=0.06; just say less choosy (or lower ability)
L 309: a developed; the ability
L 326: contrasting this with a correlation between the results of ref. 24 and this study would strengthen this observation of a lack of correlation (i.e. it is biological signal)
Fig. S1: I think the error bars are not correct – in panel S1a habitat choice at immigration seems significant for five genotypes, and error bars are different from the ones in fig. 2c (error bar for D17 is even absent). Same for panel b, seems significant for 5 genotypes when only 1 is marginally significant in Table 1.
L 329: I count 4? And not suboptimal habitat choice, but a preference for suboptimal habitat (maybe also elsewhere in the text)
L 338: isn´t the most logical explanation just a simple probability effect? Also under random diffusion fewer individuals will reach patches that are further away. Mention that, at the very least, this effect should contribute to the observed pattern. (If that is what you meant with "reduced ability to cross corridors" then make this more specific).
L 351: as an aside, you may be able to disentangle these different forms? First, determine if (long-term) exposure to specific thermal environment predicts preferred environment: if it does not, there is no imprinting. If it does, it could be imprinting but it could also be matching habitat choice - the latter if the phenotype and hence performance has changed via plasticity. Therefore, if there is an effect of past environment on habitat preference then next do an experiment to determine if performance can be improved after exposure to a specific environment (i.e. phenotypic plasticity). If not, it was not matching habitat choice but imprinting. If so, then unfortunately you can´t tell, until you can change the phenotype and thermal performance independent of confounding effects.
By the way, can we be sure that thermal performance has not changed via plasticity in this experiment? This is important, because if it does, then the assumptions of the formulae to calculate H do not hold (i.e. the f values are not constant). Mention this in the text.
L 355: ref 61 is double. I think citing (Edelaar, P, Baños-Villalba, A, Quevedo, DP, Escudero, G, Bolnick, DI & Jordán-Andrade, A 2019. Biased movement drives local cryptic coloration on distinct urban pavements. Proceedings of the Royal Society B 286: 20191343) might be worthwhile here, as they have shown the operation (and consequences) of matching habitat choice by manipulating the phenotype, independent of genetic preferences or imprinting.

L 376: you use habitat choice and habitat preference here, while referring to the same thing – be consistent in terminology. Also, suboptimal habitat preference is not the same as preference for suboptimal habitat, and we are talking about the second here. Also here and throughout the paper, be clear and consistent in terminology.

Decision letter (RSPB-2019-2818.R0)

31-Dec-2019

Dear Miss Laurent

I am pleased to inform you that your manuscript RSPB-2019-2818 entitled "Fragmentation mediates thermal habitat choice in ciliate microcosms" has been accepted for publication in Proceedings B.

The referee has recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript. 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 us know.

To revise your manuscript, log into https://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, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. 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.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will

not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.
Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

6) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

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

Sincerely,
Dr Sasha Dall
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
The reviewer agrees the MS has much improved but still has some minor issues, in particular with respect to the interpretation of the results that are presented. These would seem easy to address. The detailed list prepared by the reviewer would be an excellent guide.

Reviewer(s)' Comments to Author:

Referee: 1
Comments to the Author(s).
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interesting, especially regarding the second of the two objectives (does fragmentation affect the ability to choose habitats)? One of the surprising observations is that habitat choice at emigration becomes not weaker but stronger (counter to prediction), and with more genotypes preferring suboptimal habitats. Unfortunately this is still not mentioned in the abstract. The discussion surrounding this observation is clearer now, and the authors correctly highlight that more research is needed. The authors also find some genetic variation in these effects, which is interesting. Even though the authors have cleared up many questions and issues in the ms and/or their cover letter, a few doubts remain here and there, and I suggest that these are also addressed in any next version that the authors may prepare.

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Decision letter (RSPB-2019-2818.R1)

07-Jan-2020

Dear Miss Laurent

I am pleased to inform you that your manuscript entitled "Fragmentation mediates thermal habitat choice in ciliate microcosms" 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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Appendix A

Dear Editor,

We are very pleased to read how positive you and the reviewers were about the importance of the topic and results presented in this manuscript, and are highly grateful for constructive and very helpful reviews.

Following the reviewers' comments, we performed significant changes in the manuscript, the most important one being the change in data analysis approach. We also deeply modified the writing of the manuscript, clarified predictions, definitions of the terms used and interpretation of results, and the title, according to reviewers' comments and modifications performed.

We believe that the suggested changes greatly improved the quality and clarity of our paper, and we provided below detailed responses to all addressed concerns and comments. Especially, the change of statistical approach performed maintains the key conclusions of the study, and now provides in our opinion deeper understanding about the adaptive (or maladaptive) value of habitat choice in the context of habitat fragmentation.

We remain at your disposal for any further information you might need, and look forward regarding the suitability of our manuscript for publication in Proceedings of the Royal Society B.

Yours sincerely

Referee: 1

In this study the authors test whether an experimental elongation of the corridors linking a central release patch with two peripheral patches differing in temperature, affect the total proportion of individuals (a unicellular ciliate) arriving in any of the peripheral sites, and the relative proportions between peripheral sites.
I am very sympathetic to the argument that fragmentation not only should hamper dispersal, as is well-known, but also the ability to select patches. This is an aspect of fragmentation that has been little explored, so an experimental test of this is very relevant. However, with the design as presented and the information available to the reader, I do not think that the experiments and analyses as performed can actually reliably address these topics.
As I see it, there are several problems:

- The dispersal and immigration rates are inadequately defined, and this creates problems with analysis and interpretation (see further below)
- The corridors do not only differ in length, but likely also in temperature and maybe even nutrients gradients
- We don´t know how fast these organisms move, and how much informaton they might be able to acquire and handle. Do they really make a decision on whether to disperse or not given the distance they need to transverse through unsuitable matrix habitat? How do they know how great this is, before initiating dispersal? Or can they decide to return when in transit? Which temperature gradients could they detect? Etc.
- All genotypes seem to prefer the warmer temperatures, which is surprising but might instead be an artifact of other temperature-related processes, like movement or reproduction

Hence, it is not clear to what extent this experiment really sets out to do what it is said to do. It is possible that the data is actually useable, but more background information and re-analyses are necessary for this.

R: We greatly thank you for a very helpful review. Following your comments below, together with those of reviewer 2, about the statistical analyses and the request to link dispersal decisions to the performance of genotypes at the tested temperatures, we chose to deeply change the way we analysed the present data. Specifically, we now follow the method used in a recent paper (Jacob et al. 2018. PNAS), consisting in quantifying habitat choice of each genotype and each corridor length treatment, as the relationship between dispersal decisions (emigration and immigration rates) and the expected fitness at each temperature (growth rate relative to the performance at thermal optimum). This metric varies between -1 and 1: h = 0 for random dispersal, h > 1 for a preference for optimal habitats and h < 0 for a preference for suboptimal habitats. Habitat choice at emigration quantifies how genotypes adjust the decision to remain in the local patch depending on local temperature (including the possibility to return after prospecting neighbouring patches), and habitat choice at immigration represents the decision of where to settle if leaving the local patch.
This method allows quantifying a meaningful estimate of habitat choice for each genotype (see Methods L. 149-180), testing whether increasing inter-patch distance affects this estimate, and especially provide important knowledge about the potential adaptive (or maladaptive) value of these dispersal decisions (see Discussion).

Detailed comments:
L 44: fragmentation-induced

R: We modified this sentence following your next comment, and removed this formulation.

L 44: reverse order with L 47 (47 leads to 44)

R: We edited these two sentences as suggested.

L 77: has

R: Corrected

L 101: engage suggests initiation, but you only measured rates of individuals that did disperse, not of individuals that started dispersal but returned?

R: You are indeed right that here we quantified effective dispersal rate and not the tendency to initiate a dispersal movement. We corrected this important point here and throughout the manuscript: "We predicted that increased inter-patch distance should increase costs of dispersal and therefore reduce effective dispersal rate" (L. 102-103). We furthermore make clear that our measures of dispersal rate and habitat choice at emigration include the possibility to return after prospecting neighbouring patches (L. 151-153 & 177-180), see also our response below to your comment about L. 144).

L 99: I expect that that will also reduce the temperature gradient in the corridor, so provides different information
L 104: so for this prediction, the reduced temperature gradient is important – the information has changed

R: The experimental design we used ensure that the temperature of patches remained independent of the corridor length treatments. Consequently, when patches become more distant, the temperature gradient in the corridors should become smoother. We now included in the manuscript a discussion about the mechanisms potentially involved in cell movement and habitat choice, tackling especially the potential importance of temperature gradient in the corridor for habitat choice ability (L. 257-294).

L 127: how fast to cells swim normally, and how fast if they want to disperse? To put the lengths of the tubes and duration of a trial into perspective.
L 204: this suggests that individuals might have moved between all three patches in the time span of six hours – it that likely, given their speed of movements?

R: The relationship between cell movement ability and dispersal is indeed a very interesting point. Based on movement ability only, we would expect population sizes in the three patches to homogenize in a couple of hours: in the present study, average net speed is of 155 µm/s, meaning that cells should be able to cross the corridors in less than 5min. However, population densities very rarely reach homogenization when corridors contain no nutrients (e.g. Fronhofer et al. 2018 Nature Ecol.Evol.; Jacob et al. under review), showing that the initiation of dispersal through the corridors is not simple diffusion, but is likely to involve an active behavioural decision. Accordingly, the usually observed correlation between cell velocity and dispersal rate (e.g. Pennekamp et al. 2014 Evolution; Fronhofer & Altermatt 2015 Nature Com.) vanishes when harsh corridors are used (i.e. no nutrients; Jacob et al. under review). These results furthermore suggest that cells are probably able to prospect patches during the timing used, as expected given the existence of habitat choice at immigration in this species (Jacob et al. 2017 Nature Ecol.Evol., 2018 PNAS, this manuscript).
We added a full paragraph in the discussion to broach this point (L. 268-294).

L 130: how long does this matrix habitat remain harsh? Diffusion would introduce nutrients into the tubes? But this would take longer for the longer tube, so the long tube might have been a harsher matrix habitat than the shorter tube, in addition to being a longer one. This changes interpretations.

R: In a previous experiment, we found that the matrix remains harsh for more than 5 hours, even while using smaller size dispersal systems with corridors twice shorter that the short length used here (Jacob et al. under review). Consequently, although we did not quantify diffusion of nutrients depending on corridor length in the present experiment, we can confidently consider that the matrix conditions remained harsh during its whole duration. We now incorporated the above information in the Methods as follows: "Corridors separating habitat patches contained no resources (i.e. filled with water), to generate a harsh matrix [53,54]. In a previous experiment, we showed that this method allows maintaining matrix harshness for more than 5 hours (Jacob at al. under review)." (L. 129-132).

L 132: local and central temperature is the same?

R: You are right, we changed "local" by "central" here to keep consistent and clear: we performed 6 replicates for each corridor length and central temperature.

L 133: what do you know about the temperature in the tubes? If the air is below 27 degrees, the tubes may well be colder than any of the patches? Which would make informed dispersal more difficult?

R: Generating spatial heterogeneity of temperature in systems of connected patches was reached using a system of dry baths in incubators that we calibrated during previous studies (Jacob et al. 2017 Nature Ecol.Evol; 2018 PNAS). We didn't measured temperature in the corridors for technical reasons, but the system ensures that it is somehow intermediate between 27 and 35°C. Indeed, this system consists in placing patches in dry baths at 27°C connected to patches in 35°C incubators (and inversely, half of the replicates for each).

L 144: so dispersal rate is defined as the proportion that has arrived at another patch, not the proportion that has left the central patch (which you can also calculate if you measured central density as well, or is there much reproduction during your trials?). So, even if the same (or even a greater) proportion of individuals left the central patch, more individuals will be in transit at the end of a trial if the connecting tube is longer, and that reduces the value of dispersal rate the way you define it. You predicted: "... that increased inter-patch distance should reduce the tendency of individuals to engage in costly dispersal movements". This feels like a plastic decision of the individuals, but the way you test it, it is more the result of a physical process. Brownian diffusion of inert particles would likely yield the same result.

R: Our measure of dispersal rate is indeed defined as the proportion of cells that arrived in target patches, meaning effective dispersal rate. The proportion of individual that left the start patch (i.e. emigration rate) is impossible to measure in our experimental systems (although we are currently working on systems specifically designed to this purpose). As pointed out above, we clarified this important distinction between initiation and effective dispersal, and how informative are the measures we performed in this study (see especially L. 151-153 & 177-180).

You suggested to estimate emigration rate from the difference between inoculated cell density and the number of cells that remained in the central patch 6 hours later. Although appealing, we believe this method would lead to an inaccurate measure of emigration rate for two reasons. First, the estimation of population density using pictures is efficient and repeatable, but the inoculation of a given cell density

in dispersal systems come with some inevitable variance. Second, this species shows a latency time before growth initiation, making population growth negligible during the 6 hours of dispersal assay (Pennekamp et al. 2014 Evolution; Jacob et al. 2018 PNAS). Taken isolated, these two points (inoculation and population growth) are of negligible concern. However, even such low population growth levels might add to the above-mentioned variance in inoculation density, making the quantification of emigration rate (sensus proportion of cells initiating a dispersal movement) inaccurate in this system. We hope to soon be able to tackle this interesting aspect of the dispersal process through the specific systems we are currently working on (see above), studying the transient phase of dispersal being a challenge on its own (e.g. Clobert et al. 2009 Ecol.Lett.).

Regarding the proposed hypothesis about the role of diffusion in dispersal in these systems, we agree that this is an important hypothesis to consider. However, we previously showed that dispersal consists in active movement and not diffusion in this model system. **Tetrahymena thermophila** ***cells, as most ciliates, are covered with cilia providing high mobility to catch food and move from one location to another. It is able to orient in its environment through for instance taxic responses (reviewed in Fenchel 1987), and has been found able to adjust dispersal decisions depending on kinship (Chaine et al. 2010 Evolution), population density (Pennekamp et al. 2014 Evolution; Jacob et al. 2016 Evolution; Jacob et al. in press Am. Nat.), resources (Jacob et al. in press Am. Nat.) and temperature (Jacob et al. 2017 Nature Ecol.Evol.; 2018 PNAS). Furthermore, residents and dispersers in these microcosms differ in their phenotype, with for instance dispersers being more elongated, moving faster and in a more linear way (Pennekamp et al. 2014 Evolution, 2018; Jacob et al. 2016 Evolution, in press Am. Nat.). Finally, we previously demonstrated that these dispersal movements involve active habitat choice, and favour local adaptation instead of hindering it as would be expected under the diffusion hypothesis (Jacob et al. 2017 Nature Ecol.Evol.). This means that the contribution of passive diffusion to dispersal is likely very small in our study. We included a discussion of this important aspect in our manuscript (L. 268-294).***

L 159: this isn´t correct. Random factors are used to take into account multiple measures of the same entity (like repeated measures, which are positively correlated), but the way you defined immigration rates, they always sum to 1 so they are actually negatively correlated. As a consequence, there cannot possibly be an effect of fragmentation on average immigration rate, since the average is always 0.5 (this is nicely shown in Fig. 2.). I think you should just analyse a single immigration rate, and test for the interactions involving fragmentation treatment, and then interpret in which of the treatments the genotypes made the better decisions (based on prior knowledge about their preference of performance, which you presumably have).
L 161: why not just run the full model, and test all factors separately for their significance, so we have all results? For example, excluded and unreported variables cannot be used for meta-analyses. I am also not a fan of binary significance testing according to an arbitrary significance criterion, and therefore would prefer to see all p-values (a p-value of 0.051 tells me that something is going on with a probability only slightly lower than p=0.049 – p values were designed to allow continuous interpretation of probability!).

R: This point has been raised by both you and the second reviewer, and we greatly thank you for pointing out this important mistake in the way we performed statistical analyses. As explained above, following these comments on the statistical approach used and the request to link dispersal and performance, we changed the way we analysed the present data to match the approach used in a recent study (Jacob et al. 2018. PNAS). Please see our response above and the associated changes in the manuscript for detailed explanation of the new method and associated results.

L 191: I don´t think you can say that “individuals reduced dispersal movements when facing ...” – fewer individuals made it to the other side, which is a different (and, as I argue above, a potentially passive) thing.

R: We removed this sentence while significantly editing the discussion of our manuscript, and now specifically discuss the potential hypotheses underlying the decrease in effective dispersal rate (L. 257-267).

L 192: based on Fig. 2, it seems like the proportions are more different between temperatures when the tubes are shorter. This might look like a greater ability to choose habitat, but all genotypes arrived in a greater proportion in the warmer habitat. To what extent isn´t this also an artifact of greater movement at higher temperatures (in the matrix) by an ectothermic organism? Or greater reproduction rate? Did you expect all genotypes to prefer warm temperatures, based on their performance or preference in previous experiments? If so, why didn´t you select some genotypes which would have preferred 27 degrees to see if they could choose better with less fragmentation? If not, then how do you explain these results?

R: As explained in the general response above, following your comments on the statistical analyses and the link between dispersal and genotypes' performance, we now present habitat choice estimates that directly integrate how dispersal decisions relates to the thermal niche.
As you will see in the new results (summarized in Table 1 and Figure 1), the genotypes used cover a variability of habitat choice strategies, from preference for optimal habitats to suboptimal preferences as found previously (Jacob et al. 2018. PNAS). Very interestingly, we found that increasing corridor length while keeping the structure of patch temperatures unchanged indeed increases habitat choice at emigration, and can even turn habitat choice from a preference for optimal habitats to suboptimal habitat preference (Figure 1, L. 209-211 & 291-306; which add to the above-mentioned arguments for the low contribution of passive movement to dispersal in this system).
We deeply modified the discussion of the manuscript to specifically cover hypotheses regarding the potential adaptiveness of changes in dispersal decisions demonstrated here and the potential mechanisms underlying these patterns, as pointed out above.

L 205: here you allude to information use, and I would argue again that the shallower temperature gradient in the longer tube provides different, and less, information, independent of the possiblity for prospection

R: As exposed above, we now discuss the different hypotheses potentially underlying the patterns found in the manuscript, including taxic responses (L. 268-294).

L 335: ref incomplete

R: Corrected

L 401: here you use emigration rate, but in the text you use dispersal rate. Not only is this inconsistent, but also confusing, since emigration rate is normally the proportion of individuals leaving a focal patch, but you measured the proportion of individuals arriving in another patch. Can you now calculate and analyse both (and the proportion of individuals in transit), to get the full picture?
L 407: the way you measured dispersal, you don´t have a binary classification between stay or disperse, but three categories: stay, transit, arrival. Moreover, it is not clear why this is a decision, if individuals have no way of knowing how long a tube is? Unless they decide to go back while in transit, but is that realistic?

R: As part of the changes described above, we now modified the data analysis approach to quantify habitat choice at emigration and immigration (see above). We now make the meaning of habitat choice at emigration and immigration clear: "*Habitat choice at emigration quantifies how genotypes adjust the decision to remain in the local patch depending on local temperature (including the possibility to return after prospecting neighbouring patches), and habitat choice at immigration represents the decision of where to settle if leaving the local patch****" (L. 177-180). As exposed above, we furthermore discussed the mechanisms potentially underlying the changes in dispersal found, including return rate.***

Referee: 2

Comments to the Author(s)

This paper reports the results of an experiment in the lab, using simple metapopulation microcosms and the ciliate Tetrahymena, to test the idea that increasing distance between patches in a fragmented landscape constrains both dispersal and habitat choice. The introduction does a very good job in giving motivations for this study and placing it in the broader context of study of the effects of habitat fragmentation on dispersal and its consequences. Artificial systems with very similar design have been previously used by the authors and others to reveal interesting complex variation in dispersal and habitat choice. Four types of 3-patches metapopulations are tested, varying the distance between microcosms and the temperature of the central patch (the temperature differs between the marginal patches but is kept constant across metapopulation). This design is replicated across 6 different genotypes, with 6 repetitions per genotype. The authors ask (i) whether emigration from the central patch decreases depending on distance between patches, (ii) for those having dispersed whether choice between warmer and colder patches is affected by distance between patches. There is no clear expectation about how the central patch temperature should affect this. Given past results on this system (and other ciliates) showing a lot of variation between genotypes in their dispersal behavior and plasticity, replicating the experiment for different genotypes seems a relatively standard test of generality and robustness of conclusions.

The analysis of the data shows that emigration indeed decreases with distance between patches as expected, but only in two genotypes out of 6, the rest showing no response to distance. As found before, emigration also varies across genotypes and temperature with complex interactions between the two, but no significant interaction between the effect of temperature and fragmentation. The effect of fragmentation on habitat choice at immigration is more consistent across genotypes: warmer environment are preferred, but with less intensity when distance between patches are larger.

The paper is well written, easy to follow and straightforward, the topic is interesting and addressing a very relevant question in ecology and evolution, the conclusions are well discussed. Even if the system is very artificial, the experiment helps to disentangle the effect of evolution of reduced dispersal and plastic response of dispersal to habitat fragmentation, which can be difficult to tease apart in a natural field system. The most novel exciting aspect however is the evidence that fragmentation constrains habitat choice, which would be very difficult to show in most system, and also seems to be a stronger more general pattern, less contingent on specific genotypes. Despite all these positive aspects, I still have concerns, which should be addressed or clarified by the authors. Most of them concern the statistical analysis and its interpretation.

R: We thank you for your positive evaluation of our work and the very constructive and useful comments you provided. As detailed below, we performed thorough revisions of the statistical analyses used and the discussion of results accordingly. Especially, following comments from both you and the first reviewer, we deeply modified the way we analysed data and now follow the method used in a recent paper (Jacob et al. 2018. PNAS). This method consists in quantifying habitat choice of each genotype and

each corridor length treatment as the relationship between dispersal decisions (emigration and immigration rates) and the expected fitness at each temperature (growth rate relative to the performance at thermal optimum). This metric varies between -1 and 1: h = 0 for random dispersal, h > 1 for a preference for optimal habitats and h < 0 for a preference for suboptimal habitats. Habitat choice at emigration quantifies how genotypes adjust the decision to remain in the local patch depending on local temperature, and habitat choice at immigration represents the decision of where to settle if leaving the local patch.
This method allows quantifying a meaningful estimate of habitat choice for each genotype (see Methods L. 149-180), testing whether increasing inter-patch distance affects this estimate, and especially provide important knowledge about the potential adaptive (or maladaptive) value of these dispersal decisions (see Discussion).

1) My first comment however is more about content. Even though the experiment is solid and well replicated, I find that the paper lacks a bit of depth. This probably reflects my taste for richer contents and longer papers exploring various aspects of the question, mechanisms and further exploration in a more complete picture. I am aware that the present journal aims for short papers and that simple message are more easily communicated, but I wondered whether this was the right amount of results for this format.

R: As explained above, we deeply modified the way we analysed the results of this manuscript. Together with the new metric of habitat choice computed, we significantly developed the discussion compared to the previous version of our manuscript, including discussion about the adaptive (or maladaptive) significance of habitat choice and the mechanisms potentially underlying fragmentation effects on dispersal. Note that we still kept the manuscript relatively short in accordance with the journal guidelines.

2) I do not understand very well the idea with varying the central patch temperature, nor the expectations here, and as a consequence, I do not understand well the choices made in statistical models about this effect. When analyzing emigration rates, temperature of the local (central, starting) patch is entered as an explanatory variable in the model, this is OK to me even though I do not know what to expect. Yet, when analyzing immigration rates in exactly the same experiment, I understood from the table (and differently from the text line 156) that local temperature (meaning central temperature) was not included in the model as a fixed factor, but only neighbor temperature was (the temperature of the patch receiving the immigrants). Line 159 the authors mention “dispersal system” being included as a random effect in that model and I am not sure what a dispersal system is. Is it related to the temperature of the central patch? If so, why is it a random effect? If not, why is this random effect not in the model for emigration rate? Why do we expect that the fact that individuals prefer immigrating in a warm or cold patch does not depend on the fact that they emigrated from a warm or cold patch? Would the difference in temperature between starting and final patch be an alternative measure of habitat choice? Why would immigration decision be not related to emigration decision? Much more justification and explanation is needed here.

R: As explained above, we deeply modified the analyses performed, solving most of the points you raised here. An important one still is regarding the difference between emigration and immigration habitat choice. As you tell, habitat choice might be defined regarding to the difference between starting and final patch temperature. However, we believe the distinction between emigration and immigration decisions is a very important aspect of dispersal decisions, as advanced previously (e.g. Clobert et al. 2009 Ecol.Lett.; see Jacob et al. 2015 Ecol.Evol for a discussion specific of habitat choice). Accordingly, habitat preferences at emigration and immigration did not significantly correlate in this study, neither

with standard nor long corridors (t = 0.67; p = 0.54; t = -0.22; p = 0.84; resp.). Furthermore, while only one genotype over the six tested here under standard conditions show a preference for suboptimal habitats at emigration, three show suboptimal habitat choice at immigration. We now included these additional aspects of the results and discuss them (L. 289-306; Figure S1).

3) I fail to understand the structure of the statistical model for immigration rates. Given the definition of the immigration rates as a proportion of all migrants immigrating at cold or warm temperature, those proportions must sum to one within a dispersal assay (as figure 2 actually shows) and yet they seem to be treated as independent observations in the statistical model. So basically all the information is in the proportion immigrating in cold patches, temperature of the patch receiving the migrants should not be in the model (but temperature of the central patch should be). I am quite worried by the structure and degrees of freedom of this model unless I am very confused by the explanations of the model structure given by the authors; maybe the definition of local and neighbor temperature should be clarified and this is the source of my confusion.

R: This point has been raised by both you and the first reviewer, and we greatly thank you for pointing out this important mistake in the way we performed statistical analyses. As explained above, following these comments on the statistical approach used and the request for linking dispersal and performance (reviewer 1), we changed the way we analyzed data following a recent study (Jacob et al. 2018. PNAS). Please see our response above and the associated changes in the manuscript for detailed explanation of the new method and associated results.

4) Given that all the explained variables are proportions I was a bit surprised that linear or mixed linear models were used everywhere and not generalized models with a logit link for instance as is typical for proportions; could the authors explain why they did not need to do this?

R: As explained above, we restart all the analyses following a completely different method.

5) It is a bit confusing and somehow leading to contradictory statements that authors discuss patterns per genotype even though they find no evidence for an interaction when testing globally for it. For instance for immigration rates, there is no evidence for any genotype effect or interaction with genotype: then what is the point of showing and discussing the variation of patterns among genotypes as in figure 2 if the global analysis says that none of this variation is statistically supported and something else than noise; similarly, the analysis for emigration rates concludes that the triple interaction is not significant, yet the discussion of joint effects of temperature and fragmentation as in line 174 differing across genotypes suggests an interaction worth looking at. It would make it much simpler to understand the significant interaction and ignore the non significant ones, if not only the raw data was plotted but the predictions of the best model or the model with only the significant interaction terms; this would help to see the patterns that are statistically supported and separate it from noise.

R: The analyses presented in the previous version of our manuscript were inappropriate, as pointed by you and the first reviewer. The new method we use provide evidence for differences among genotypes in habitat choice strategies and fragmentation effects on these strategies. For instance, while four genotypes similarly increase emigration habitat choice when facing longer corridors, one does so by preferring optimal habitats while the three others invert their strategy, preferring suboptimal habitats over better ones (Figure 1). Together with solving an important statistical aspect, this new method therefore provides additional information to understand the value of fragmentation-induced changes in

habitat choice, as we now discuss in the manuscript. We hope the modifications we performed clarified this important aspect of our study.

6) The title, abstract and discussion put emphasis on the fact that emigration rate decreases with fragmentation; yet it is found only in two genotypes out of 6. Therefore for the majority of genotypes fragmentation does not affect emigration. I therefore think that this conclusion is not so well supported by the present data and the emphasis could have been on the absence of an effect rather than on the minority of cases. Much more nuanced conclusion is required here.

R: We accordingly edited the title, abstract and discussion to clarify what we think is a very interesting aspect of this study: the existence of intraspecific variability in fragmentation effects on dispersal and habitat choice (see especially L. 295-306).

7) Concerning the interpretation of the reduced (but not abolished) habitat discrimination with increasing distance between patches, the authors seem to favor the interpretation that exploration and prospection is made more difficult by distance (with the idea that individuals would go and prospect different sites and return if they do not like it?). Yet, alternatively, is it possible that a temperature gradient establishes in the corridor and that with long corridors this gradient is necessarily shallower and therefore information about difference in temperature between patches more difficult to get; if this is the case, is this mechanism a bit artificial meaning that the reduced habitat choice may not be seen in more natural systems? In other words is the issue here the limits to movement of individuals or the limits to diffusion of cues about habitat quality through space?

R: We agree that the mechanisms underlying changes in habitat choice observed here are important to study. Accordingly, we now included two paragraphs to discuss specifically this aspect (L. 257-294). Still, we are convinced that increasing inter-patch distance can naturally decrease diffusion or reduce gradients of cues from one patch to another, and thus limit information acquisition and habitat choice. We now also mentioned this point in the discussion (L. 280-285).

8) Some more explanation would be necessary to understand how you can tease apart dispersal and population growth (and differential reproduction at different temperature) at the time scale of the experiment given that all estimates of dispersal are based on density counts.

R: This species shows a latency time before growth initiation [at 27 °C: mean ± SE = 17.34 ± 1.80h; at 35 °C: 9.97 ± 2.49h; Jacob et al. 2018 PNAS], meaning that population growth is negligible during the 6h of dispersal assay and thus, does not affect estimates of dispersal rates as previously shown (Pennekamp et al. 2014 Evolution). We now included this important methodological aspect in the manuscript (L. 145-148).

9) Line 253: I disagree that this experiment shows that habitat selection is counter-selected in fragmented landscapes: the experiment shows that habitat selection is constrained, not that genetic variants with higher discrimination would be selected against in comparison to less discriminating genotypes; more generally I think that in the introduction and discussion it would be good to distinguish more clearly between plasticity (fragmentation impacts the expression of dispersal and habitat selection) and evolution of these traits in a fragmented landscapes through selection on it; the present experiment says nothing about selection. Papers showing that dispersal evolves (dfferent genotypes are selected for) in fragmented landscapes are not cited (e.g Cheptou et al. 2008) and could be, just to make the point that these processes are different even if connected.

R: We absolutely agree about this important distinction. The present experiment was indeed not designed to determined which would be the most important between selection from genetic variants and plasticity in affecting fragmentation-induced changes in dispersal and habitat choice. As part of the changes introduced above, we now included a discussion of the mechanisms underlying fragmentation effects on dispersal in the discussion (L. 257-267; including key references), and modified the sentences in the introduction and discussion that incorrectly read as evidence for either hypothesis.

Appendix B

Review of ms RSPB-2019-1733

Fragmentation mediates thermal habitat choice in ciliate microcosms
by Laurent E., Schtickzelle N. and Jacob S.

In this manuscript, the authors report on an experimental test of the influence of one of the environmental parameters affected by fragmentation, namely inter-patch distance, on habitat choice ability in a ciliate. Using a simple three-patch system in which the length of the corridors separating patches is manipulated (comparing a 'standard' and an elongated situation), they show that dispersal decisions of different genotypes of *T. thermophila* are globally affected by the manipulation of corridor length. Although results in such simple systems can of course not be directly generalized to more complex *in natura* systems, I believe that this type of experiments bridging theory and empirical evidence is of very high interest and importance to test theoretical predictions and shed light on factors affecting the evolution of dispersal, here related to habitat fragmentation. Dispersal in this system is well-known and the authors (and their group) have already conducted different studies that constitute a solid background for the present experiment and help validating the results, especially the measure of habitat selection chosen here that accounts for thermal preference of the different genotypes. Thus I believe that this study is of high importance and deserves consideration in a high-impact journal such as *Proceedings of the Royal Society B*. Yet, I had several main concerns when reading the manuscript that I believe need to be addressed before the manuscript can be accepted for publication, even though this is already a revised version. These concerns relate to the statistics and presentation of results and to the protocol itself. I detail them below and hope that the authors will find these comments useful.

I did not review the first version of this manuscript, but I agree that the changes in the statistical approach and in particular the use of a new response variable accounting for the variation in thermal optimum among genotypes (and correcting for the use of previously non-independent arrival patch choice variables) are welcome and far better adapted to address the question of interest compared to the variables previously used. Here, the authors focus on two 'independent' variables (but see below) reflecting the decision to leave a patch and, for individuals that left, the choice of the patch to settle in. Yet, I have been very confused by the statistical section, especially regarding how habitat choice has been quantified.

First, the section from l. 171 to 180 is particularly unclear and needs clarification. I do not understand how habitat choice parameters can be derived from a model in which dispersal rate (= response variable) depends on expected fitness but also *habitat choice* and *dispersal propensity* (=explanatory variables)?? How can dispersal rate be both a response and an explanatory variable? I guess that dispersal propensity is derived from another measure, but it is not clear which. Dispersal propensity is defined here as the dispersal rate at expected fitness = 0.5, but how is this measured here? Was it measured during the course of this experiment, in which case it would need to be described in full, or was it determined during the thermal optimum measurement, and in this case how? The authors cite one of the articles of their group to justify this, but given the importance of this variable here, we have to be able to understand the way it was computed without referring to an external article here. In the same model, the term habitat choice is not clear: this is not a given parameter, so how can this be included as an explanatory variable in a model? Do we talk about

temperature? I am lost here. Finally, the text mentions 'this metric' of habitat choice l.175, but we do not know of which metric we talk about here, since only the model is (badly) described and we do not know what parameter is extracted from this model. I really believe that this whole description needs to be rewritten so that we can understand what has been done here.

Second, the choice of terms lacks coherence throughout the manuscript and this does not help to understand what we are talking about. For example, the text mentions 'dispersal rate at emigration and immigration' in the method section (l. 172-173), but the first line of the statistics and result sections mention 'dispersal rate' (l. 183 and l. 194). I guess that dispersal rate is here the fraction of individuals leaving the central patch, so why not calling it emigration rate as done l. 151? We also have 'habitat choice at emigration and immigration' (the h metric), but in what does emigration rate differs from habitat choice at emigration? If habitat choice at emigration is for the choice of the arrival patch while emigration rate is for the total number of individuals leaving the central patch to any of the peripheral ones, then in what does habitat choice at emigration differs from habitat choice at immigration? Although we kind of intuitively follow the results overall, my point here is that the use of terms that kind of mix-up makes it difficult to easily grasp the main results. Importantly, it is very difficult for me even after reading the results several times to understand the difference made between habitat choice at emigration and dispersal rate, as they are presented in different analyses. To formulate it another way, we have only two independent decisions / variables (whether individuals stay or not = emigration, and where they go if they decide to emigrate = immigration), so how can it be that we in the end still have three analyses (dispersal rate l. 194-199), habitat choice at emigration (l. 200-211) and habitat choice at immigration (l. 212-219)? I am extremely confused here about the difference made between emigration rate and habitat choice at emigration (see l. 177-178: how does this differ from emigration rate?). I must have misunderstood something here, but I feel that many readers would probably be as confused as I am, and because these are the main results of the study, this really needs a big clarification. Should the results about emigration rate (without accounting for thermal preference) not simply be deleted?

Third, the result section mentions (and fig. 1 shows) 'absolute values of habitat choice' that apparently range from 0 to 1 (given axes of fig. 1): unless I missed it (but I looked for it three times in the text), the computation of this variable is absolutely not described. What does this correspond to? Why comparing the h metric to this variable? Given the difference between fig a and b, it is really important that we understand what this is. From the text we somehow understand that absolute values are computed without accounting for expected fitness at the temperature considered (l. 206-207), but how is this done? A simple ratio of what over what? As it stands now, this prevents readers from understanding the results reported on fig. 1b. Also, because no statistical test is performed on these absolute values, it is hard to assess their relevance on top of their meaning.

Fourth, the choice of the statistical procedure for testing the effect of fragmentation may be better explained: why using t-tests rather than a global LMM for repeated measures in the two experimental conditions ('standard' vs. long corridors) including a random factor for replicates? This may be because of the limited number of genotypes tested, but in this case this should be clearly mentioned. Such a general model would test directly the effect of fragmentation over all genotypes (including a genotype x treatment interaction of course to test for variation among genotypes) rather than leaving readers with 6 different tests in each case (each on 12 values in total).

Finally, I found it very hard to relate the text to the figures, with several cases where I could not find the illustration of the result reported in the text. For instance, l. 209-210 (and 248-249) mention that two genotypes turned habitat choice to the suboptimal habitat, but on fig 1a, I can see

three dots below the h= 0.5 line for long corridors. Or is the one closer to the center not significant? But table 1a reports four significant statistical tests…? Given that there are only 6 points on each figure, one way to make it easier for the readers would be to label each point on the figures (e.g. 1 to 6 or a to f) and mention clearly in the text (and table 1) which points are concerned.

My second major concern relates to the *T. thermophila* genotypes chosen. The experiment tests 6 different genotypes, but (unless I missed it) nothing is said about how these genotypes were chosen, and why 6 were chosen rather than 4, 8, 10… Regarding the genotypes themselves, given the large experience of the authors' group with this system, I had the feeling – but this could be wrong so correct me in this case – that many characteristics are known for a high number of genotype lines kept in their laboratory, and therefore, I was wondering why the authors did not choose either (i) very contrasted genotypes in terms of their thermal preference (e.g. 3 genotypes preferring low temperatures and 3 preferring high temperatures), or (ii) a gradient of genotypes along the thermal preference, ranging between the two temperature chosen for the experiment. Instead, I feel when reading the manuscript that these 6 genotypes were chosen at random: is this the case? If yes, why not choosing according to known characteristics identified in previous studies? If no, then how were they chosen exactly? I believe that this needs to be clarified. The main reason for this is that, if among the chosen genotypes, some show a thermal preference just in between 27 and 35 °C (if this is possible?), they are not expected to show any habitat selection at all to start with, in any experimental condition ('standard' vs. long corridors). At least 3 genotypes remain close to h=0 in both experimental conditions (fig. 1a), and such non-choosiness could be due to their intermediate thermal preference. I would therefore have expected, for such an experiment, that highly contrasted genotypes are used, to make sure that habitat choices will be observed. Of course, along with thermal preference, dispersal ability may play a main role here, and this is why we would need to know more about the genotypes selected for the experiment. We currently have no information about the chosen genotypes other than the overall range of expected fitness in 'optimal' vs. 'non-optimal' thermal conditions, but no detailed information per genotype (e.g. are the fitness estimates correlated in optimal vs. non-optimal temperature? what is the thermal preference of each genotype and the related fitness estimates? etc.). If there were many, many genotypes used, then knowing about their characteristics would not be important. But with 'only' 6 genotypes, and given the variation in the response of the different genotypes to the experimental set-up, this question becomes quite crucial. I would therefore suggest adding (at least as supplementary material) a table reporting the different characteristics of interest for the 6 genotypes chosen (thermal optima, expected fitness in both experimental conditions…) and possibly the reasons for choosing them.

This brings me to the next point: why were 6 genotypes used and not more? I understand that such experiments may take time, but again, given the variation in the response to temperature and corridor treatment among genotypes, it could have been a good idea to use more genotypes. This would in particular have allowed making patterns of response (or gradients) and correlations appear where we currently have a few scattered points. I really believe that such an experiment conducted on a wide range of genotypes would bring extremely relevant information regarding the question of interest: it seems here that not all genotypes respond to fragmentation, and the responding ones show reversed reactions. It would be very useful to be able to do statistics on the responses themselves, using more genotypes. Were the experiments very long or difficult to conduct? Given the relatively simple experimental set-up, this would not seem so at first sight from an external point of view. But if the number of genotypes used was very limiting due to experimental

constraints, I believe that it would be important to mention so. And if not, to justify why only 6 genotypes were used here (together with their characteristics).

In relation to this, I also wondered about how the number of replicates conducted was decided? Was a power analysis conducted given previous information about the selected genotypes to assess the strength of the response and determine the number of replicates? It seems that within-genotype variation can be quite high (e.g. for immigration decisions), thus more information on this point would be good.

On a more minor (but still important) note, there are quite a number of repetitions in the text that may be related to the rewriting of several parts of the manuscript. E.g. l. 43-44 and l. 46-47, l. 55-57 and l. 63-65 (even though these two sentences illustrate selection vs. plasticity, they read as highly redundant and should be rephrased to put the emphasis on this difference in the underlying process; something like 'increased costs in terms of time or energy during the dispersal movement should lead to a reduced probability to disperse, either by selection or by phenotypic plasticity' – I do not think that a very detailed distinction between an evolutionary vs. plastic response is needed so early in the manuscript), l. 50-51 and 222-223, l. 307-309. Furthermore, some parts of the discussion are not well structured, with different ideas put together that do not seem to be much related to each other; in particular l. 268-294: the first part of the paragraph discusses movement speed (and to my opinion, may advantageously be moved to the method section), then the text moves to other cues that individuals may use outside direct prospecting, and the paragraph finished in considering differences between emigration and immigration decisions – basically three pretty unrelated ideas. I think this section should be rewritten with clear transitions (or links) between ideas (see below for suggestions to reorganize this section).

Other comments

- l. 42: strictly speaking, the change in the use of landscape is not fragmentation, but fragmentation may result from this change.
- l. 56 and 65: replace tendency by probability - tendency may be a confusing term.
- l. 63: here the authors could make the effort to cite a few more varied examples covering more taxa…
- l. 70: are these costs really indirect?
- l. 76: it seems a bit strange to oppose random dispersal to habitat choice directly; I would mention random dispersal vs. informed dispersal or non-random dispersal due to habitat choice (i.e. the two terms do not seem to act on the same level to me) – this is actually what authors write l. 79: maybe just write it similarly here for coherence.
- l. 76-77: replace by 'non-random dispersal due to habitat choice may in particular favour rather than hinder local adaptation even with high dispersal rates' (or something like this).
- l. 111: is it really useful to mention the genotype codes here if no other information is given about them? see also main comments above.
- l. 115: why were sterile conditions needed?
- l. 125-129: maybe a graph showing the 4 different treatments (2 temperatures and 2 corridor lengths) would be useful, not only to illustrate the experimental set-up but also the statistical comparisons made later to assess the effect of the manipulation on habitat choice (i.e. first comparing both temperatures to assess habitat choice and then comparing both corridor

lengths to assess the change in habitat choice due to the manipulation – but see above the main comments on statistics)

- l. 128 and later: unless a corridor length of 5 cm is a classical 'standard' length that relates to a known biological function, why not use the more straightforward terms 'short' and 'long' to define the treatments (i.e. replace 'standard' by 'short' throughout the text)?
- l. 129: I first started to ask myself about the movement ability of this species here. For readers non familiar with this biological model, it is difficult to assess the strength of the manipulation based on these two measurements only, without biological information about the species' movement ability. Then I saw that this information was given in the discussion section (l. 268-271). Because this is important for interpreting results, I think that this information should be moved to the methods section, when describing the design of the patches.
- l. 149: this sentence is not very clear: based on it, I understand that for a genotype that would disperse unconditionally through e.g. a random diffusion process (i.e. same emigration and/or immigration rates whatever the temperature), habitat choice 'at emigration' (but see above regarding the terms chosen here) would yield different estimates because fitness differs at both temperatures…? I think that the phrasing here would deserve rewriting to make sure we understand what is done.
- l. 204-205: here, it is not mentioned that one of the genotypes actually increased its habitat choice for the optimal patch in the long corridor condition (i.e. the opposite result from what is expected due to a potentially more difficult patch quality assessment). This is discussed later on but I believe it would be important to mention already here.
- l. 207-208 (and 244): I do not think that 'increase their habitat choice ability is correct'; their ability has probably not changed but their decisions have; delete ability?
- l. 219: labelling panels with a, b, c and d could be easier to follow.
- l. 224-227: cut this sentence in two; otherwise 'a dimension of fragmentation' may relate to habitat choice, which is not what you mean here.
- l. 228: I would start by discussing results on emigration, to keep the same order as in the rest of the manuscript.
- l. 228 and 243: you manipulated inter-patch distance, not fragmentation itself (distance is only one parameter of fragmentation). It would be worth being more precise here.
- l. 233-235: the mention of temperature gradients that appears later on (l. 280-285) should be moved already here.
- l. 240-242: I am not sure that I agree information transferred through immigrants may be less affected. As the number of immigrants will decrease with increasing inter-patch distance, and their nature may change (e.g. only individuals in better condition may afford to disperse), this information may well be affected as well. However, other information may remain less affected, such as acoustic / chemical / long-distance visual cues. I would suggest rephrasing here with such examples.
- l. 241-242: changing the type of information used should in any case have consequences.
- l. 253: I do not agree here: ecological traps relate to situations where the meaning of information has changed unexpectedly such that the relation between information cue and expected fitness is altered (possibly reversed). In any case, preferring suboptimal habitats in itself does not define ecological trap. This is not the case here; information is simply more difficult to obtain. Please delete.

- l. 257: see above for the confusing choice of terms. Replace here by ‘dispersal decisions’?
- l. 257-267: this whole section could be an introductory section, there is no link with results at all. I would suggest strongly reducing or moving to the introduction (where these ideas are already presented I think).
- l. 268-271: move this section to the methods to justify the design.
- l. 270-273: I found this argument quite disturbing. If individuals can cross short corridors in less than 5 min, they could in theory cross long ones in less than 10 min, which is far below the duration of the experiment. In this case, how can the effect of lengthening corridors be interpreted in terms of lower ability to prospect? Even if individuals would prospect both sides and come back to the central patch, this could (in theory) take them as little as lets’ say one hour… Maybe their willingness to engage in prospecting would be more likely to be affected, which could be related to different temperature gradients, rather than their movement ability. I think that this may deserve a bit more discussion here, as the authors seem to favour the interpretation of lower information availability from the start (see abstract l. 29-30 that may need to be changed to present alternative mechanisms).
- l. 311: ‘facing habitat fragmentation’: rewrite
- l. 312: prospecting
- l. 316: but partly resulting in increasing optimal habitat choice: why ignore this variation here and report only the results that go in the expected direction?
- l. 318: how can fragmentation modify the adaptive significance of dispersal? I do not understand. Here in particular, I do not think that the adaptive significance is concerned (no change in expected fitness with corridor length): delete.
- l. 320: landscape
- l. 325: maybe ‘predict the fate of biodiversity’ is a bit too ambitious…
- fig. 1: grey dashed lines seem to have disappeared.

Appendix C

Dear Editor,

Please find here a revised version of our manuscript entitled "Fragmentation mediates thermal habitat choice in ciliate microcosms", revised following the recommendations of the Associate Editor and two reviewers.

We thank the reviewers for their positive evaluation of this study and the modifications performed at the previous reviewing step, and especially for their very deep reviews that helped us clarifying multiple core aspects of this study. As detailed in our response letter, we greatly revised the description of the methods to clarify how we computed habitat choice, which are the input and estimated variables, and the terminology used throughout the manuscript. We furthermore significantly edited the abstract, introduction and discussion to clarify the hypotheses and key results.
To summarize, here we demonstrated experimentally that habitat choice is affected by fragmentation. The observed decreased dispersal rate and habitat choice at immigration directly follow the predictions. Interestingly, habitat choice at emigration also appeared affected by fragmentation, with genotypes becoming choosier in their decision to either stay or leave their patch when obtaining information about neighboring patches get harder. Finally, we found that this increased choosiness can come with a preference for suboptimal habitats, another very interesting (and stimulating) result of the present study to our opinion.

We are convinced that the clarity and quality of our manuscript improved a lot following this review process, and hope that you and the reviewers will agree.

We remain at your disposal for any further information you might need, and look forward regarding the suitability of our manuscript for publication in Proceedings of the Royal Society B.

Yours sincerely

Associate Editor Board Member

The revised MS has been seen by two reviewers, one who commented on the original MS and a new one. The first reviewer concluded that the MS has definitely improved, but not up to the point where it should be accepted by the Proceedings. This reviewer's main issue is that the MS is confused about how the findings relate to the original hypotheses. This reinforces to some extent the views expressed by the new reviewer. This reviewer likes the problem and experimental methodology, but got confused by the analysis. There is not only some confusing sloppiness in the terminology used, but also some deep issue about what are explained and explanatory variables in the study.
My personal feeling is that if the authors paid more attention to what kind of information might be gained by the experimental animals (and where!) it might help them to resolve some of these issues. But whatever the case, the MS definitely needs more work.

Referee: 1

I was also a reviewer of a previous version. Compared to that one, the ms has much improved, especially regarding the statistical analyses. The topic remains interesting, especially regarding the second of the two objectives (does fragmentation affect the ability to choose habitats)? While the authors do find this affect, it is not quite how we expect it to play out: with greater fragmentation the ciliates seem to prefer more strongly the habitats to which they are less adapted, i.e. habitat choice becomes not weaker but stronger (counter to prediction), and in a maladaptive way. While the authors provide some discussion (speculation) on why this might be expected, perhaps I did not understand it but I was not so convinced. The authors also find some genetic variation in these effects, which is interesting. This leaves me a bit conflicted about the paper. While the title is corrent, it does not reflect this unexpected result. The same is true for the abstract. This suggests to me that the authors also do not really know what to make of their results, and are unwilling to stress or even mention the full results. Hence, much remains to be understood, and so perhaps this paper isn´t for the Proceedings which often publishes papers with more final conclusions.
Even though the authors have cleared up many questions and issues in the ms and/or their cover letter, a few doubts remain here and there, and I suggest that these are also addressed in any next version that the authors may prepare.

R: In this study, we demonstrated experimentally that habitat choice is affected by fragmentation (i.e. inter-patch distance). The results obtained regarding dispersal rate and habitat choice at immigration directly follow the predictions, both being reduced by an increase of inter-patch distance. Interestingly, habitat choice at emigration also appeared affected by fragmentation, with genotypes becoming choosier in their decision to either stay or leave their patch when obtaining information about neighbouring patches get harder. Although this point was initially not expected, this result is to our opinion not counter intuitive. That such increased choosiness comes with a preference for suboptimal habitats is another very interesting (and stimulating) result of the present study. All these results are mentioned and discussed in the manuscript, it has never been our intention to under-mention any part of the results. We significantly modified the abstract and discussion following the comments of the two reviewers (see especially. L. 32-36 & L. 290-356).

L 44: patches, and thereby inflate
R: We removed this sentence as part of the revisions performed.

L 72: from your description, with patch selection depending on the match with phenotype, it seems you are talking about matching habitat choice? I would be good if you make clear here and in the discussion which

type(s) of habitat choice your ciliates employ using the classification in Akcali & Porter 2017 Evolution, since the dynamics and predictions can be quite different.
R: You are right that the habitat choice mechanism involved in this species might match the definition of "matching habitat choice" as defined by Akcali & Porter. However, an observed match between preference and expected fitness is not sufficient to rule out alternative mechanisms, since natal imprinting and genetic preference should lead to the same match between preference and fitness (Berner & Thibert-Plante 2015; Ackali & Porter 2017). We therefore keep using the general term "habitat choice" throughout the manuscript, and now discuss the potential habitat choice mechanisms in the Discussion (L. 338-356).

L 76:... , in general habitat choice
R: We modified this sentence following comments from reviewer 3 as follows: "*In contrast to random dispersal, informed dispersal leading to habitat choice may especially favour rather than hinder local adaptation even with high dispersal rates [19,22,28], a prediction that has recently been experimentally demonstrated [13]****" (L. 70-73).***

L 100+129, and maybe elsewhere: corridors that were twice as long.
R: Modified accordingly.

L 134: you still talk about local temperature when you mean central temparature? Change throughout ms
R: Corrected accordingly.

L 152: dispersed out of the
R: We removed this sentence as part of the changes performed following reviewer 3 comments.

L 163: explain what is a gam, or at least give the entire model name
R: We now provide the full model name, the reference for the package used, and explain briefly what it is (L. 189-193).

L 167: most optimal of the two thermal
R: Corrected.

L 173: is that the expected fitness of both patches? And how does habitat choice enter into a habitat choice parameter? Basically, you cannot just cite another paper here, you need to explain how you obtain this critical parameter, and why it varies between -1 and 1.
R: We now provide detailed information on how we computed habitat choice (part "Habitat choice characterization" in the methods, and especially L. 200-236). We hope these changes clarified this key metric of the study.

L 175: explain what the nls function is
R: We clarified the model used (L. 214-215) as part of the changes performed in the Methods (see above).

L 180: the decision of where to settle depends on leaving the local (do you mean central?) patch. This means that settlement is a function of both donor and recipient patch. How is that incorporated in your metric?
R: We here quantified habitat choice at immigration only within dispersers, i.e. the decision of where to settle for individuals who leaved the central patch (now clarified L. 176-180). This allows exploring the patterns of covariation of habitat choice between emigration and immigration, an interesting aspect of this study that we tackle in the Discussion (L. 323-331).

L 181: I´m surprised you don´t fit or investigate any potential nuisance/error/confounding terms, like date, block, batch, spatial location, observer, etc. Perhaps you are sure that all replicates are completely

comparable, but it would be good to see that that is indeed the case, especially since some results are hard to understand and might reflect spurious results.
R: All treatments and genotypes were performed under exactly the same laboratory conditions, with the same experimenters allocated to each task (EL and SJ), standardized procedures to take digital pictures and automatic analysis of these pictures. Replicates of each treatment and genotype where perfectly randomized among days, thus ensuring that all treatments and genotypes effects are strong, not resulting from any bias or confounding factor. We clarified this important point in the Methods (L. 137-140).

L 185+195: genotype by fragmentation
R: Corrected accordingly.

L 196: I think you don´t report any effect for central temperature?
R: The central temperature is only included as a covariate here, since this first model aimed at testing whether an increased inter-patch distance reduced dispersal rate irrespective of temperature. Results for effects of the central temperature on dispersal decisions are presented for habitat choice at emigration.

L 202: given that you talk about emigration, maybe say avoidance of the least optimal temperature?
R: Whether habitat choice results from preference of some habitats or avoidance of others, and whether the importance of preference/avoidance of some conditions can differ between emigration and immigration decisions is an interesting and general question. We would however prefer to keep using "preference" here, to stay consistent between steps of dispersal.

L 208: instead of choice ability, maybe say choosiness? The ability to choose might be high even when organisms don´t choose.
R: We removed "ability", as also suggested by reviewer 3.

L 209: omit Interestingly.
R: Done.

L 226: I suggest ... a behaviour that can mediate the ... consequences of fragmentation.
R: We removed this sentence as part of the revisions performed.

L 228: dispersal rate in 2 out of 6 genotypes, ...
R: Modified accordingly.

L 231: you say indeed, but I´m not sure if you ever made this prediction explicit in the introduction. If not, do so. I agree with that prediction. At the same time, I´m not sure if there is any a priori prediction for what would happen at emigration (given that they don´t know what the length of the tube and the quality of the patch at the other side will be?). If so, make this lack of a prediction also clear in the introduction. You also allude to this in L 254-256, suggesting it is not clear what would happen or why it happened.
R: Regarding the expected decreased habitat choice at immigration when facing increased fragmentation, the prediction is provided L. 78-81 & 100-102 of the introduction. Regarding habitat choice at emigration, we now make clear that this is an open question in the introduction (L. 98-100). We discuss this interesting result in Discussion, allocating the full second paragraph for it (L. 290-304).

L 248: I think it is three?
R: Corrected.

L 268: remove Interestingly
R: Modified accordingly. Note that we moved this part to the Methods following reviewer 3 comments.

L 269: was this speed measured in your experiment, or inferred from previous studies?
R: As stated above, we moved this part in the methods and clarified that this measure was performed in this study.

L 301: if that is an explanation, then you could correlate your results to the movement patterns of the 6 genotypes, to test if that is indeed so.
R: Which characteristics of the genotypes mediate effects of fragmentation on habitat choice, such as for instance dispersal propensity, prospecting ability or thermal generalism, is a highly interesting question. However, as also pointed out by reviewer 3, testing for correlations between fragmentation effects and the characteristics of the genotypes will require far more than 6 points. Reiterating the experiment described in the present manuscript with a higher number of genotypes to explore the correlates of fragmentation effects on habitat choice is a very interesting next step that we will certainly perform soon, but this objective fall beyond the aims of the present study.

Referee: 3

In this manuscript, the authors report on an experimental test of the influence of one of the environmental parameters affected by fragmentation, namely inter-patch distance, on habitat choice ability in a ciliate. Using a simple three-patch system in which the length of the corridors separating patches is manipulated (comparing a 'standard' and an elongated situation), they show that dispersal decisions of different genotypes of T. thermophila are globally affected by the manipulation of corridor length. Although results in such simple systems can of course not be directly generalized to more complex in natura systems, I believe that this type of experiments bridging theory and empirical evidence is of very high interest and importance to test theoretical predictions and shed light on factors affecting the evolution of dispersal, here related to habitat fragmentation. Dispersal in this system is well-known and the authors (and their group) have already conducted different studies that constitute a solid background for the present experiment and help validating the results, especially the measure of habitat selection chosen here that accounts for thermal preference of the different genotypes. Thus I believe that this study is of high importance and deserves consideration in a high-impact journal such as Proceedings of the Royal Society B. Yet, I had several main concerns when reading the manuscript that I believe need to be addressed before the manuscript can be accepted for publication, even though this is already a revised version. These concerns relate to the statistics and presentation of results and to the protocol itself. I detail them below and hope that the authors will find these comments useful.

1- I did not review the first version of this manuscript, but I agree that the changes in the statistical approach and in particular the use of a new response variable accounting for the variation in thermal optimum among genotypes (and correcting for the use of previously non-independent arrival patch choice variables) are welcome and far better adapted to address the question of interest compared to the variables previously used. Here, the authors focus on two 'independent' variables (but see below) reflecting the decision to leave a patch and, for individuals that left, the choice of the patch to settle in. Yet, I have been very confused by the statistical section, especially regarding how habitat choice has been quantified.
First, the section from l. 171 to 180 is particularly unclear and needs clarification. I do not understand how habitat choice parameters can be derived from a model in which dispersal rate (= response variable) depends on expected fitness but also habitat choice and dispersal propensity (=explanatory variables)?? How can dispersal rate be both a response and an explanatory variable? I guess that dispersal propensity is derived from another measure, but it is not clear which. Dispersal propensity is defined here as the dispersal rate at expected fitness = 0.5, but how is this measured here? Was it measured during the course of this experiment, in which case it would need to be described in full, or was it determined during the thermal optimum

measurement, and in this case how? The authors cite one of the articles of their group to justify this, but given the importance of this variable here, we have to be able to understand the way it was computed without referring to an external article here. In the same model, the term habitat choice is not clear: this is not a given parameter, so how can this be included as an explanatory variable in a model? Do we talk about temperature? I am lost here. Finally, the text mentions 'this metric' of habitat choice l.175, but we do not know of which metric we talk about here, since only the model is (badly) described and we do not know what parameter is extracted from this model. I really believe that this whole description needs to be rewritten so that we can understand what has been done here.

R: We now provide detailed information on how we computed habitat choice (part "Habitat choice characterization" in the methods, and especially L. 200-236), instead of summarizing a method detailed elsewhere (Jacob et al. 2018. PNAS). The Methods section now especially clarifies which are the input variables (expected fitness $\bar{f}$ and dispersal rate D_e) and which are those estimated from them (habitat choice h and dispersal propensity Dp_e; L. 215-217). We hope that these modifications clarified this core metric of our study.

2- Second, the choice of terms lacks coherence throughout the manuscript and this does not help to understand what we are talking about. For example, the text mentions 'dispersal rate at emigration and immigration' in the method section (l. 172-173), but the first line of the statistics and result sections mention 'dispersal rate' (l. 183 and l. 194). I guess that dispersal rate is here the fraction of individuals leaving the central patch, so why not calling it emigration rate as done l. 151? We also have 'habitat choice at emigration and immigration' (the h metric), but in what does emigration rate differs from habitat choice at emigration? If habitat choice at emigration is for the choice of the arrival patch while emigration rate is for the total number of individuals leaving the central patch to any of the peripheral ones, then in what does habitat choice at emigration differs from habitat choice at immigration? Although we kind of intuitively follow the results overall, my point here is that the use of terms that kind of mix-up makes it difficult to easily grasp the main results. Importantly, it is very difficult for me even after reading the results several times to understand the difference made between habitat choice at emigration and dispersal rate, as they are presented in different analyses. To formulate it another way, we have only two independent decisions / variables (whether individuals stay or not = emigration, and where they go if they decide to emigrate = immigration), so how can it be that we in the end still have three analyses (dispersal rate l. 194-199), habitat choice at emigration (l. 200-211) and habitat choice at immigration (l. 212- 219)? I am extremely confused here about the difference made between emigration rate and habitat choice at emigration (see l. 177-178: how does this differ from emigration rate?). I must have misunderstood something here, but I feel that many readers would probably be as confused as I am, and because these are the main results of the study, this really needs a big clarification. Should the results about emigration rate (without accounting for thermal preference) not simply be deleted?

R: We accordingly work throughout the manuscript to homogenize the terms used. We now clarified the definitions of these three variables in the Methods (see below), and carefully keep on using these terms throughout the manuscript (especially removing all occurrences of "emigration/immigration rates" that were indeed misleading. This results in the following variables:

- ***Dispersal rate: proportion of individuals leaving the central patch (would be equivalent to emigration rate; L. 165-167).***
- ***Habitat choice at emigration: how genotypes adjust the decision to remain in the local patch depending on local temperature (L. 176-178).***
- ***Habitat choice at immigration: how genotypes adjust the decision of where to settle if leaving the local patch depending on neighbouring temperatures (L. 178-180).***

Importantly, you are right in saying that we here investigated two steps of the dispersal process: emigration and immigration. From these data, we can however test the effects of fragmentation on three variables: dispersal rate, habitat choice at emigration, and habitat choice at immigration. Dispersal rate

indeed differs from habitat choice at emigration: while dispersal rate is the proportion of individuals that leave the central patch, habitat choice at emigration is the reaction norm of dispersal rate along temperature. We hope that the modifications we performed in the manuscript (especially in the methods) clarified these important aspects.

3- Third, the result section mentions (and fig. 1 shows) 'absolute values of habitat choice' that apparently range from 0 to 1 (given axes of fig. 1): unless I missed it (but I looked for it three times in the text), the computation of this variable is absolutely not described. What does this correspond to? Why comparing the h metric to this variable? Given the difference between fig a and b, it is really important that we understand what this is. From the text we somehow understand that absolute values are computed without accounting for expected fitness at the temperature considered (l. 206- 207), but how is this done? A simple ratio of what over what? As it stands now, this prevents readers from understanding the results reported on fig. 1b. Also, because no statistical test is performed on these absolute values, it is hard to assess their relevance on top of their meaning.

R: We clarified the method to compute habitat choice (see above), and now explain how and why we compute absolute values of habitat choice in the Methods as follows: "*The two metrics of habitat choice (he and hi) computed vary between -1 and 1: h = 0 for random dispersal, h > 0 for a preference for optimal habitats (i.e. where expected fitness is higher) and h < 0 for a preference for suboptimal habitats (i.e. where expected fitness is lower). In addition, we computed the absolute value of these habitat choice metrics to denote the intensity of habitat choice, irrespective of its direction or adaptive significance.****" (L. 237-241). These absolute values of the habitat choice metrics h_e and h_i, are computed to remove the information about the direction of choice in order to better highlight the effects of fragmentation on the intensity of habitat choice.***

4- Fourth, the choice of the statistical procedure for testing the effect of fragmentation may be better explained: why using t-tests rather than a global LMM for repeated measures in the two experimental conditions ('standard' vs. long corridors) including a random factor for replicates? This may be because of the limited number of genotypes tested, but in this case this should be clearly mentioned. Such a general model would test directly the effect of fragmentation over all genotypes (including a genotype x treatment interaction of course to test for variation among genotypes) rather than leaving readers with 6 different tests in each case (each on 12 values in total).

R: Following the changes of statistical procedure performed during the previous step of revision, we adopted an approach leading to one estimate of habitat choice for each genotype. Although the method allows testing for its significance, as presented in Table 1, it does not result in one measure per replicate. This method is therefore incompatible with a global model approach. Furthermore, we moved to this approach exactly because a mixed model approach with replicate as a random factor was unsuitable to account for the dependence of proportions of cells in the two neighbouring patches (whose sum to 1).

5- Finally, I found it very hard to relate the text to the figures, with several cases where I could not find the illustration of the result reported in the text. For instance, l. 209-210 (and 248-249) mention that two genotypes turned habitat choice to the suboptimal habitat, but on fig 1a, I can see three dots below the h= 0.5 line for long corridors. Or is the one closer to the center not significant? But table 1a reports four significant statistical tests...? Given that there are only 6 points on each figure, one way to make it easier for the readers would be to label each point on the figures (e.g. 1 to 6 or a to f) and mention clearly in the text (and table 1) which points are concerned.

R: We are sorry for these misunderstandings. We now clarified the text as follows: "*This resulted in three genotypes with a preference for suboptimal habitats in the fragmented treatment, while one genotype with optimal preference show increased habitat choice (Figure 2a)****" (L. 271-273) and "****Interestingly, our results*

suggest that this increased habitat choice at emigration can come with suboptimal decisions: three of the four genotypes that increased their choosiness with fragmentation also showed a preference for the suboptimal temperature over the most optimal one (Figure 2a)" ***(L. 294-297). We furthermore modified the figure as suggested (now figure 2).***

6- My second major concern relates to the T. thermophila genotypes chosen. The experiment tests 6 different genotypes, but (unless I missed it) nothing is said about how these genotypes were chosen, and why 6 were chosen rather than 4, 8, 10... Regarding the genotypes themselves, given the large experience of the authors' group with this system, I had the feeling – but this could be wrong so correct me in this case – that many characteristics are known for a high number of genotype lines kept in their laboratory, and therefore, I was wondering why the authors did not choose either (i) very contrasted genotypes in terms of their thermal preference (e.g. 3 genotypes preferring low temperatures and 3 preferring high temperatures), or (ii) a gradient of genotypes along the thermal preference, ranging between the two temperature chosen for the experiment. Instead, I feel when reading the manuscript that these 6 genotypes were chosen at random: is this the case? If yes, why not choosing according to known characteristics identified in previous studies? If no, then how were they chosen exactly? I believe that this needs to be clarified. The main reason for this is that, if among the chosen genotypes, some show a thermal preference just in between 27 and 35 °C (if this is possible?), they are not expected to show any habitat selection at all to start with, in any experimental condition ('standard' vs. long corridors). At least 3 genotypes remain close to h=0 in both experimental conditions (fig. 1a), and such non-choosiness could be due to their intermediate thermal preference. I would therefore have expected, for such an experiment, that highly contrasted genotypes are used, to make sure that habitat choices will be observed. Of course, along with thermal preference, dispersal ability may play a main role here, and this is why we would need to know more about the genotypes selected for the experiment. We currently have no information about the chosen genotypes other than the overall range of expected fitness in 'optimal' vs. 'non- optimal' thermal conditions, but no detailed information per genotype (e.g. are the fitness estimates correlated in optimal vs. non-optimal temperature? what is the thermal preference of each genotype and the related fitness estimates? etc.). If there were many, many genotypes used, then knowing about their characteristics would not be important. But with 'only' 6 genotypes, and given the variation in the response of the different genotypes to the experimental set-up, this question becomes quite crucial. I would therefore suggest adding (at least as supplementary material) a table reporting the different characteristics of interest for the 6 genotypes chosen (thermal optima, expected fitness in both experimental conditions...) and possibly the reasons for choosing them.

R: Given the strong connections between this question and the next one, we provide below a common answer.

7- This brings me to the next point: why were 6 genotypes used and not more? I understand that such experiments may take time, but again, given the variation in the response to temperature and corridor treatment among genotypes, it could have been a good idea to use more genotypes. This would in particular have allowed making patterns of response (or gradients) and correlations appear where we currently have a few scattered points. I really believe that such an experiment conducted on a wide range of genotypes would bring extremely relevant information regarding the question of interest: it seems here that not all genotypes respond to fragmentation, and the responding ones show reversed reactions. It would be very useful to be able to do statistics on the responses themselves, using more genotypes. Were the experiments very long or difficult to conduct? Given the relatively simple experimental set-up, this would not seem so at first sight from an external point of view. But if the number of genotypes used was very limiting due to experimental constraints, I believe that it would be important to mention so. And if not, to justify why only 6 genotypes were used here (together with their characteristics).

R: Following previous studies providing evidence for temperature-dependent habitat choice in **T. thermophila,** ***here we aimed at testing whether habitat fragmentation affects habitat choice. Initially, we***

envisaged to use only one genotype for this new experiment, since it would be sufficient for the focal question. However, we decided to replicate the experiment using several genotypes to explore whether genotypes differ or not in their response to fragmentation. We choose to replicate the experiment with 6 genotypes, thus keeping the design feasible while tackling the question of intraspecific variability in fragmentation response. To do so, we choose 5 genotypes from the 12 previously characterized (Jacob et al. 2018), plus one new genotype. Within the first five genotypes, all performed habitat choice at emigration, and we choose two genotypes that performed habitat choice at immigration, and 3 that did not.
The resulting experiment revealed that habitat choice can be affected by fragmentation, and that this response can differ among genotypes. Which characteristics of the genotypes mediate effects of fragmentation on habitat choice, such as for instance dispersal propensity, prospecting ability or thermal generalism, is indeed a highly interesting question. However, as pointed out by the reviewer, testing for correlations between fragmentation effects and the characteristics of the genotypes will require far more than 6 points. Reiterating the experiment described in the present manuscript with a higher number of genotypes to explore the correlates of fragmentation effects on habitat choice is a very interesting next step that we will certainly perform in the future, but also falls beyond the aims of the present study.
We now clarified the choice of the 6 genotypes in the Methods section (L. 109-112). We however don't provide further information about genotypes characteristics (other than habitat choice parameters): as explained above, and pointed out by the reviewer, exploring correlations with other traits of genotypes would require more than 6 genotypes, and we would prefer not to mislead readers about the conclusions that this study can provide.

8- In relation to this, I also wondered about how the number of replicates conducted was decided? Was a power analysis conducted given previous information about the selected genotypes to assess the strength of the response and determine the number of replicates? It seems that within- genotype variation can be quite high (e.g. for immigration decisions), thus more information on this point would be good.

R: Previous studies in the team classically performed 5 replicates, which provided repeatable estimates of the focal traits (e.g. Jacob et al. 2018 PNAS) and even of ecological dynamics (e.g. Jacob et al. 2019 Am.Nat.). Since we did not aim at testing for complex interactions here, keeping with 5 replicates would be sufficient. We still increased to 6 replicates because it proved feasible given the experimental design.

9- On a more minor (but still important) note, there are quite a number of repetitions in the text that may be related to the rewriting of several parts of the manuscript. E.g. l. 43-44 and l. 46-47, l. 55-57 and l. 63-65 (even though these two sentences illustrate selection vs. plasticity, they read as highly redundant and should be rephrased to put the emphasis on this difference in the underlying process; something like 'increased costs in terms of time or energy during the dispersal movement should lead to a reduced probability to disperse, either by selection or by phenotypic plasticity' – I do not think that a very detailed distinction between an evolutionary vs. plastic response is needed so early in the manuscript), l. 50-51 and 222-223, l. 307-309. Furthermore, some parts of the discussion are not well structured, with different ideas put together that do not seem to be much related to each other; in particular l. 268-294: the first part of the paragraph discusses movement speed (and to my opinion, may advantageously be moved to the method section), then the text moves to other cues that individuals may use outside direct prospecting, and the paragraph finished in considering differences between emigration and immigration decisions – basically three pretty unrelated ideas. I think this section should be rewritten with clear transitions (or links) between ideas (see below for suggestions to reorganize this section).

R: We thank the reviewer for pointing out these repetitions and flaws in the structure. We removed the repetitions L. 44-46, L. 51-60, edited the sentence L. 368-370, and restructured parts of the discussion as suggested (especially the points about prospecting and population sizes that were moved to the Methods L. 150-162, and taxic responses moved L. 308-316).

Other comments

- l. 42: strictly speaking, the change in the use of landscape is not fragmentation, but fragmentation may result from this change.
R: We modified these sentences as follows: "*Natural ecosystems are increasingly converted into agricultural or urban areas for human activities [1,2], turning landscapes into smaller and more distant patches [3,4]. Beyond overall habitat loss, the resulting habitat fragmentation is expected to reduce dispersal movements among patches as a result of increased inter-patch distances [1,4–6]****" (L. 43-46)***

- l. 56 and 65: replace tendency by probability - tendency may be a confusing term.
R: Corrected.

- l. 63: here the authors could make the effort to cite a few more varied examples covering more taxa...
R: Here we focus on papers that synthesized these concepts and reviewed evidence for them. Since reviewing empirical evidence for informed dispersal is beyond the scope of the present manuscript, we removed the empirical studies that were inappropriately included here, and now only refer to reviews (L. 60).

- l. 70: are these costs really indirect?
R: These costs are indeed only "other" costs (than costs of movement* per se*). We removed "indirect" here.

- l. 76: it seems a bit strange to oppose random dispersal to habitat choice directly; I would mention random dispersal vs. informed dispersal or non-random dispersal due to habitat choice (i.e. the two terms do not seem to act on the same level to me) – this is actually what authors write l. 79: maybe just write it similarly here for coherence.
R: We replaced "habitat choice" by "informed dispersal" accordingly.

- l. 76-77: replace by 'non-random dispersal due to habitat choice may in particular favour rather than hinder local adaptation even with high dispersal rates' (or something like this).
R: Changed accordingly.

- l. 111: is it really useful to mention the genotype codes here if no other information is given about them? see also main comments above.
R: Following the above comment, we link these genotypes to previous studies on habitat choice, and included these codes in the figures.

- l. 115: why were sterile conditions needed?
R: Manipulating* T. thermophila *genotypes under sterile conditions is required to avoid contaminations of cultures by environmental bacteria or fungi, especially in axenic conditions as used here (L. 114-116). We now explain this point in the manuscript (L. 116-118).

- l. 125-129: maybe a graph showing the 4 different treatments (2 temperatures and 2 corridor lengths) would be useful, not only to illustrate the experimental set-up but also the statistical comparisons made later to assess the effect of the manipulation on habitat choice (i.e. first comparing both temperatures to assess habitat choice and then comparing both corridor lengths to assess the change in habitat choice due to the manipulation – but see above the main comments on statistics)
R: We now added an illustration of the dispersal systems used and treatments performed (new figure 1).

- l. 128 and later: unless a corridor length of 5 cm is a classical 'standard' length that relates to a known biological function, why not use the more straightforward terms 'short' and 'long' to define the treatments (i.e. replace 'standard' by 'short' throughout the text)?

R: We used the term "standard" because this corridor length was the one used in previous studies (Jacob et al. 2017 NEE; 2018 PNAS). If possible, we would prefer to keep this term: we indeed believe that it makes clearer the connections between this study and the results of previous ones. We clarified this point in the methods as follows: "*We manipulated habitat fragmentation by using corridors separating habitat patches either of the standard length used in previous studies (hereafter named "standard corridors"; 5 cm long, meaning ~2500 times the size of cells; [13,24]) or twice longer in the case of the fragmented treatment (Figure 1)****" (L. 129-133).***

- l. 129: I first started to ask myself about the movement ability of this species here. For readers non familiar with this biological model, it is difficult to assess the strength of the manipulation based on these two measurements only, without biological information about the species' movement ability. Then I saw that this information was given in the discussion section (l. 268-271). Because this is important for interpreting results, I think that this information should be moved to the methods section, when describing the design of the patches.

R: As suggested, we moved this point in the methods (L. 150-155).

- l. 149: this sentence is not very clear: based on it, I understand that for a genotype that would disperse unconditionally through e.g. a random diffusion process (i.e. same emigration and/or immigration rates whatever the temperature), habitat choice 'at emigration' (but see above regarding the terms chosen here) would yield different estimates because fitness differs at both temperatures...? I think that the phrasing here would deserve rewriting to make sure we understand what is done.

R: We deeply modified this part of the methods to clarify the measure of habitat choice (L. 200-236; see also above). We hope that this point is now clear.

- l. 204-205: here, it is not mentioned that one of the genotypes actually increased its habitat choice for the optimal patch in the long corridor condition (i.e. the opposite result from what is expected due to a potentially more difficult patch quality assessment). This is discussed later on but I believe it would be important to mention already here.

R: We now mention this point more clearly in the results (L. 271-273).

- l. 207-208 (and 244): I do not think that 'increase their habitat choice ability is correct'; their ability has probably not changed but their decisions have; delete ability?

R: We accordingly removed "ability" here and elsewhere.

- l. 219: labelling panels with a, b, c and d could be easier to follow.

R: Changed as suggested.

- l. 224-227: cut this sentence in two; otherwise 'a dimension of fragmentation' may relate to habitat choice, which is not what you mean here.

R: We clarified this sentence as follows: "*Here we tested experimentally whether habitat choice is affected by an increase of inter-patch distance, a dimension of habitat fragmentation that can mediate its ecological and evolutionary consequences.****" (L. 287-289).***

- l. 228: I would start by discussing results on emigration, to keep the same order as in the rest of the manuscript.

R: We changed the ordering of these parts accordingly.

- l. 228 and 243: you manipulated inter-patch distance, not fragmentation itself (distance is only one parameter of fragmentation). It would be worth being more precise here.
R: We changed for "inter-patch distance".

- l. 233-235: the mention of temperature gradients that appears later on (l. 280-285) should be moved already here.
R: We accordingly moved this part L. 308-316.

- l. 240-242: I am not sure that I agree information transferred through immigrants may be less affected. As the number of immigrants will decrease with increasing inter-patch distance, and their nature may change (e.g. only individuals in better condition may afford to disperse), this information may well be affected as well. However, other information may remain less affected, such as acoustic / chemical / long-distance visual cues. I would suggest rephrasing here with such examples.
R: We agree that this is purely speculative and requires specific investigation, and are sorry if this sentenced didn't read as such. We now modified the sentence to include long distance cues and clearly state that this is a trail for future research (L. 318-322).

- l. 241-242: changing the type of information used should in any case have consequences.
R: We modified this sentence as explained above.

- l. 253: I do not agree here: ecological traps relate to situations where the meaning of information has changed unexpectedly such that the relation between information cue and expected fitness is altered (possibly reversed). In any case, preferring suboptimal habitats in itself does not define ecological trap. This is not the case here; information is simply more difficult to obtain. Please delete.
R: We deleted this point accordingly.

- l. 257: see above for the confusing choice of terms. Replace here by 'dispersal decisions'?
R: Modified accordingly. See also above our response regarding to the changes performed in the terms used.

- l. 257-267: this whole section could be an introductory section, there is no link with results at all. I would suggest strongly reducing or moving to the introduction (where these ideas are already presented I think).
R: We reduced the point on selection versus plasticity, and instead develop a discussion about the mechanisms and consequences of different habitat choice mechanisms, following a suggestion of reviewer 1 (L. 338-356).

- l. 268-271: move this section to the methods to justify the design.
R: We moved this part accordingly, as explained above.

- l. 270-273: I found this argument quite disturbing. If individuals can cross short corridors in less than 5 min, they could in theory cross long ones in less than 10 min, which is far below the duration of the experiment. In this case, how can the effect of lengthening corridors be interpreted in terms of lower ability to prospect? Even if individuals would prospect both sides and come back to the central patch, this could (in theory) take them as little as lets' say one hour... Maybe their willingness to engage in prospecting would be more likely to be affected, which could be related to different temperature gradients, rather than their movement ability. I think that this may deserve a bit more discussion here, as the authors seem to favour the interpretation of lower information availability from the start (see abstract l. 29-30 that may need to be changed to present alternative mechanisms).

R: If cells would go straight in the corridors, they would indeed make it to the neighbouring patches in a few minutes. However, we know they don't: dispersal in this species is not limited to movement ability but involve active behavioral decisions. As part of the changes performed as required above, we moved this part in the methods, and modified it to try clarifying this point as follows: "*Importantly however, less than three hours are usually not enough to observe dispersers in these systems (Jacob; personal observation), and population sizes in this experiment did not homogenize even after six hours, which would be the result of a very high level of movement. Furthermore, we previously found that the usually observed correlation between cell velocity and dispersal rate (e.g. [35,47,48]) vanishes when harsh corridors are used (i.e. no nutrients; [44]). Dispersal in this experimental system is therefore not a simple diffusion process mediated only by movement ability, but should involve active behavioural decisions****" (L. 155-162). We hope that this point is now clearer. We furthermore clarified the potential existence of mechanisms other than reduced movement ability (L. 28-29).***

- l. 311: 'facing habitat fragmentation': rewrite

R: Modified as follows: "*Here we provided experimental evidence for decreased dispersal rate and reduced habitat choice at immigration under habitat fragmentation****" (L. 370-371).***

- l. 312: prospecting

R: Corrected.

- l. 316: but partly resulting in increasing optimal habitat choice: why ignore this variation here and report only the results that go in the expected direction?

R: We corrected this point as follows: "*Furthermore, we found that increasing inter-patch distance also modified the decision to either stay or leave the local patch (i.e. habitat choice at emigration), resulting in either increased optimal habitat choice or the rise of suboptimal habitat preferences****" (L. 375-377).***

- l. 318: how can fragmentation modify the adaptive significance of dispersal? I do not understand. Here in particular, I do not think that the adaptive significance is concerned (no change in expected fitness with corridor length): delete.

R: We deleted "its adaptive significance" accordingly.

- l. 320: landscape

R: Corrected.

- l. 325: maybe 'predict the fate of biodiversity' is a bit too ambitious...

R: We modified this sentence as follows: "*Investigating the consequences of habitat fragmentation for populations and communities while accounting for changes in the different facets of dispersal (e.g. dispersal rate, habitat choice, but also for instance dispersal syndromes and distance) is consequently an important next step to understand the dynamics of biodiversity facing current environmental changes****" (L. 382-386).***

- fig. 1: grey dashed lines seem to have disappeared.

R: The grey dashed lines should appear only in panels a & c, since they indicate the random dispersal hypothesis (h=0). We checked on the submitted pdf and dashed lines rightly appear on panels a & c, but we can submit another version of the figure if any problem persists.