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PROCEEDINGS B

Frequency-dependent fitness and reproductive dynamics contribute to habitat segregation in sympatric Jewelflowers

Kyle Christie and Sharon Strauss

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Original submission: 19 July 2019
1st revised submission: 12 March 2020
2nd revised submission: 15 April 2020
Final acceptance: 16 April 2020

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

Review History

RSPB-2019-1665.R0 (Original submission)

Review form: Reviewer 1

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?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

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?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This study evaluated abiotic niche partitioning, local adaptation, and a part of species interaction, to explain the local micro-parapatry distributions of the two Stereptanthus species.
I admire their really elaborated investigations, examining different kinds of candidates that may cause the paratatry carefully, presenting the results clearly, and discussing the results with a wide range of reference including not only plant but also animal studies. Although this study is a sort of one part of a serial studies, this paper itself can be treated as an independent study to discuss the effect of the resource competitions or environmental preferences on the distributional relationships between closely related plant species. I, therefore, enjoyed reading this paper, and like to read other papers the authors previously presented or are preparing for the two Streptanthus species.
Only one thing I am concerned is the statistical analysis: I am afraid that results for the seed viability should be analyzed with not GLM but GLMM (and if possible, I hope the authors add the data for their NMDS). Otherwise, I did not find any other things to complain!
I am really looking forward to seeing this paper in the journal, and also seeing their studies completed in near future.

Review form: Reviewer 2 (Karl Hülber)

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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N/A

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Is it adequate?
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Comments to the Author
The paper compares fitness of two parapatric Streptanthus species between their resident and foreign habitats to test for home-site advantages. It uses a multitude of experimental approaches to gain results for a comprehensive set of fitness components. The topic is highly interesting and I highly appreciate such a work. However, I recommend a major revision for the following reasons.

In contrast to the excellent introduction and discussion, the methods and results of the experiments are presented in a highly confusing way – both in terms of the methodological description as well as the illustration of results.

Due to the splitting of the description of experimental designs between i) the main text and the supplementary material and ii) between different sections (e.g. local adaptation – seed viability; germination – local adaptation) within both parts leaves it to the reader to collect and digest the necessary pieces of information. Thus, it is extremely hard for the reader to grasp the design of single experiments as well as the reasoning why they were performed. This problem is further enhanced i) by the redundancies emerging from the splitting and ii) by the fact that a bulk of necessary methodological details are simply kept secret. Thus, I strongly recommend to re-think the structuring of the method section in the main text and I further question the use of the supplementary materials to provide descriptions of experiments and analyses. I suggest a consequent structuring either along experiments, fitness components or questions.

The presentation of the results needs to be revised too. The allocation of results (text as well as the figures) to the main text and the supplementary material is not clear to me. Both include information of similar relevance considering the main aim of the study (see line 77-86): e.g. Question 1 and 2 are answered in the main text and the supplement, respectively. Moreover, the text of the result section includes many statements/sentences (e.g. those following "suggesting …") which are clearly interpretations and, hence, should be shifted to the discussion section. In addition, the most important results are given in the section "factors shaping spatial isolation" (including Table 1), which is not mentioned in the methods and not described at all. Hence, it is really hard to grasp its content to get the main message of the paper.
Concerning figures, I question the use of a (bold) line connecting the treatments/classes to

illustrate the "reaction norm" in Figure 1, S3, S4, S5 for two reasons: i) These lines indicate a transition or at least a connection between the treatments which simply does not exist. Furthermore, you have no clue about the values of intermediate states (if they exist), but they certainly need not to be intermediate; ii) the slope of these lines depends on the scales of the axes, which are highly arbitrary (particularly but not only for the x-axis). So, I recommend to find another illustration, remove the lines or at least chose a less eye-catching style (i.e. less than those for the points and whiskers). Moreover, I prefer to see raw data instead of model predictions in all figures. Furthermore, the captions of most figures describe the pattern illustrated in the respective panels, but methodological details necessary or desirable for the understanding of the figure content are frequently missing.

For all the points raised above, see numerous detailed comments below.

Potential non-independence of measurements obtained from individuals/seeds within one site are considered for by using site as fixed effect in the regression analyses. However, potential non-independence of individuals/seeds within a block (within a site) in the transplantation experiment(s) are disregarded. Does this require the use of nested random-effects in a GLMM?

Detailed comments to the main text:
L10: replace "lack" by "scarcity"
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L79: include the family (=Brassicaceae)
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L145: "…in which a species has higher fitness at the home site". Personally I would prefer the use of the terms "resident/migrant" over "home/away" throughout the paper. I suggest not to mix these terms to avoid synonymy.
l146-149: It seems that germination was intended to be measured as character of the local adaptation experiment, which is perfectly right. It might happen that is a (nearly) total failure in one character, rendering additional setups or changes in the analyses necessary. Nevertheless, I suggest to present germination as part (4th character apart from survival, growth and reproduction) of the intended experiment – of course by providing the details (deviating from other characters).
L153: Please specify at which stage (seed, seedling, juvenile, adult) these migrants were transplanted. It should also be clear for which stages the term migrant is used: only for (adult) plants or also for seeds (compare e.g. Line 153 "migrants" to Line 136 "away seeds") -> unify if necessary
L155: replace "~" by "on average"
L160ff: I miss the details of the design: e.g. How many migrants? Why 41 blocks? Did you use 2 sites of each species (3*2= 6 sites)? Specify whether the 6 sites are the same as used in other experimental approaches of this study; "outcrop" seems to be synonym to "site" -> unify; The use of the term "reciprocal transplant experiment" is confusing and ambiguous (compare Line 169). Find unique terms for each of your experiments (e.g. reciprocal seed sowing experiment; adult transplantation experiment)
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L189-190: "We grew native genotypes…" -> be more detailed (from seeds, where, how long,

etc.?); in particular, define (or avoid) the term genotype: Did you really produced clones, which were used in each treatment? Or did you simply grow individuals/plants?
L198-200: I do not see a relevant difference between "differences in abiotic niches" and "habitat partitioning" in this study, with the exception that the former and latter refer to the Adonis-test and NMDS-ordination, respectively. In line 130 you refer to the same test as "as species-level habitat differences". Please unify the terminology.
L203: There is no test for differences in niche breadth.
L207-208, 212-213, 234-235, 236-244, 254-255,257-258, 261-262, 265-266, 268-269, 271-272, 273-274, 300-303: interpretation -> shift to the discussion
L214: the p-value does not match those in the table ($p < 0.05$). If you provide it here, you should goals give the p-values for other factors like plant height (L218). Generally, I prefer to see the exact p-values instead of classes (<0.05, <0.01, <0.001).
L219-220: rewrite
Figure 1: It is not clear, whether 1D is based on values of all plants or only those with 7 or 8 conspecific neighbors per block.
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L285-287: According to Fig. S6, plant height of S. hesperidis does not (significantly) depend on the presence/absence of a competitor, which strongly contrasts this statement- even if it is numerically correct (i.e. the mean is lower with competitors)!
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L298-300: Is this statement deduced from the fact that only "seed viability" has "***"? give a more comprehensive description of the table!
Table 1 is very hard to understand: The meaning of all the values is dubious at first glance. It seems that the home and away fitness represent predictions of models for each component and the relative fitness is the latter divided by the former. Both need to be explained. Units should be given for all components: by the way values for germination and survival represent proportions (range:0-1) not % (range:0-100). Furthermore, the order of components is not clear and it is hard to connect the fitness components mentioned in the table to the respective experiments and/or sections of the methods. In addition, results from the greenhouse germination experiment are not included in Table 1: Are they not important? If so, remove the experiment from the manuscript, otherwise show its results with the same priority as that for survival, growth and fertility.
L319: Isn´t "competition" inherent to "local adaptation"? If so I suggest to remove it here. Otherwise I appreciate to include it into the questions (equivalent to the other factors: niche partitioning, local adaptation and rarity)

Detailed comments to the supplement:
L50: This contrasts line 98 ff: six of these sites are intermixed
L55: subscript "3"
Figure S1: remove titles (not necessary, not complete); I expect some (significant) differences in single variables. They might be shortly mentioned or illustrated. A methodological description of the chemical and texture analyses is appreciated.
L61: "abiotic site conditions" are referred to as "physical site attributes" in line 129 -> unify; replace "locations" with "sites"
L63: How is niche breadth defined?
L69: "… at randomly…"
L70-71: remove "10 each for…each site" because it is confusing: site-species-combinations are unique
L74: give the formula

Figure S2: mention the test used for obtaining the significance levels (also in subsequent figures)
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L90: give n of seeds sown
l94: 240 per site/species/in total?
L95: Give the interval of observation, the conditions (temperature, light, humidity, etc.)
Figure S3: Should "soil" be replaced with "outcrop"; significance levels are nit indicates
L109: Give the number of sown seeds
L113: at the end of the growing season?
L115: provide these numbers: at least for each species at each site
L121: give the conditions of the lathhouse (temperature, etc.); give the date of scoring
L124: add "(see main text)" at the end of the sentence.
Figure S4: remove titles
L132: "…transplant experiment conducted…"
L137: Specify conditions and the design of this experiment
L140: "and while keeping ...(D40 Deepots)" shift beyond "McLaughlin" at line 143
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Figure S6: Test at the 90% confidence level are not necessary -> omit
Supplementary tables: avoid abbreviations (GH, NJ, QV, etc.); improve the adjustment of values; give the exact p-values; might be merged to a single table

Decision letter (RSPB-2019-1665.R0)

06-Sep-2019

Dear Dr Christie:

I am writing to inform you that your manuscript RSPB-2019-1665 entitled "Frequency-dependent fitness and reproductive interference contribute to habitat segregation in sympatric Jewelflowers" 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,
Dr Sasha Dall
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
I have now received two reviews of the manuscript “Frequency-dependent fitness and reproductive interference contribute to habitat segregation in sympatric Jewelflowers.” The manuscript explores a number of different ecological factors that may maintain habitat segregation in two incompatible species. Although several factors contributed modestly, the major explanatory factor for habitat segregation was that low frequency migrants suffered poor seed quality (likely due to receipt of heterospecific pollen). The work addresses important issues of coexistence, in particular, whether being a minority a benefit or a detriment.

Both reviews were fairly positive, suggesting that the manuscript has potential to be a strong contribution. One review recommended relatively modest changes, while the other had more substantial recommendations, in particular to the organization of the methods and results. In addition, both reviewers raised statistical questions.

A substantial revision could produce a manuscript that makes a strong contribution to our understanding of local-scale habitat segregation.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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I am really looking forward to seeing this paper in the journal, and also seeing their studies completed in near future.

Referee: 2

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Figure S6: Test at the 90% confidence level are not necessary -> omit
Supplementary tables: avoid abbreviations (GH, NJ, QV, etc.); improve the adjustment of values; give the exact p-values; might be merged to a single table

Author's Response to Decision Letter for (RSPB-2019-1665.R0)

See Appendix A.

RSPB-2020-0559.R0

Review form: Reviewer 1

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.
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?
Yes

Is it clear?
Yes

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
I appreciate that the authors improved their manuscript with more detailed explanation and rearrangement of the story to make their aim of the study, methods, and results clearer and easier to follow. I have nothing more to say, except for the part of the reproductive interaction (Question 4).

I understand that the authors combined their data for the reproductive interference (which the authors had described as "in review" in the previous manuscript) and the data for the other competition into this revised manuscript. I am glad to read the comprehensive study in one paper, but some improvement is now needed for the part of the study on reproductive interference.

We cannot determine whether shortage of conspecific pollen or reproductive interference from the heterospecific pollen affected reproductive success of the focal species, from the results written in the main manuscript and shown in Fig. 4, Table S3.3 or S3.4. To evaluate the reproductive interference, the experimental crosses (for Fig. S1) is a much better indicator rather than the experiment for Fig. 4. I recommend that the authors use Fig. S1 for the main text (not supplementary information) rather than Fig. 4, with detailed explanation of the experiment design and statistics (GLMM with including individuals as a random effect). Or, do the authors intend to use the data for Fig. S1 for the other paper? If so, it would be good to mention "in review" when they show the figure, since we do not know whether we have a way to access to the details of the results for Fig. S1 in somewhere in future.

As for the experiment for Question 4 (Table S3.3, S3.4, Table S5), I have two things to recommend that the authors should reconsider. First, please include individual plant as a random effect for their statistics. As far as I read the manuscript, the authors included only experimental block as a random effect, just the same way for the other analyses. However, since each individual should have several flowers or fruits, I believe that they need to use GLMM with including not only the block but also individual (therefore, individual nested in block) as a random effect for this results. Second, please show us the statistics independently for each species instead of the inclusive one as they show in Table S5. Since the authors suggest there is an asymmetrical effect in the reproductive interference between the two species, I believe we need to evaluate the effect of number of conspecifics on the seed viability independently for each species.

By the way, I also have one very minor question: what is "ESM" ? (e.g., P4 L116)

That's all for my comments.

Decision letter (RSPB-2020-0559.R0)

11-Apr-2020

Dear Dr Christie

I am pleased to inform you that your manuscript RSPB-2020-0559 entitled "Frequency-dependent fitness and reproductive dynamics contribute to habitat segregation in sympatric Jewelflowers" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' 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:
I appreciate the thorough revision of this manuscript; I enjoyed reading it! The new statistics and the reorganization of the manuscript have resulted in a clear and compelling story. The reviewer has some recommendations for additional changes. Please address these before submitting a final version. I will say, I think it is your discretion whether you include Fig 4 or Fig S1 in the main text.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
I appreciate that the authors improved their manuscript with more detailed explanation and rearrangement of the story to make their aim of the study, methods, and results clearer and easier to follow. I have nothing more to say, except for the part of the reproductive interaction (Question 4).

I understand that the authors combined their data for the reproductive interference (which the authors had described as “in review” in the previous manuscript) and the data for the other competition into this revised manuscript. I am glad to read the comprehensive study in one paper, but some improvement is now needed for the part of the study on reproductive interference.

We cannot determine whether shortage of conspecific pollen or reproductive interference from the heterospecific pollen affected reproductive success of the focal species, from the results written in the main manuscript and shown in Fig. 4, Table S3.3 or S3.4. To evaluate the reproductive interference, the experimental crosses (for Fig. S1) is a much better indicator rather than the experiment for Fig. 4. I recommend that the authors use Fig. S1 for the main text (not supplementary information) rather than Fig. 4, with detailed explanation of the experiment design and statistics (GLMM with including individuals as a random effect). Or, do the authors intend to use the data for Fig. S1 for the other paper? If so, it would be good to mention “in review” when they show the figure, since we do not know whether we have a way to access to the details of the results for Fig. S1 in somewhere in future.

As for the experiment for Question 4 (Table S3.3, S3.4, Table S5), I have two things to recommend that the authors should reconsider. First, please include individual plant as a random effect for their statistics. As far as I read the manuscript, the authors included only experimental block as a random effect, just the same way for the other analyses. However, since each individual should have several flowers or fruits, I believe that they need to use GLMM with including not only the block but also individual (therefore, individual nested in block) as a random effect for this results. Second, please show us the statistics independently for each species instead of the inclusive one as they show in Table S5. Since the authors suggest there is an asymmetrical effect in the reproductive interference between the two species, I believe we need to evaluate the effect of number of conspecifics on the seed viability independently for each species.

By the way, I also have one very minor question: what is “ESM” ? (e.g., P4 L116)

That’s all for my comments.

Author's Response to Decision Letter for (RSPB-2020-0559.R0)

See Appendix B.

Decision letter (RSPB-2020-0559.R1)

16-Apr-2020

Dear Dr Christie

I am pleased to inform you that your manuscript entitled "Frequency-dependent fitness and reproductive dynamics contribute to habitat segregation in sympatric Jewelflowers" 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.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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

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

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

March 11th, 2020

Dear Editor,

Please accept revisions to our manuscript, now entitled *"Frequency-dependent fitness and reproductive dynamics contribute to habitat segregation in sympatric Jewelflowers"* (RSPB-2019-1665). We thank the referees for their thoughtful critiques, particularly the detailed review provided by Referee 2. We have thoroughly addressed all of the referees' comments, and as a result, have significantly improved the quality of the manuscript. We believe the revised manuscript will be of great interest to a broad readership at *Proc. B*.

The referees consistently identified several important areas for improvement; accordingly:

1.) We have substantially re-organized both the Methods and Results sections of the revised manuscript. Following the advice of Referee 2, we have now structured the manuscript based on the primary research questions of the study. Each research question now has independent and aligned Methods and Results sections, as well as an independent figure. We believe the new linear organization of the manuscript greatly improves its clarity. Additionally, we have moved many of the most salient methodological details into the main text (please see specific details in our responses to Referee 2 below). All of the most crucial details and findings now appear uniquely in the main text of the revised manuscript, while background and supporting data are presented uniquely in the ESM section.

Due to the revised format of the manuscript, with Question 2 addressing the effects of habitat-specific establishment, survival, and growth, and Question 3 addressing immigrant inviability, including habitat-specific fecundity, we have also added a small amount of new data on seed production in experimental migrants. Previously we only analyzed seed viability of experimental migrants, but given the new structure of the manuscript, we felt including both seed production and seed viability provided a more complete picture of overall fecundity in migrants.

2.) As recommended by both referees, we now employ GLMMs (random-effect models) as opposed to GLMs (fixed-effect models) for analyses of our field transplant experiment (Question 2 and Question 3), now treating experimental block as a random effect. Similarly for Question 4, we now employ a GLMM with a random effect for block. For this final analysis, as we were interested in factors influencing seed viability in experimental migrants, we first employed a model selection approach to identify the most explanatory model, and then present the best-fitting model, as well as Table S4 describing all adequate models.

Due to the substantial re-structuring of the revised manuscript, a "track changes" version became overly cumbersome. As an alternative, we have provided detailed responses to each of the referee's comments below, including references to line numbers in the revised manuscript. The referee's original comments are provided in italicized text, and our revisions are indented in standard text.

Associate Editor
Board Member: 1
Comments to Author:
I have now received two reviews of the manuscript "Frequency-dependent fitness and reproductive interference contribute to habitat segregation in sympatric Jewelflowers." The manuscript explores a number of different ecological factors that may maintain habitat segregation in two incompatible species. Although several factors contributed modestly, the major explanatory factor for habitat segregation was that low frequency migrants suffered poor seed quality (likely due to receipt of heterospecific pollen). The work addresses important issues of coexistence, in particular, whether being a minority a benefit or a detriment.

Both reviews were fairly positive, suggesting that the manuscript has potential to be a strong contribution. One review recommended relatively modest changes, while the other had more substantial recommendations, in particular to the organization of the methods and results. In addition, both reviewers raised statistical questions.

A substantial revision could produce a manuscript that makes a strong contribution to our understanding of local-scale habitat segregation.

Please see our summary comments above.

Referee: 1

Comments to the Author(s)
This study evaluated abiotic niche partitioning, local adaptation, and a part of species interaction, to explain the local micro-parapatry distributions of the two Stereptanthus species. I admire their really elaborated investigations, examining different kinds of candidates that may cause the paratatry carefully, presenting the results clearly, and discussing the results with a wide range of reference including not only plant but also animal studies. Although this study is a sort of one part of a serial studies, this paper itself can be treated as an independent study to discuss the effect of the resource competitions or environmental preferences on the distributional relationships between closely related plant species. I, therefore, enjoyed reading this paper, and like to read other papers the authors previously presented or are preparing for the two Streptanthus species. Only one thing I am concerned is the statistical analysis: I am afraid that results for the seed viability should be analyzed with not GLM but GLMM (and if possible, I hope the authors add the data for their NMDS). Otherwise, I did not find any other things to complain! I am really looking forward to seeing this paper in the journal, and also seeing their studies completed in near future.

1.) We have added a more thorough description of the data used to assess habitat differences to the main Methods section of the revised manuscript.

This section now reads: "We quantified multiple physical site attributes that might influence habitat affinity including: elevation, slope, aspect, soil depth, soil color (a proxy for chemical composition and thus the relative weathering rate of serpentine parent materials), ground cover, and substrate rockiness at 32 sites at McLaughlin. We also analyzed soil chemistry (NO_3, P, K, Na, Ca, Mg, boron, cation exchange capacity, organic matter, pH, and saturation percentage) and soil texture (% sand, % silt, % clay) at 20 sites. We visualized niche breadth via NMDS ordination (*metaMDS* function in the *vegan* package[59] in R[60]), and tested for abiotic niche differences using a multi-dimensional permutation approach (*adonis2* function in *vegan*). We also measured soil moisture at sites occupied by each species by measuring gravimetric water content of soils throughout the season, and assessed difference using t-tests and Mann-Whitney tests (*ESM - Field soil moisture*)." (Lines 126-136)

We have also added the raw data used for the NMDS ordination in Figure 1A to a new table (Table S1).

2.) We now use GLMMs in Question 2 and Question 3 to analyze data from our field transplant experiments. We have retained the use of GLMs to analyze greenhouse experiments, as we randomized pot and rack locations bi-weekly throughout these experiments, thus there was no block/location effect.

For our analyses in *Question 4 - What factors influence seed viability in experimental migrants?*, we now utilize a GLMM (again with a random effect for experimental block) combined with a model selection approach (based on AICc) to determine the models and predictor variables that best explain variation in seed viability among experimental migrants.

Initially we planted the reciprocal transplant experiment solely as a test of local adaptation. We did not originally intend to assess variation in seed viability among experimental migrants; however, upon exploring these data, we realized there was considerable variability that merited analysis. There are several important factors that might influence seed viability in migrants, including: 1) the local density of residents, 2) the local density of migrants, 3) plant size and fruit number, which might be influenced by resource availability and/or competition with residents, and 4) microsite quality. We were interested in which of these factors might best explain the observed variation in seed viability. Unfortunately we did not measure resident density in the field, but we were able to fit a GLMM with the other predictor variables, including possible factors associated with local density dependence, local resource availability and/or the effects of competition, as well as local microsite quality.

Because we did not *a priori* set out to test the relative effects of each predictor, we employed an iterative model selection approach to determine the best-fitting model explaining variation in seed viability. We present findings from this GLMM (with a random effect for block) in the revised manuscript, as well as a model selection table (Table S4), describing all of the adequate models.

Referee: 2

Comments to the Author(s)
The paper compares fitness of two parapatric Streptanthus species between their resident and foreign habitats to test for home-site advantages. It uses a multitude of experimental approaches to gain results for a comprehensive set of fitness components. The topic is highly interesting and I highly appreciate such a work. However, I recommend a major revision for the following reasons.

In contrast to the excellent introduction and discussion, the methods and results of the experiments are presented in a highly confusing way – both in terms of the methodological description as well as the illustration of results.

Due to the splitting of the description of experimental designs between i) the main text and the supplementary material and ii) between different sections (e.g. local adaptation – seed viability; germination – local adaptation) within both parts leaves it to the reader to collect and digest the necessary pieces of information. Thus, it is extremely hard for the reader to grasp the design of single experiments as well as the reasoning why they were performed. This problem is further enhanced i) by the redundancies emerging from the splitting and ii) by the fact that a bulk of necessary methodological details are simply kept secret. Thus, I strongly recommend to re-think the structuring of the method section in the main text and I further question the use of the supplementary materials to provide descriptions of experiments and analyses. I suggest a consequent structuring either along experiments, fitness components or questions.

1.) We have substantially re-organized the revised manuscript following your suggestions. The Methods and Results sections are now organized by the major research questions presented in the Introduction. Individual questions and sub-questions of the Methods and Results are now aligned with complementary sections describing both the Methods and Results for each specific question. Each portion of the Results also now has its own individual figure, illustrating the main findings relating to that question. We believe this makes the manuscript much more linear and easier for readers to digest.

We have moved as many of the methodological details as possible into the main text of the revised manuscript, and have moved supporting experiments and data into the ESM. To improve clarity, we have also removed redundant text and figures from the ESM section such that the information presented in both the main text and ESM is unique. Additionally, we have attempted to be as explicit as possible about experimental details throughout the methods sections following your specific suggestions below.

The presentation of the results needs to be revised too. The allocation of results (text as well as the figures) to the main text and the supplementary material is not clear to me. Both include information of similar relevance considering the main aim of the study (see line 77-86): e.g. Question 1 and 2 are answered in the main text and the supplement, respectively. Moreover, the text of the result section includes many statements/sentences (e.g. those following "suggesting ...") which are clearly interpretations and, hence, should be shifted to the discussion section. In addition, the most important results are given in the section "factors shaping spatial isolation" (including Table 1), which is not mentioned in the methods and not described at all. Hence, it is really hard to grasp its content to get the main message of the paper.

We have re-structured the manuscript such that most salient results and figures are presented in the Results section of the main manuscript, and supporting or corroborating data and figures are presented only in the ESM. We hope that this re-structuring presents a clearer message to the reader, and highlights the main findings without unnecessary distractions.

We have removed interpretations from the Results section, and now provide only concise and numerical summaries of our analyses. Interpretations have been moved to the revised Discussion.

We have removed Table 1 from the revised manuscript, as it was previously unclear. We have attempted to highlight the relative contributions of different factors in contributing to spatial segregation in two ways – both by presenting an independent summary figure for each question (see all figures in the revised manuscript), and by verbally describing the relative contributions of each factor in the revised Discussion. (Lines 338-359)

Concerning figures, I question the use of a (bold) line connecting the treatments/classes to illustrate the "reaction norm" in Figure 1, S3, S4, S5 for two reasons: i) These lines indicate a transition or at least a connection between the treatments which simply does not exist. Furthermore, you have no clue about the values of intermediate states (if they exist), but they certainly need not to be intermediate; ii) the slope of these lines depends on the scales of the axes, which are highly arbitrary (particularly but not only for the x-axis). So, I recommend to find another illustration, remove the lines or at least chose a less eye-catching style (i.e. less than those for the points and whiskers). Moreover, I prefer to see raw data instead of model predictions in all figures. Furthermore, the captions of most figures describe the pattern illustrated in the respective panels, but methodological details necessary or desirable for the understanding of the figure content are frequently missing.

We have changed the formatting and line width in the figures to be much less bold. We have retained the "reaction norm" lines (although following your suggestion we no longer use this term in the main text), as we believe many readers interested in local adaptation will be familiar with the type of figure (advocated in the seminal review by Kawecki and Ebert 2004, and used in many related papers since then).

We have included mean model predictions and error bars representing 90% confidence intervals to facilitate easy interpretation of the figures, and to maintain consistency between different types of figures throughout the revised manuscript. Following your suggestion, we have also added the raw data to all of the revised figures in the main text.

We have attempted to provide important methodological details in the Methods sections following your specific comments below, and have moved explanations of patterns to the associated Results sections. Figure captions now provide concise descriptions and associated information needed to interpret the figures.

For all the points raised above, see numerous detailed comments below.

Potential non-independence of measurements obtained from individuals/seeds within one site are considered for by using site as fixed effect in the regression analyses. However, potential non-independence of individuals/seeds within a block (within a site) in the transplantation experiment(s) are disregarded. Does this require the use of nested random-effects in a GLMM?

As suggested, we now employ GLMMs for analyses of the field transplant experiment, now adding a random effect for experimental block (Question 2 and Question 3). We also now use a GLMM for our analyses in Question 4 (again including block as a random effect). Please see our response to Referee 1 above, which details the mixed model and model selection approach used here.

Detailed comments to the main text:

L10: replace "lack" by "scarcity"

This sentence now reads: "We find some evidence for abiotic niche partitioning and local adaptation, however differential survival across habitats cannot fully explain a scarcity of coexistence." (Lines 8-10)

L12-15: rewrite the sentence: "low-frequency migrants" is likely to be misunderstood and opens the question: What about "high-frequency migrants"

This sentence now reads: "Experimental migrants suffered reduced seed production and seed viability in sites occupied by heterospecifics, and we infer that heterospecific pollen transfer by shared pollinators contributes to reproductive interference, resulting in wasted gametes when the two congeners come into close contact." (Lines 12-14)

L17-20: This sentence is very general and unspecific. I encourage one that is more pronounced and bases more on the concrete results of the study

This sentence now reads: "Differential adaptation and resource competition have often been evoked as primary drivers of habitat segregation in plants, yet negative reproductive interactions – including reproductive interference and decreased fecundity among low-frequency migrants – may also contribute to non-overlapping distributions of related species along local tension zones." (Lines 16-20)

L32: remove the space after "scales"

This sentence now reads: "The complex interplay between geologic, evolutionary, and dispersal histories shapes species ranges at broad scales[1,2], while adaptation to environmental conditions[3,4] and random chance associated with community assembly[5,6] can shape species distributions at local scales." (Lines 31-22)

L79: include the family (=Brassicaceae)

This sentence now reads: "The reproductively isolated California Jewelflowers, *Streptanthus breweri* and *S. hesperidis* (Brassicaceae) are restricted to similar serpentine habitats and occur within meters of one another in the sympatric portion of their ranges, yet rarely coexist in intermixed patches." (Lines 79-81)

L142: to underline the character of a fitness component, plant height might be referred to as plant growth and fruit production as fertility.

We now refer to "plant growth" throughout the revised manuscript.

This particular sentence now reads: "We quantified survival, plant growth (which reflects both the intrinsic ability of a plant to grow in a foreign habitat, as well as the potential effects of resource competition with residents occurring at ambient densities), and fruit production." (Lines 162-164)

L145: "…in which a species has higher fitness at the home site". Personally I would prefer the use of the terms "resident/migrant" over "home/away" throughout the paper. I suggest not to mix these terms to avoid synonymy.

We now use the terms "resident" and "migrant", as consistently as possible throughout the revised manuscript, particularly when referring to individual plants. When referring to sites, we use "resident" and "migrant" when possible, and only in cases when that usage is awkward do we retain the terms "home" and "away" to refer to specific sites or habitats.

This sentence now reads: "Here, local adaptation is demonstrated by a significant species*habitat interaction[61], in which the resident species has higher fitness at the site it naturally occupies." (Lines 166-168)

L146-149: It seems that germination was intended to be measured as character of the local adaptation experiment, which is perfectly right. It might happen that is a (nearly) total failure in one character, rendering additional setups or changes in the analyses necessary. Nevertheless, I suggest to present germination as part (4th character apart from survival, growth and reproduction) of the intended experiment – of course by providing the details (deviating from other characters).

Question 2 now asks whether local adaptation associated with establishment, survival, and growth can explain patterns of spatial distribution at a fine-scale. Thus, we now present data on field germination here (Fig. 2A), together with the other data pertaining to local adaptation for survival (Fig. 2B), plant growth (Fig. 2C), and fruit production (Fig. 2D).

L153: Please specify at which stage (seed, seedling, juvenile, adult) these migrants were transplanted.

It should also be clear for which stages the term migrant is used: only for (adult) plants or also for seeds (compare e.g. Line 153 "migrants" to Line 136 "away seeds") -> unify if necessary

We planted seeds for all field experiments.

These sections now reads: "Using adjacent population pairs replicated at three sites, we transplanted seeds approximately 10-100m from their native sites to an adjacent serpentine patch, or to an adjacent portion of the same patch occupied by their congener." (Lines 140-142)

"In our field transplant however, we planted migrant seeds into experimental blocks with resident seeds, and into habitat patches dominated by native residents occurring at ambient densities (on average four individuals/m^2)." (Lines 193-195)

L155: replace "~" by "on average"

See above.

*L160ff: I miss the details of the design: e.g. How many migrants? Why 41 blocks? Did you use 2 sites of each species (3*2= 6 sites)? Specify whether the 6 sites are the same as used in other experimental approaches of this study; "outcrop" seems to be synonym to "site" -> unify; The use of the term "reciprocal transplant experiment" is confusing and ambiguous (compare Line 169). Find unique terms for each of your experiments (e.g. reciprocal seed sowing experiment; adult transplantation experiment)*

The description of our field experiment now reads: "At each site (three paired sites, n = 6 total), we established ten experimental blocks (1.0m x 0.5m) of 50 seeds (25 of each species) planted in a checkerboard pattern (n = 3000 seeds total). " (Lines 144-146)

Initially we planted seeds into 60 experimental blocks, however migrants only survived in 40 of these blocks (we erroneously previously reported that migrants had survived in 41 blocks), thus analyses of survivors are based on individuals from these experimental blocks.

We now use the independent terms "field transplant experiment" and "lathhouse soil transplant" to differentiate between our field experiment, and our follow-up experiment conducted with field soils in the lathhouse.

To further clarify that we conducted a single field experiment to address Question 2, Question 3, and Question 4, our description of Question 4 now reads: "At the end of our field transplant experiment, we assessed whether surviving migrants (i.e., individuals transplanted into foreign habitats) suffered decreased seed production and seed viability compared to residents (i.e., conspecific individuals growing in their native habitats). We quantified seed production and seed viability in 120 surviving migrants by harvesting 609 fruits and 7,411 seeds from six sites and 40 blocks." (Lines 201-205)

We have unified the language throughout the revised manuscript to use the term "site" when referring to experimental locations, and "outcrop" only when specifically referring to rocky serpentine patches on the landscape.

L181: "number of fruits per home plant" is not exactly defined: There is more than a single home plant. Do you mean the mean of all home plants?

Yes, we used the mean number of fruits of all residents. This section now reads: "We first fit a full GLMM (glmer function) predicting seed viability of all surviving migrants (n = 119 with known fruit number) with species, site, number of conspecifics per block (to account for local density dependence), fruit number (to account for plant size and potential resource limitation), average number of fruits per resident within the block (a proxy for microsite quality), and a species*number of conspecifics interaction as fixed effects, with experimental block as a random effect." (Lines 223-228)

L183-184: This rule for excluding individuals is not convincing for me. Stress likely reduces viability in all plants – those above and below the 5% threshold. So, why to exclude the one but include the other? Is there an indication that some individuals are particularly affected to be excluded? If so, mention your reasons to do so and ***give the proportion of excluded plants****. If not, use all plants. Did you exclude plants also from other analyses?*

We now include all survivors in the model. This section now reads: "We first fit a full GLMM (glmer function) predicting seed viability of all surviving migrants...." (Lines 223-224)

L189-190: "We grew native genotypes…" -> be more detailed (from seeds, where, how long, etc.?); in particular, define (or avoid) the term genotype: Did you really produced clones, which were used in each treatment? Or did you simply grow individuals/plants?

We grew individuals/plants. This section now reads: "Here, we grew field-collected seeds in potting soil, either alone, with a single conspecific competitor, or with a single heterospecific competitor (n = 20 per species per treatment)." (Lines 178-180).

L198-200: I do not see a relevant difference between "differences in abiotic niches" and "habitat partitioning" in this study, with the exception that the former and latter refer to the Adonis-test and NMDS-ordination, respectively. In line 130 you refer to the same test as "as species-level habitat differences". Please unify the terminology.

The revised Methods section now reads: "We visualized niche breadth via NMDS ordination (*metaMDS* function in the *vegan* package[59] in R[60]), and tested for abiotic niche differences using a multi-dimensional permutation approach (*adonis2* function in *vegan*)." (Lines 132-134)

The revised Results section now reads: "We found no significant differences in the multi-dimensional abiotic niches of the two species at McLaughlin (*adonis2* permutation test, p = 0.56)…." (Lines 236-237)

L203: There is no test for differences in niche breadth.

This section now reads: "We found no significant differences in the multi-dimensional abiotic niches of the two species at McLaughlin (*adonis2* permutation test, p = 0.56), however *S. breweri* occupies a qualitatively broader abiotic niche than *S. hesperidis* (Fig. 1A), consistent with geographic range differences, in which the smaller-ranged *S. hesperidis* is nested within the larger-ranged *S. breweri*[56]." (Lines 236-240)

L207-208, 212-213, 234-235, 236-244, 254-255,257-258, 261-262, 265-266, 268-269, 271-272, 273-274, 300-303: interpretation -> shift to the discussion

We have shifted all interpretations to the Discussion section of the revised manuscript.

L214: the p-value does not match those in the table (p < 0.05). If you provide it here, you should goals give the p-values for other factors like plant height (L218). ***Generally, I prefer to see the exact p-values instead of classes (<0.05, <0.01, <0.001).***

We now provide exact p-values throughout the revised manuscript (except for those that are < 0.001, in which, for example, extremely small p-values such as "2E-16" become cumbersome). Exact p-values are provided in the ESM tables for each model.

L219-220: rewrite

This sentence now reads: “In the field, survivors of both species grew to similar heights in both habitats (Fig. 2C; species*habitat interaction, p = 0.79;Table S2.4), and produced an equivalent number of fruits in both habitats (Fig. 2D; species*habitat interaction, p = 0.15; Table S2.5).” (Lines 261-263)

Figure 1: It is not clear, whether 1D is based on values of all plants or only those with 7 or 8 conspecific neighbors per block.

These data are now presented in Fig.3D, and include all surviving migrants regardless of the local density in each block.

This section now reads: “In the field, experimental migrants (i.e., all surviving individuals growing at sites occupied by heterospecifics) had significantly reduced seed production (species*habitat interaction $p < 0.001$; Fig. 3C) and seed viability (species*habitat interaction $p < 0.001$; Fig. 3D) compared to native plants growing outside the immediate influence of heterospecifics.” (Lines 295-298)

L223: Define or better avoid “reaction norm”

This figure caption now reads: “Average fitness associated with survival and growth of residents and migrants in a field transplant experiment.” (Lines 265-266)

L229: remove “in which … adaptation)”

This section now reads: “When replicating our field experiment in the lathhouse, growing plants in intact field soils, we did not find evidence of a home-soil survival advantage (Fig. S4A; species*soil type interaction, p = 0.45; Table S2.6).” (Lines 271-273)

L230: I suggest to avoid “largely consistent” in case of having an opposite pattern in 1 out of 3 comparisons. -> add “largely consistent … experiment for plant growth and fertility (…). In contrast, …”

The language in this section is now explicit, and reads: “When replicating our field experiment in the lathhouse, growing plants in intact field soils, we did not find evidence of a home-soil survival advantage (Fig. S4A; species*soil type interaction, p = 0.45; Table S2.6). Here, S. breweri qualitatively survived better in S. hesperidis soils (92% vs. 70% in its own soils) which have a higher water-holding capacity (Fig. 1B), indicating physiologically at least, that S. breweri may be able to thrive in those soils commonly occupied by S. hesperidis. Results for plant growth (Fig. S4B; Table S2.7) and flower production (Fig. S4C; Table S2.8) were consistent with findings from the field experiment, in which there was no evidence for a home-site or a home-soil advantage.” (Lines 271-278)

L261: the 50:50 and details of the design are lacking in the method. Description

These details are now provided in the Methods section, and read: “To determine if intrinsic soil properties influenced seed production or viability of migrants, we grew plants (n = 152; n = 76 per species) in a mesh-covered lathhouse at UC Davis, in intact soil cores collected from

the six sites used in the field experiment (*ESM - Lathhouse soil transplant*)." (Lines 184-188)

Figure 2: For me, panels B and C do not show very important -> could be moved to the supplement

The panels have been removed from the revised figure (Fig. 4).

L277: "fitness" is unspecific -> replace by "seed viability"

This figure caption now reads: "Density-dependent seed viability of experimental migrants…." (Lines 322)

L285-287: According to Fig. S6, plant height of S. hesperidis does not (significantly) depend on the presence/absence of a competitor, which strongly contrasts this statement- even if it is numerically correct (i.e. the mean is lower with competitors)!

Thank you for pointing this out. This section now reads: "*:* In the greenhouse competition experiment, in which individuals grew within 1-2cm of one another in the same pots, patterns for plant growth (species*competitor interaction, $p = 0.12$; Fig. S5A) were consistent with patterns from the field (Fig. 2B), in which both species reached similar heights in both competitive environments. We found no differences in the relative effects of intraspecific compared to interspecific competition on plant growth for either species (Fig. S5B; Fig. S5C)." (Lines 280-285)

L299: "seems" is not a good choice in the results section

This section has been removed from the revised manuscript.

*L298-300: Is this statement deduced from the fact that only "seed viability" has "***"? give a more comprehensive description of the table!*

This table has been removed. A verbal discussion now appears in the Discussion section. (Lines 338-359)

Table 1 is very hard to understand: The meaning of all the values is dubious at first glance. It seems that the home and away fitness represent predictions of models for each component and the relative fitness is the latter divided by the former. Both need to be explained. Units should be given for all components: by the way values for germination and survival represent proportions (range:0-1) not % (range:0-100). Furthermore, the order of components is not clear and it is hard to connect the fitness components mentioned in the table to the respective experiments and/or sections of the methods. In addition, results from the greenhouse germination experiment are not included in Table 1: Are they not important? If so, remove the experiment from the manuscript, otherwise show its results with the same priority as that for survival, growth and fertility.

This table has been removed from the revised manuscript; see above.

L319: Isn´t "competition" inherent to "local adaptation"? If so I suggest to remove it here. Otherwise I appreciate to include it into the questions (equivalent to the other factors: niche

partitioning, local adaptation and rarity)

The revised manuscript indicates that the effects of competition might manifest in the field transplant experiment, as described in the Methods section for Question 2: "We quantified survival, plant growth (which reflects both the intrinsic ability of a plant to grow in a foreign habitat, as well as the potential effects of resource competition with residents occurring at ambient densities), and fruit production." (Lines 162-164).

Fig. 2C depicts plant growth in the field, and captures the effects of competition in an ecologically realistic context (i.e., fairly low density of individuals).

Additionally, we assessed the relative effects of intra- and interspecific competition through a separate greenhouse experiment, in which plants were grown immediately adjacent to one another. We include these experiments as a part of Question 3 ("*Do migrants experience immigrant inviability due to: (a) competition…).*

We unify these findings in the Results section: "In the greenhouse competition experiment, in which individuals grew within 1-2cm of one another in the same pots, patterns for plant growth were consistent with patterns from the field (Fig. 2B), in which both species reached similar heights in both competitive environments (species*competitor interaction, $p = 0.12$; Fig. S5A). We found no differences in the relative effects of intraspecific compared to interspecific competition on plant growth for either species (Fig. S5B; Fig. S5C)." (Lines 280-285)

Detailed comments to the supplement:
L50: This contrasts line 98 ff: six of these sites are intermixed

A description of the habitat characterizations has been moved to the main manuscript. We surveyed 32 total sites for the overall site characterizations; soils from 20 sites were tested for texture and chemistry. (see Lines 126-136 of main text)

L55: subscript "3"

Change made.

Figure S1: remove titles (not necessary, not complete); ***I expect some (significant) differences in single variables. They might be shortly mentioned or illustrated. A methodological description of the chemical and texture analyses is appreciated.***

Titles have been removed from the revised figure (now Fig. S2). We have also added Table S1, which summarizes differences in single variables.

L61: "abiotic site conditions" are referred to as "physical site attributes" in line 129 -> unify; replace "locations" with "sites"

We have unified this language; this sentence now reads: "NMDS ordination of 33 physical site attributes, including soil texture and soil chemistry, for 20 *S. breweri* (n = 10) and *S. hesperidis* (n = 10) sites at McLaughlin." (Lines 138-139 ESM)

L63: How is niche breadth defined?

We defined niche breadth using the *ordiellipse* function from the *vegan* package in R, which draws convex hulls around points in an ordination, using the standard deviation of point scores, and a 95% confidence limit.

L69: "… at randomly…"

This sentence now reads: "At each site at each time point, we collected 10 soil cores from immediately beneath native plants at randomly selected microsites (n = 60 total samples per time point)." (Lines 71-73 ESM)

L70-71: remove "10 each for…each site" because it is confusing: site-species-combinations are unique

See above.

L74: give the formula

This sentence now reads: "We used the weights of field-wet soils and dried soils to calculate gravimetric water content (Θ_d = [weight of wet soil - weight of dry soil] / weight of dry soil)." (Lines 76-77 ESM)

Figure S2: mention the test used for obtaining the significance levels (also in subsequent figures)

The previous Figure S2 now appears as Fig. 1B in the main text. This portion of the Methods section now reads: "We also quantified soil moisture at sites occupied by each species by measuring gravimetric water content of soils throughout the season, and assessed difference using t-tests and Mann-Whitney tests (ESM - Field soil moisture)." (Lines 134-136 main text)

L84: "…S. breweri (blue) and S. hesperis (green)…"

We have added a legend to the revised Fig. 1B.

L88: The experimental design of "germination" and "local adaptation" is highly related/intermingled. It would more readable when they are described together; some details are lacking

Details of the field experiment are now unified in the main Methods section of the paper (Question 2). The follow-up germination experiment (using seeds and soils from all six experimental sites) is now described independently in the ESM. (Lines 86-96 ESM).

L90: give n of seeds sown

The methods for assessing germination success in the field are now described in the main Methods and Results sections. (Lines 147-155 of the main text)

This methods section now reads: “We initially transplanted seeds in the winter of 2014-2015, however the vast majority (n = 2800) of seeds failed to germinate likely due to a winter drought. Some seeds did germinate at two sites in the spring of 2015 however (n = 200 of 1000 seeds planted at these sites), and here we assessed germination success to determine if site-specific germination might prevent each species from becoming established.” . (Lines 147-155 of the main text)

l94: 240 per site/species/in total?

This section now reads: “Here, we first collected field soil from immediately under native plants, then planted field-collected seeds (n = 240 total) into field soils in germination trays, and placed trays under a mist-bench in the greenhouse.” (Lines 90-92 ESM).

L95: Give the interval of observation, the conditions (temperature, light, humidity, etc.)
Figure S3: Should “soil” be replaced with “outcrop”; significance levels are nit indicates

Details have been added to this section. (Lines 94-96 ESM).

L109: Give the number of sown seeds

These details are now clarified in the main Methods section of the revised manuscript.

L113: at the end of the growing season?

These details are now clarified in the main Methods section of the revised manuscript.

L115: provide these numbers: at least for each species at each site

These details are now clarified in the main Methods section of the revised manuscript.

L121: give the conditions of the lathhouse (temperature, etc.); give the date of scoring

See above.

L124: add “(see main text)” at the end of the sentence.

This sentence now reads: “We assessed patterns of local adaptation to specific soils using GLMs with species, soil type and a species*soil type interaction as fixed effects.” (Lines 108-109 ESM).

Figure S4: remove titles

We have removed the titles from this figure.

L132: “…transplant experiment conducted…”

This figure caption now reads: “Average fitness associated with survival and growth in habitat-specific soils in the lathhouse.” (Lines 161 ESM)

L137: Specify conditions and the design of this experiment

This experiment is now described in the "Lathhouse soil transplant section". (Lines 98-114 ESM)

L140: "and while keeping ...(D40 Deepots)" shift beyond "McLaughlin" at line 143

This section now reads: "While keeping the soil structure as undisturbed as possible, we transferred soil cores into cylindrical greenhouse pots (D40 Deepots), and then transplanted greenhouse-grown germinants (n = 76 per species) into site-specific soils." (Lines 100-102 ESM)

Figure S5: panel A seems to be a copy-paste of Figure 1D. thus, I suggest to add panel B to Figure S4 (in accordance with the style of Figure 1). Maybe the text "Field soil and seed viability" can be integrated into "local adaptation"

This redundant figure has been removed from the ESM; data on seed production and seed viability in both the field and lathhouse experiments now appear in the revised Fig. 3.

Figure S6: Test at the 90% confidence level are not necessary -> omit

All tests are now conducted at $\alpha = 0.05$. These details are now provided in the main Methods section. (Lines 180-183 of main text).

Supplementary tables: avoid abbreviations (GH, NJ, QV, etc.); improve the adjustment of values; give the exact p-values; might be merged to a single table

We have removed abbreviations for sites in these tables, and now provide exact p-values.

Appendix B

April 14th, 2020

Dear Editor,

Please accept final revisions to our manuscript, entitled *"Frequency-dependent fitness and reproductive dynamics contribute to habitat segregation in sympatric Jewelflowers"* **(**RSPB-2020-0559). We describe our final revisions below:

Editor's comments

1.) We have elected to retain Fig. 4 in the main body of the manuscript, and Fig. S1 in the electronic supplementary material (ESM). We believe that the data presented in Fig. 4 are a crucial part of the story and corroborate other findings of the study. Specifically, the data presented in Question 3/Fig. 3 show that rare migrants suffer fitness costs, and Fig. 4 further builds on these findings, demonstrating that the relative degree of migrant rarity is also correlated fitness (i.e., more rare migrants suffer greater fitness costs than less rare migrants).

Reviewer's comments

2.) We have followed the reviewer's suggestions concerning the analyses associated with our pollination experiment (the data presented in Fig. S1). We now use a GLMM with random effects for maternal and paternal individuals in our analysis, and we have added an additional table summarizing this model (Table S0). We have also added additional methodological details describing this experiment to the supplement (ESM Lines 66-74).

Given the page constraints in *ProcB*, we have elected to retain the crossing data in the supplement and not in the main body of the manuscript. While these data are crucial to a complete understanding of the reproductive dynamics occurring in the field, they corroborate previously published findings showing that *S. breweri* and *S. hesperidis* are reproductively isolated by intrinsic barriers. These data add to previous findings by showing the intrinsic reproductive isolation exists between the specific *S. breweri* and *S. hesperidis* populations where we conducted the field experiment. Additionally, the pollination experiment also shows that individuals receiving mixed pollen (likely what is occurring in intermixed patches in the field) also suffer reduced seed viability compared to individuals receiving pure conspecific pollen.

We agree that both pollen limitation and reproductive interference could affect the reproductive success of the focal species. Pollen limitation might reduce overall seed production of migrants (Table S3.3), however there is no reason to believe that it alone would also decrease seed viability (Table S3.4). Thus, the findings presented in Figure 3D appear more consistent with reproductive interference, however given the nature of our experiment, we cannot definitively determine the relative contributions of reproductive interference and pollen limitation in driving these patterns. As the reviewer indicates, both phenomena might be contributing to the pattern shown in Fig.4. We verbally address this possibility in Lines 360-362 and Lines 418-419.

3.) In terms of the analyses for Question 4, individual plants were our experimental units here. For each individual plant, we counted the number of fruits, and the total number of viable and inviable seeds in all fruits combined (i.e., points in Fig. 4 indicate seed viability of individual plants, not of individual fruits within plants). We do not have seed viability data on a fruit-by-fruit basis, thus are unable to include individual plant as a random effect in this model.

The best-fitting model for this analysis did not include a term for species (Table S4), thus this factor is absent from Table S5. For the same reason, we fit a single line in Fig. 4, instead of two lines for species-specific model predictions. Our discussion of the possible asymmetries between species is meant to connect with the broader literature on reproductive interference and frequency-dependence, and we are explicit that any asymmetry in *S. breweri – S. hesperidis* is qualitative, and not statistically significant in the best-fitting model. However, because a species term was present in three of seven of the top models, and qualitatively apparent in the data (see both extremes in Fig. 4), we devote a small portion of the discussion to this possibility.

Lines 409 – 415 read: *"Here, few of the best-fitting models explaining variation in seed viability in migrants included a significant species effect (Table S4); however qualitatively, some S. breweri occurring alone (n = 1 in Fig. 4) still produced seeds with high viability. This suggests that species-specific pollinator constancy or conspecific pollen precedence might promote assortative mating in mixed-species patches. On the other hand, low-density S. hesperidis individuals may lack equally well-developed mechanisms enabling high reproductive fitness when rare."*

4.) "ESM" refers to "Electronic Supplementary Material".

Since we retained Figure 4 in the main body of the manuscript and addressed the reviewer's additional comments in the supplemental methods section, the main text remains substantively unchanged since the previous submission. We have made several small clerical corrections.

We thank the editor and referees for their time, effort, and insightful comments throughout the review process.