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The evolution and fate of diversity under hard and soft selection

Patrick Chen and Rees Kassen

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Original submission: 13 May 2020
Revised submission: 31 July 2020
Final acceptance: 6 August 2020

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

Review History

RSPB-2020-1111.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?
Excellent

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

Is the length of the paper justified?
Yes

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

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

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

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors verified the hypothesis that diversity is maintained under soft selection but not hard selection using bacterial populations under a laboratory work. They found that both resistant and sensitive types for an antibacterial agent were coexisted under negative frequency dependent soft selection for a long time. Their experiments were well designed and its results well supported basic ecological hypothesis. This study can be published in Proceeding B. However, presentation of results should be revised before publication.
L101–105, many readers of Proceeding B including me may not be so familiar with bacterial experiments. Moreover, experimental design of hard and soft selection is an important point of this study, but a little bit hard to understand. Therefore, it is better that the experimental design is shown in a schematic figure to help the understanding of readers.
L122–123, there are lacking of the value of F1,19 in the final frequency.
L132, evolved populations -> populations
In Figs 1–4, the authors used two different symbols, filled circle and triangle for hard and soft in figs 1 and 2, but for resistant and sensitive in Figs 3 and 4, respectively. This causes some confusion and these two symbols are hard to distinguish when their sizes are too small, e.g., in Figs 2 and 4. It is better to use different symbol sets for selection and resistant types.
In Figs 2 and 4, although the authors used shaded region for drawing SE, many readers will expect CI when they will see the shaded region. Moreover, when the regression coefficient is not significant, it may be better not to show regression line and shaded area, i.e., day 40 of hard selection in Fig. 4.
In Fig. 1, it is better to fit more smooth curve by adding more predictive points on the X axis.
In Fig. 2, please add explanations of MIC and LB.

Review form: Reviewer 2

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?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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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.

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The authors compare effects of hard vs soft selection on the fate of diversity in an evolution experiment in which each population adapts to a regime with two spatial components, one with and one without antibiotic, but in which dispersal between environments either varies with patch productivity or is held equal. Three significant effects of the manipulation are found, first on the final frequency of antibiotic-resistant bacteria in the context of their evolution experiment, second on the temporal dynamics of fitness increase by antibiotic-sensitive (but not ab-resistant) bacteria and third and most importantly, on the qualitative evolutionary fate of negative-frequency dependence of fitness initially present early in evolution under both hard and soft selection but which disappears over time under hard selection while being maintained under soft selection.

Minor points:

L48 - delete ‘are’

L57-L58 - Here isn't the meaning "resulting in the eventual fixation of the type that is fittest in the the niche that contributes most to the population"?

L122 - missing F value

This study appears to be well conducted and is a significant contribution to our understanding of eco-evolutionary mechanisms of diversity maintenance. The documentation of real-time evolutionary disappearance of negative-frequency dependence is intriguing and, as the authors point out, of significance for ecologically-informed models of diversity evolution.

Decision letter (RSPB-2020-1111.R0)

15-Jul-2020

Dear Mr Chen:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and Associate Editor find a lot to like about your manuscript, however they have raised some concerns about presentation and the degree to which your hard selection regieme matches that outlined by the classic models (all outlined in more detail below). Thus, I invite you to revise your manuscript to address these concerns.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

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

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the 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.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

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If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

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In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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

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].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This ms reports an interesting microbial evolution experiment, testing the divergent predictions of "hard" and "soft" selection in spatially heterogenous environment (one patch with, the other without an antibiotic). The authors find that, as predicted, soft selection is more conductive to the maintenance of polymorphism with respect to antibiotic resistance. Through additional experiments they demonstrate the continuing existence of negatively frequency-dependent selection under the soft selection treatment, as expected under this mode of population regulation. Somewhat unexpectedly, they also find polymorphism and negative frequency-dependence under their hard selection regime, although this disappears in the course of the experiment.

Both reviewers find this ms interesting and suitable for Proc B, and I agree. Nonetheless, both reviewers point to a number of issues in the presentation that need be addressed; to those I add a couple below.

I also have a more substantial criticism and a resulting suggestion to amend the interpretation of the study. In the population genetic models that inspired these experiments, hard selection is defined as characterized by a "global" form of population regulation, with no density regulation operating in each patch. Thus, if the mean fitness in a given patch doubles, the absolute contribution of that patch to the migrant pool also doubles (thus if initially each patch contributed 50% to the migrant pool, with doubling of mean fitness in the first patch the relative contributions would be 67% and 33%). By the authors own admission, this is not the case in their "hard selection" regime: the cultures are growing until the stationary phase; thus there is competition and density do take place to some degree in each patch separately. It is thus important to clarify that the authors' regime does not quite correspond to the assumptions of the classic models of Dempster 1955 or Christiansen 1975. Indeed, it is hard to imagine a spatially heterogeneous scenario under which selection is completely hard; it would thus be more useful to present the hard-soft selection as a continuum rather than as two qualitative alternatives.

Minor comments:
References should be consistently formatted according to the journal requirements.
Figure 3: reiterate in the caption that this is done in drug-free environment.

Reviewer(s)' Comments to Author:

Referee: 1

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In Fig. 1, it is better to fit more smooth curve by adding more predictive points on the X axis.

In Fig. 2, please add explanations of MIC and LB.

Referee: 2

Comments to the Author(s)
The authors compare effects of hard vs soft selection on the fate of diversity in an evolution experiment in which each population adapts to a regime with two spatial components, one with and one without antibiotic, but in which dispersal between environments either varies with patch productivity or is held equal. Three significant effects of the manipulation are found, first on the final frequency of antibiotic-resistant bacteria in the context of their evolution experiment, second on the temporal dynamics of fitness increase by antibiotic-sensitive (but not ab-resistant) bacteria and third and most importantly, on the qualitative evolutionary fate of negative-frequency dependence of fitness initially present early in evolution under both hard and soft selection but which disappears over time under hard selection while being maintained under soft selection.

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L122 - missing F value

This study appears to be well conducted and is a significant contribution to our understanding of eco-evolutionary mechanisms of diversity maintenance. The documentation of real-time evolutionary disappearance of negative-frequency dependence is intriguing and, as the authors point out, of significance for ecologically-informed models of diversity evolution.

Author's Response to Decision Letter for (RSPB-2020-1111.R0)

See Appendix A.

Decision letter (RSPB-2020-1111.R1)

06-Aug-2020

Dear Mr Chen

I am pleased to inform you that your manuscript entitled "The Evolution and Fate of Diversity Under Hard and Soft Selection" has been accepted for publication in Proceedings B. Thank you for a very nice revision of your manuscript, and I look forward to seeing your paper in press.

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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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.

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,
Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
The authors did a good job addressing the reviewers' comment.
T. J. Kawecki

Appendix A

Dr. Sarah Brosnan July 31, 2020

Editor, Proceedings B

Dear Dr. Brosnan,

First, we would like to extend our thanks for the time and effort that the associate editor and the two anonymous referees have invested in reviewing our manuscript. We found their comments helpful and we trust that, by incorporating their suggestions in our edits, our manuscript has been noticeably improved.

Below we include the full text of the original reviews along with our responses (italicized and coloured light blue). A copy of our original submission with our edits marked with Track Changes is also included following our responses. Please do not hesitate to contact us should you require any further information or clarification. We look forward to the outcome of this review process.

Our best regards,

Patrick Chen & Rees Kassen

Associate Editor

Board Member: 1

Comments to Author:

This ms reports an interesting microbial evolution experiment, testing the divergent predictions of "hard" and "soft" selection in spatially heterogenous environment (one patch with, the other without an antibiotic). The authors find that, as predicted, soft selection is more conductive to the maintenance of polymorphism with respect to antibiotic resistance. Through additional experiments they demonstrate the continuing existence of negatively frequency-dependent selection under the soft selection treatment, as expected under this mode of population regulation. Somewhat unexpectedly, they also find polymorphism and negative frequency-dependence under their hard selection regime, although this disappears in the course of the experiment.

Both reviewers find this ms interesting and suitable for Proc B, and I agree. Nonetheless, both reviewers point to a number of issues in the presentation that need be addressed; to those I add a couple below.

We are pleased that our work has been well-received. We thank you and the two referees for the time and effort put in to reviewing our work; it is greatly appreciated!

I also have a more substantial criticism and a resulting suggestion to amend the interpretation of the study. In the population genetic models that inspired these experiments, hard selection is defined as characterized by a "global" form of population regulation, with no density regulation operating in each patch. Thus, if the mean fitness in a given patch doubles, the absolute contribution of that patch to the migrant pool also doubles (thus if initially each patch contributed 50% to the migrant pool, with doubling of mean fitness in the first patch the relative contributions would be 67% and 33%). By the authors own admission, this is not the case in their "hard selection" regime: the cultures are growing until the stationary phase; thus there is competition and density do take place to some degree in each patch separately. It is thus important to clarify that the authors' regime does not quite correspond to the assumptions of the classic models of Dempster 1955 or Christiansen 1975. Indeed, it is hard to imagine a spatially heterogeneous scenario under which selection is completely hard; it would thus be more useful to present the hard-soft selection as a continuum rather than as two qualitative alternatives.

This is a fair and important point, and we thank the AE for raising it. We have now included an additional paragraph in the discussion on this issue, pointing out that, in our experiment, the distinction between hard and soft selection should be interpreted as one of degree rather than kind.

Minor comments:

References should be consistently formatted according to the journal requirements.

Done.

Figure 3: reiterate in the caption that this is done in drug-free environment.

Done.

Referee 1:

Comments to the Author(s)

The authors verified the hypothesis that diversity is maintained under soft selection but not hard selection using bacterial populations under a laboratory work. They found that both resistant and sensitive types for an antibacterial agent were coexisted under negative frequency dependent soft selection for a long time. Their experiments were well designed and its results well supported basic ecological hypothesis. This study can be published in Proceeding B. However, presentation of results should be revised before publication.

L101–105, many readers of Proceeding B including me may not be so familiar with bacterial experiments. Moreover, experimental design of hard and soft selection is an important point of this study, but a little bit hard to understand. Therefore, it is better that the experimental design is shown in a schematic figure to help the understanding of readers.

We have added a schematic of our experimental design as a figure that is included in the revised manuscript (the new Fig 1).

L122–123, there are lacking of the value of F1,19 in the final frequency.

This was a mistake on our part. The appropriate value reported should be a χ^2 statistic and its associated P-value, given the nature of the test (a generalized linear mixed model). This has now been done.

L132, evolved populations -> populations

Done.

In Figs 1–4, the authors used two different symbols, filled circle and triangle for hard and soft in figs 1 and 2, but for resistant and sensitive in Figs 3 and 4, respectively. This causes some confusion and these two symbols are hard to distinguish when their sizes are too small, e.g., in Figs 2 and 4. It is better to use different symbol sets for selection and resistant types.

Thank you for pointing this out. We have revised the symbols to be consistent and clear. We have also slightly enlarged the point size in Fig 2 and 4 for clarity.

In Figs 2 and 4, although the authors used shaded region for drawing SE, many readers will expect CI when they will see the shaded region. Moreover, when the regression coefficient is not significant, it may be better not to show regression line and shaded area, i.e., day 40 of hard selection in Fig. 4.

In fact, the shaded regions in the figure do represent the 95% CI and not the SE. We have corrected the figure legend appropriately. Note that the error bars from Fig 1 and Fig 3 do represent standard error as stated in the caption.

While we recognize that a regression line that is not significant is, in the strictest sense, no line at all, the regressions in Fig 4 represent the strength of the frequency-dependent fitness function, and so have biological meaning. We therefore prefer to keep the lines in the figure, even if non-significant, so the reader can more easily contrast how this fitness function has changed over time.

In Fig. 1, it is better to fit more smooth curve by adding more predictive points on the X axis.

While this would be nice to do, we feel it is unnecessary. The statistical tests (at least for final frequency) are significant and adding more points would only produce a smaller P-value and not change the interpretation. Moreover, given the constraints on laboratory space and time presented by the COVID-19 pandemic, we are reluctant to do additional work that adds at best only marginal value to the results.

In Fig. 2, please add explanations of MIC and LB.

These are defined in the main text already and so their interpretation in the legend of Fig 2 should already be clear. Nevertheless, to avoid any confusion we have clarified our use of the terms in the figure legend.

Referee 2:

Comments to the Author(s)

The authors compare effects of hard vs soft selection on the fate of diversity in an evolution experiment in which each population adapts to a regime with two spatial components, one with and one without antibiotic, but in which dispersal between environments either varies with patch productivity or is held equal. Three significant effects of the manipulation are found, first on the final frequency of antibiotic-resistant bacteria in the context of their evolution experiment, second on the temporal dynamics of fitness increase by antibiotic-sensitive (but not ab-resistant) bacteria and third and most importantly, on the qualitative evolutionary fate of negative-frequency dependence of fitness initially present early in evolution under both hard and soft selection but which disappears over time under hard selection while being maintained under soft selection.

This is a great summary of the paper, thank you.

Minor points:

L48 - delete ‘are’

Done.

L57-L58 - Here isn't the meaning "resulting in the eventual fixation of the type that is fittest in the the niche that contributes most to the population"?

Yes. We have replaced “replacement” with “fixation”.

L122 - missing F value

See our comment to reviewer 1 above, who noted the same mistake. This has now been corrected.

This study appears to be well conducted and is a significant contribution to our understanding of eco-evolutionary mechanisms of diversity maintenance. The documentation of real-time evolutionary disappearance of negative-frequency dependence is intriguing and, as the authors point out, of significance for ecologically-informed models of diversity evolution.

Thank you for your kind review, it is greatly appreciated!