THE ROYAL SOCIETY PUBLISHING

PROCEEDINGS B

Experimental evidence that matching habitat choice drives local adaptation in a wild population

Carlos Camacho, Alberto Sanabria-Fernández, Adrián Baños-Villalba and Pim Edelaar

Article citation details
Proc. R. Soc. B **287**: 20200721.
http://dx.doi.org/10.1098/rspb.2020.0721

Review timeline

Original submission:	15 November 2019
1st revised submission:	31 March 2020
2nd revised submission:	23 April 2020
Final acceptance:	23 April 2020

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

Review History

RSPB-2019-2671.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?
Good

Is the length of the paper justified?
Yes

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

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

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

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors show that a color polymorphic grasshopper match the color of their body with background. This has been shown with natural coloration and after having manipulated body coloration.

I have no major comments. I liked the study which is straightforward. Here are two minor typos:
Line 104 : remove ,
Line 132 : were not where

Review form: Reviewer 2 (Rafael Duarte)

Recommendation
Major revision is needed (please make suggestions in comments)

If yes, please enter your name here as it should appear.
Rafael C. Duarte

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

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?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Evaluation of the manuscript: "Experimental evidence that matching habitat choice drives local adaptation in a wild population".

Overview
This manuscript investigates the occurrence of matching habitat choice in a colour polymorphic grasshopper species living in an urban area. While the experiments and analysis are simple, the theoretical background from which the authors put their work is very clear and interesting. By painting the body coloration of animals, the authors show that grasshopper are able to change their choice towards a new matching background in order to maintain cryptic. This experimental manipulation is very promising since it enables to alter animal's phenotype without changing their own genotype, which opens a range of new questions to be addressed on this and other biological systems. The authors discuss their findings based on matching habitat choice theory on which animals may detect the substrate where their fitness is enhanced. This is a new and little studied issue from the camouflage theory and studies like this are important to point new directions at this growing research area. The manuscript is well written and all statistical analysis appropriated, although I found some parts in the Methods section hard to follow, especially during the explanation of the statistical models used (I suggest below the inclusion of a table in order to show all variables included in each model). Still in Methods, I found the way the authors measure grasshopper colour very subjective (estimating a ranking of blackness), mainly because we currently have many different and easy methods to record animal coloration accurately. Even not possible to have these accurate measurements in this study, I think the authors can discuss the probable shortcomings of this kind of subjective ranking. In addition, I missed some citations both in the Introduction and in Discussion, mainly because the authors have focused on studies about grasshoppers and did not generalize their findings to other taxa or ecosystems (especially to marine habitats). Finally, I missed some alternative explanation about the possible sensory cues grasshoppers may use to perceive their own phenotype (aside vision), especially some related to thermal cues. Details are given below:

Introduction
1 – How different is your work compared to that of Edelaar et al. (2019) [REF 45]? I found both studies very similar, including the experimental alteration of grasshopper colour. Would the big difference this work being conducted entirely in the field while the other in laboratorial conditions? This needs to be clear in your Introduction in order to show that you are not repeating the same protocols from the Edelaar et al. (2019) paper.
2 – Lines 38-39: What type of trade-offs do you expect here?

3 – I suggest you include a new citation as an example of phenotype-environment match mediate by habitat choice in marine habitats, as a way to show how widespread this mechanisms can be in nature (Green et al 2019: http://www.nature.com/articles/s42003-019-0465-8)
4 – Line 61: Survival is for individuals, maybe for population, the best term to use is "population persistence".
5 – Better to combine the 4th and 5th paragraphs in a single one since both focus in the colour traits of the grasshopper species and how such variation is important to the context of your research questions.
6 – Lines 89-90: The REF 38 is about the role of animal behaviour influencing camouflage. Here, when talking about background matching, I suggest you cite the Merilaita & Stevens (2011) chapter about crypsis by background matching (REF 55).

Methods
7 – The method you used to measure grasshopper colour is very subjective. How does this blackish scale work? Do you have any correlation between your assignment and the real colour (measured as brightness or colour reflectance) of the animal?
8 – Line 139: Does the pen marking persist even after grasshopper moulting? How do you control possible moulting of the marked animals?
9 – Lines 141-143: I did not understand whether you recapture marked individuals or not. You explain that you recaptured grasshoppers without handling them because you observed the marked animals with binoculars, right? Please, make this clearer in the text.
10 – Have you compared the colour of painted grasshopper with the correspondent original coloration of the animals? Is the coloration of painted grasshoppers similar to the natural colour of animals? Do you have the brightness value of painted animals?
11 – Have you painted both adults and nymphs? Have you recorded the size of individuals? You need to be careful about the importance of animal's size controlling mechanisms of habitat selection.
12 – The explanation about the recapture model is hard to follow since you have many different variables. I suggest you summarize all factors you included in both models (model 1 – capture; model 2 – recapture) in a table or schematic design.
13 – It is not clear for me what is your dependent variable for both models. Is it the substrate chosen by grasshoppers? Make it explicit for the readers.

Results
14 – Lines 231-233: Please provide the standard deviation for your mean estimate of grasshopper colour.
15 – The number of recaptured painted grasshoppers is very different according to the treatment. You recaptured the double of pale-painted animals compared to the black-painted. Do you think this is a result of differential mortality between treatments? I think this need to be included somewhere in the Discussion.
16 – In Figure 2, does the shaded grey area represent the 95% CI? Put this information in the legend.

Discussion
17 – Line 264: All papers you cite here showed indirectly that background matching confers higher survival of camouflaged individuals. I suggest you include the work of Duarte et al. (2018) - https://www.nature.com/articles/s41598-018-34470-z - in which the authors show experimentally that better camouflaged marine prawns are less predated by seahorses confirming visual modelling predictions.
18 – Line 267: I think here you can add an observation: "the mechanism causing this association is unknown (but see… and include some references)". I suggest you cite here the Green et al. 2019 paper.
19 – Do you have any alternative explanation about how grasshoppers "know" their actual coloration? Do they have colour vision? Aside colour assessment by vision, is it possible they use "thermal" cues to perceive their coloration (black grasshoppers feel warmer than pale ones and

this “feeling” guide their habitat choice)?

Decision letter (RSPB-2019-2671.R0)

07-Jan-2020

Dear Dr Camacho:

I am writing to inform you that your manuscript RSPB-2019-2671 entitled "Experimental evidence that matching habitat choice drives local adaptation in a wild population" 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 Maurine Neiman
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Dear authors,

Two reviewers and myself have read your MS. We all like your MS and appreciate the elegance of your simple experiment and the clear results. It is also well written. However, reviewer 2 and myself do have a substantial list of points that need to be clarified (some are overlapping, see specific comments). Furthermore, the MS should explain much clearer already in the Introduction already how it advances on previous work on other species and previous experiments on the

same species on this topic, now this is introduced too late in the Discussion and it is not explained specifically enough what the added value (and thereby novelty) of this study is. Hence my recommendation 'to 'Reject & allow resubmission'.

Major comments Associate editor:
In the Introduction the state of the art is only very superficially described. It states: " No study to date has adopted such a comprehensive approach with wild animals in a natural situation and, therefore, a conclusive demonstration that matching habitat choice operates in natural populations has remained elusive." It would be good to explain in a separate paragraph what has been done before (on what type of species in what way) and what we have and have not learned from it specifically. For example, is the experimental manipulation or the natural setting that sets your study apart, or both (does this refer to the "comprehensive" bit)? Have previous experimental manipulations been done, or studies in a natural setting? In fact I was quite surprised to read only in the Discussion that (some of) the authors have previously conducted a similar experiment on the same species. (L289), which made me wonder what the added value of the current experiment is. In L311 we read about two more previous experimental studies. It should be crisp and clear in the introduction already how this MS advances on previous work and what the novelty is.

Minor comments Associate editor:
1. L50 "performance trade-offs" Why is "trade-off" included, would performance not suffice?
2. L70 "Next, to decouple phenotype from genotype (and any habitat preference alleles) and from past experience (and any habitat imprinting), …" Reword, complex sentence with lots of "and"s.
3. L80 "novel" Why relevant? If unexplained here, then the word novel raises more questions than it answers.
4. Recapture and marking protocol timing not specific, in suppl.?
5. Figure 1 It would be good to show examples of all possible unmanipuated and manipulated phenotypes"(unmanipulated individuals across the range from pale to dark, pale manipulated to dark, pale manipulated to pale, dark manipulated to dark, dark manipulated to pale) in order to see how well manipulation reflects the natural range of colour morphs/patterns. As well as the range of colour backgrounds encountered. Currently the example photos are a bit confusing. In (a) I suspect we see a pale(?) morph on light asphalt, but this looks similar in colour the "dark" asphalt in (b).
6. L230: "Grasshoppers distributed themselves across the general study area regardless of their sex and colour." It is not clear on what data/analysis this results is based.
7. Figure 2 only shows the fitted model, not the data, which makes it hard to asses model fit. Could you also visualize the data, eg. by binning individuals in darkness 3-5 classes (of course analysis should be done on unbinned data)?
8. L242: "but with a minor remaining significant effect" I do not think you show the effect size and that is minor.
9. Table 1b: the authors show an effect of the original and new colour. If unmanipulated dark morphs occur more on dark asphalt (see fig. 2) then would we not expect an interaction between new and original colour?
10. L264: but in L103 you state that there is no survival difference.
11. L267-270. But does it exclude that other mechanisms are also at work?
12. L280 phenotypically plastic colour change?
13. L284-306 seem better placed in the Introduction, also because t does not discuss any results of the current study.
14. A limitation of this study is that it remains unclear what fitness benefits there are of habitat matching. It was unclear to me due to potentially conflicting wording (this may just been me misreading it) whether there are any survival differences. If not it would be good to hypothesize which other fitness components could be at work, especially given the recurrent referral to performance trade-off which suggest to that some fitness components benefit and other suffer from habitat matching(?).

15. Conclusion section seems to be mainly summarizing the results and reiterating points from the Introduction, what new insights are provided here to justify this section?

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
The authors show that a color polymorphic grasshopper match the color of their body with background. This has been shown with natural coloration and after having manipulated body coloration.

I have no major comments. I liked the study which is straightforward. Here are two minor typos:
Line 104 : remove ,
Line 132 : were not where

Referee: 2

Comments to the Author(s)
Evaluation of the manuscript: "Experimental evidence that matching habitat choice drives local adaptation in a wild population".

Overview
This manuscript investigates the occurrence of matching habitat choice in a colour polymorphic grasshopper species living in an urban area. While the experiments and analysis are simple, the theoretical background from which the authors put their work is very clear and interesting. By painting the body coloration of animals, the authors show that grasshopper are able to change their choice towards a new matching background in order to maintain cryptic. This experimental manipulation is very promising since it enables to alter animal's phenotype without changing their own genotype, which opens a range of new questions to be addressed on this and other biological systems. The authors discuss their findings based on matching habitat choice theory on which animals may detect the substrate where their fitness is enhanced. This is a new and little studied issue from the camouflage theory and studies like this are important to point new directions at this growing research area. The manuscript is well written and all statistical analysis appropriated, although I found some parts in the Methods section hard to follow, especially during the explanation of the statistical models used (I suggest below the inclusion of a table in order to show all variables included in each model). Still in Methods, I found the way the authors measure grasshopper colour very subjective (estimating a ranking of blackness), mainly because we currently have many different and easy methods to record animal coloration accurately. Even not possible to have these accurate measurements in this study, I think the authors can discuss the probable shortcomings of this kind of subjective ranking. In addition, I missed some citations both in the Introduction and in Discussion, mainly because the authors have focused on studies about grasshoppers and did not generalize their findings to other taxa or ecosystems (especially to marine habitats). Finally, I missed some alternative explanation about the possible sensory cues grasshoppers may use to perceive their own phenotype (aside vision), especially some related to thermal cues. Details are given below:

Introduction
1 – How different is your work compared to that of Edelaar et al. (2019) [REF 45]? I found both studies very similar, including the experimental alteration of grasshopper colour. Would the big difference this work being conducted entirely in the field while the other in laboratorial conditions? This needs to be clear in your Introduction in order to show that you are not repeating the same protocols from the Edelaar et al. (2019) paper.
2 – Lines 38-39: What type of trade-offs do you expect here?

3 – I suggest you include a new citation as an example of phenotype-environment match mediate by habitat choice in marine habitats, as a way to show how widespread this mechanisms can be in nature (Green et al 2019: http://www.nature.com/articles/s42003-019-0465-8)
4 – Line 61: Survival is for individuals, maybe for population, the best term to use is "population persistence".
5 – Better to combine the 4th and 5th paragraphs in a single one since both focus in the colour traits of the grasshopper species and how such variation is important to the context of your research questions.
6 – Lines 89-90: The REF 38 is about the role of animal behaviour influencing camouflage. Here, when talking about background matching, I suggest you cite the Merilaita & Stevens (2011) chapter about crypsis by background matching (REF 55).

Methods
7 – The method you used to measure grasshopper colour is very subjective. How does this blackish scale work? Do you have any correlation between your assignment and the real colour (measured as brightness or colour reflectance) of the animal?
8 – Line 139: Does the pen marking persist even after grasshopper moulting? How do you control possible moulting of the marked animals?
9 – Lines 141-143: I did not understand whether you recapture marked individuals or not. You explain that you recaptured grasshoppers without handling them because you observed the marked animals with binoculars, right? Please, make this clearer in the text.
10 – Have you compared the colour of painted grasshopper with the correspondent original coloration of the animals? Is the coloration of painted grasshoppers similar to the natural colour of animals? Do you have the brightness value of painted animals?
11 – Have you painted both adults and nymphs? Have you recorded the size of individuals? You need to be careful about the importance of animal's size controlling mechanisms of habitat selection.
12 – The explanation about the recapture model is hard to follow since you have many different variables. I suggest you summarize all factors you included in both models (model 1 – capture; model 2 – recapture) in a table or schematic design.
13 – It is not clear for me what is your dependent variable for both models. Is it the substrate chosen by grasshoppers? Make it explicit for the readers.

Results
14 – Lines 231-233: Please provide the standard deviation for your mean estimate of grasshopper colour.
15 – The number of recaptured painted grasshoppers is very different according to the treatment. You recaptured the double of pale-painted animals compared to the black-painted. Do you think this is a result of differential mortality between treatments? I think this need to be included somewhere in the Discussion.
16 – In Figure 2, does the shaded grey area represent the 95% CI? Put this information in the legend.

Discussion
17 – Line 264: All papers you cite here showed indirectly that background matching confers higher survival of camouflaged individuals. I suggest you include the work of Duarte et al. (2018) - https://www.nature.com/articles/s41598-018-34470-z - in which the authors show experimentally that better camouflaged marine prawns are less predated by seahorses confirming visual modelling predictions.
18 – Line 267: I think here you can add an observation: "the mechanism causing this association is unknown (but see… and include some references)". I suggest you cite here the Green et al. 2019 paper.
19 – Do you have any alternative explanation about how grasshoppers "know" their actual coloration? Do they have colour vision? Aside colour assessment by vision, is it possible they use "thermal" cues to perceive their coloration (black grasshoppers feel warmer than pale ones and this "feeling" guide their habitat choice)?

Author's Response to Decision Letter for (RSPB-2019-2671.R0)

See Appendix A.

RSPB-2020-0721.R0

Review form: Reviewer 2 (Rafael Duarte)

Recommendation
Accept with minor revision (please list in comments)

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

General interest: Is the paper of sufficient general interest?
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.
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?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Evaluation of the manuscript: "Experimental evidence that matching habitat choice drives local adaptation in a wild population".

Overview

This new version of the manuscript was clearly improved by the authors mostly in terms of being clearer on the existent gaps in the topic it addresses and the benefits it provides to the area, and by clarifying important questions in the methodology and results. All the major and minor concerns I had raised in my first review were answered and the necessary changes provided. I have only minor points that needs consideration:

Minor points

1 – Line 144: Do you know for how long the urban-like habitat at your sampling has been there? In the text, you define it as "recent", but how much is this?
2 – Line 222: Better to list "pale" first (since it is the 0 code).
3 – Line 249: You say here that you Z-transformed all continuous variables, but do you have more than one variable of this type? I have understood that your single continuous variable was the original grasshopper colour (in %). Is it right?
4 – Lines 289, 293: "table" instead of "Table".
5 – Line 296: Would not it be "time since manipulation"?
6 – Line 322: Order the references here.
7 – Lines 341-345: Indeed, I think this is a good explanation about the observed bias in the number of resighted animals. A higher mortality of black-painted grasshoppers maybe have occurred since they tended to be associated to darker substrate (asphalt), which possibly has caused an overheating of the animals.
8 – Line 380: change to "trichromatic colour vision" and add a reference for this information. Is the visual system of grasshoppers conserved among different species?

Decision letter (RSPB-2020-0721.R0)

21-Apr-2020

Dear Dr Camacho

I am pleased to inform you that your manuscript RSPB-2020-0721 entitled "Experimental evidence that matching habitat choice drives local adaptation in a wild population" 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 Maurine Neiman
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).
Evaluation of the manuscript: "Experimental evidence that matching habitat choice drives local adaptation in a wild population".

Overview

This new version of the manuscript was clearly improved by the authors mostly in terms of being clearer on the existent gaps in the topic it addresses and the benefits it provides to the area, and by clarifying important questions in the methodology and results. All the major and minor concerns I had raised in my first review were answered and the necessary changes provided. I have only minor points that needs consideration:

Minor points

1 – Line 144: Do you know for how long the urban-like habitat at your sampling has been there? In the text, you define it as "recent", but how much is this?
2 – Line 222: Better to list "pale" first (since it is the 0 code).
3 – Line 249: You say here that you Z-transformed all continuous variables, but do you have more than one variable of this type? I have understood that your single continuous variable was the original grasshopper colour (in %). Is it right?
4 – Lines 289, 293: "table" instead of "Table".
5 – Line 296: Would not it be "time since manipulation"?
6 – Line 322: Order the references here.
7 – Lines 341-345: Indeed, I think this is a good explanation about the observed bias in the number of resighted animals. A higher mortality of black-painted grasshoppers maybe have occurred since they tended to be associated to darker substrate (asphalt), which possibly has caused an overheating of the animals.
8 – Line 380: change to "trichromatic colour vision" and add a reference for this information. Is the visual system of grasshoppers conserved among different species?

Author's Response to Decision Letter for (RSPB-2020-0721.R0)

See Appendix B.

Decision letter (RSPB-2020-0721.R1)

23-Apr-2020

Dear Dr Camacho

I am pleased to inform you that your manuscript entitled "Experimental evidence that matching habitat choice drives local adaptation in a wild population" 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 9 pages long. Our Production Office will be able to confirm the exact length at proof stage.

Open Access
You are invited to opt for Open Access, making your freely available to all as soon as it is ready for publication under a CCBY licence. Our article processing charge for Open Access is £1700. Corresponding authors from member institutions (http://royalsocietypublishing.org/site/librarians/allmembers.xhtml) receive a 25% discount to these charges. For more information please visit http://royalsocietypublishing.org/open-access.

Paper charges
An e-mail request for payment of any related charges will be sent out shortly. The preferred payment method is by credit card; however, other payment options are available.

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.

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit https://royalsociety.org/journals/ethics-policies/media-embargo for more information.

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

MS RSPB-2019-2671: Experimental evidence that matching habitat choice drives local adaptation in a wild population

Associate Editor
Board Member: 1

Comments to Author:

Dear authors,

Two reviewers and myself have read your MS. We all like your MS and appreciate the elegance of your simple experiment and the clear results. It is also well written. However, reviewer 2 and myself do have a substantial list of points that need to be clarified (some are overlapping, see specific comments). Furthermore, the MS should explain much clearer already in the Introduction already how it advances on previous work on other species and previous experiments on the same species on this topic, now this is introduced too late in the Discussion and it is not explained specifically enough what the added value (and thereby novelty) of this study is. Hence my recommendation 'to 'Reject & allow resubmission'.

Response: Thank you for your critical comments on our manuscript and for the opportunity to submit a revised version. We have revised the manuscript thoroughly based on your comments and those of the two reviewers, and address them below on a point-by-point basis. Changes made to the original document have also been pasted after each response to facilitate assessment. Note that line numbers mentioned in our answers refer to the revised manuscript with track-changes.

Major comments Associate editor:

In the Introduction the state of the art is only very superficially described. It states: " No study to date has adopted such a comprehensive approach with wild animals in a natural situation and, therefore, a conclusive demonstration that matching habitat choice operates in natural populations has remained elusive." It would be good to explain in a separate paragraph what has been done before (on what type of species in what way) and what we have and have not learned from it specifically. For example, is the experimental manipulation or the natural setting that sets your study apart, or both (does this refer to the "comprehensive" bit)? Have previous experimental manipulations been done, or studies in a natural setting? In fact I was quite surprised to read only in the Discussion that (some of) the authors have previously conducted a similar experiment on the same species. (L289), which made me wonder what the added value of the current experiment is. In L311 we read about two more previous experimental studies. It should be crisp and clear in the introduction already how this MS advances on previous work and what the novelty is.

Response: We apologize for not including a detailed explanation of the added value of the current work in the first draft. This has now been clarified in the revised version – please, see our response to the same comment by Reviewer 2 for a detailed explanation of the major advances and novelty of the present work.

Minor comments Associate editor:

1. L50 “performance trade-offs” Why is “trade-off” included, would performance not suffice?

Response: For simplicity, we have replaced ‘performance trade-offs across environments’ by ‘local performance across environments’.

2. L70 “Next, to decouple phenotype from genotype (and any habitat preference alleles) and from past experience (and any habitat imprinting), …” Reword, complex sentence with lots of “and”s.

Response: This sentence has been reformulated as follows:

> (L.76-79): “Next, to decouple the effect of phenotype from that of direct genetic preference or imprinting, an experimental manipulation of phenotypes should be performed”.

3. L80 “novel” Why relevant? If unexplained here, then the word novel raises more questions than it answers.

Response: We really meant ‘recently-developed’, so the term ‘novel’ has been replaced by the latter term.

4. Recapture and marking protocol timing not specific, in suppl.?

Response: as noted by reviewer 2, the use of the term ‘recapture’ to make reference to records of previously captured and marked individuals is misleading, because

> (L.170-174): “Marked grasshoppers were not recaptured, because in virtually all cases the letter code could be identified from a distance using binoculars. For visual recaptures, we also recorded the type of substrate upon encounter, coordinates, date, and time of resighting using the same field methods as for first captures”.

5. Figure 1. It would be good to show examples of all possible unmanipulated and manipulated phenotypes”(unmanipulated individuals across the range from pale to dark, pale manipulated to dark, pale manipulated to pale, dark manipulated to dark, dark manipulated to pale) in order to see how well manipulation reflects the natural range of colour morphs/patterns. As well as the range of colour backgrounds encountered. Currently the example photos are a bit confusing. In (a) I suspect we see a pale(?) morph on light asphalt, but this looks similar in colour the “dark” asphalt in (b).

Response: Great suggestion, thank you! Figure 1 now includes a set of photographs showing unmanipulated individuals across the natural range from pale to dark, and Figure 3 includes examples of manipulation to dark and to pale. Colour backgrounds encountered are restricted to the ones shown in the figure (dark asphalt in the centre, and pale sidewalks (tiles) and parking lots (cement) on both sides), and so the picture of the study area remains the same. Finally, it is important to note that our manipulation is not intended to reflect the natural range of colour morphs, but the extremes of the colour range, as it is in these extreme phenotypes where the effects of matching habitat choice on are expected to be most pronounced (Camacho & Hendry 2020).

6. L230: “Grasshoppers distributed themselves across the general study area regardless of their sex and colour.” It is not clear on what data/analysis this result is based.

Response: This statement was based on a visual assessment of the plotted locations. We have decided to remove it from the text because it is not directly relevant to the main results of the paper and an additional spatial analysis would unnecessarily enlarge the text.

7. Figure 2 only shows the fitted model, not the data, which makes it hard to asses model fit. Could you also visualize the data, eg. by binning individuals in darkness 3-5 classes (of course analysis should be done on unbinned data)?

Response: Figure 2 now includes the individual data points, so that the model fit can be visually evaluated, even though this is always harder with binomial data.

8. L242: "but with a minor remaining significant effect" I do not think you show the effect size and that is minor.

Response: Thank you for pointing this out. The term 'minor' should indeed read as '*relatively* minor' because the differences in AICc of the most satisfactory model to the models excluding 'new colour' and 'original colour' (Table 1b) indicate that the model including 'new colour' alone performs *relatively* better than the model including 'original colour' alone. Therefore, the effect of 'original colour' on substrate use is *relatively* minor compared to the effect of new colour. To make this point clearer, we have modified the text in the results as follows:

> (L.293-296): "Black-painted and white-painted individuals aggregated together on the dark asphalt and pale pavement, respectively (figure 3), although model comparisons indicated that there was a relatively minor remaining significant effect of their original colour on substrate use (Table 1b)".

More convincing (quantitative) evidence of the greater importance of 'new colour' relative to 'original colour' can be obtained by quantifying the loss of explanatory power associated with the exclusion of each variable. We used the package MuMIn (Barton & Barton 2019) to calculate the theoretical R^2 for binomial GLMMs, following Nakagawa et al. (2017) J. R. Soc. Interface.14: 20170213. We first computed the % of variance explained by the model including both variables and by the models lacking 'original colour' and 'new colour', respectively, and then compared the R^2 values of the three models.

Excluding 'original colour' from the most satisfactory model implied a loss of explanatory power of 9.4%, whereas the exclusion of 'new colour' implied a loss of 60.8%. This difference confirms that the effect of 'original colour' on the response variable is smaller than that of 'new colour'.

This approach has not been incorporated into the MS to avoid overly long explanations, but we would be happy to do it if requested.

9. Table 1b: the authors show an effect of the original and new colour. If unmanipulated dark morphs occur more on dark asphalt (see fig. 2) then would we not expect an interaction between new and original colour?

Response: No such interaction is expected because (*i*) phenotype-dependent movement should be guided by the current phenotype, regardless of the magnitude of the change from the original to the current phenotype, and (*ii*) the interaction between new and original colour would occur if we analysed a change in grasshopper numbers, but here we analyse the probability of changing substrate, so the numerical distribution of phenotypes across environments before the manipulation is not relevant.

10. L264: but in L103 you state that there is no survival difference.

Response: Certainly, the survival benefits associated with background colour matching are not readily detectable in our study area, likely because predation pressure is mild compared to non-urban, natural areas where predators are more abundant. Therefore, even though the link between crypsis and survival applies to many other analogous systems, we have removed this part of the sentence to avoid confusion.

11. L267-270. But does it exclude that other mechanisms are also at work?

Response: By manipulating the phenotype of wild-caught grasshoppers and by including the term 'original colour' in the models analysing habitat use by colour-manipulated individuals, our study *accounts for* the effects of past experience and genetic background. This sentence has been modified to better reflect this point:

> (L.328-332): "Here, the experimental manipulation of the colour of wild, free-ranging grasshoppers allowed us to demonstrate that differential habitat use in response to individual colour was responsible for the observed phenotype-environment match in unmanipulated individuals, after accounting for the effects of genetic background and imprinting".

12. L280 phenotypically plastic colour change?

Response: Changed, thanks.

13. L284-306 seems better placed in the Introduction, also because it does not discuss any results of the current study.

Response: Right, thank you. This paragraph has entirely been transferred to the introduction so that the readers can get an idea of what have been done so far, the main inferential limitations of previous studies compared to ours and thereby, also, the added value of the current experiment.

14. A limitation of this study is that it remains unclear what fitness benefits there are of habitat matching. It was unclear to me due to potentially conflicting wording (this may just been me misreading it) whether there are any survival differences. If not it would be good to hypothesize which other fitness components could be at work, especially given the recurrent referral to performance trade-off which suggest to that some fitness components benefit and other suffer from habitat matching(?)

Response: The only reason we can think of why grasshoppers would want to increase similarity in colour between themselves and their substrate is to provide greater crypsis, because in their natural habitat this is expected to decrease detection by predators and, therefore, increase survival probability. As it turns out, in our study area we do not find an effect of crypsis on survival probability (see Edelaar et al. 2019), probably because the predator community on streets is severely reduced (numerically and in diversity). However, as explained in electronic supplementary material 2, we have strong evidence (e.g. reduced detectability on matching backgrounds, increased crypsis due to plasticity under experimentally induced predation risk, and behavioural responses by less cryptic individuals suggesting they feel less safe) that background colour matching improves crypsis and thereby protects grasshoppers from being eaten. It seems reasonable to expect that grasshoppers consistently aim for increased crypsis (e.g. the nymphs show phenotypic plasticity in the lab when exposed to different backgrounds, even if this does not increase survival), because this is something that has worked for them for millions of years, and may be a rather inflexible behaviour, that is, not responsive to individual assessments of predator density, for example.

With respect to the trade-offs, we did not mean trade-offs between different kinds of benefits, but that an individual that is more cryptic in one habitat will be less cryptic in a different habitat (especially here, with only two types of habitats). To clarify this, we have added the following to the introduction:

> (L.125-128): "(iv) previous evidence indicates that they are indeed more colour-matched on their local urban pavement than they are on alternative pavements, supporting the potential for a trade-off in survival between distinctly coloured substrates [35, 43]".

15. Conclusion section seems to be mainly summarizing the results and reiterating points from the Introduction, what new insights are provided here to justify this section?

Response: This section has been deleted, so that additional text can be added where requested.

Reviewer(s)' Comments to Author:

REFEREE: 1

Comments to the Author(s)

The authors show that a color polymorphic grasshopper match the color of their body with background. This has been shown with natural coloration and after having manipulated body coloration.

I have no major comments. I liked the study which is straightforward. Here are two minor typos:

Line 104 : remove ,

Response: Done, thanks!

Line 132 : were not where

Response: Changed, thanks!

REFEREE: 2

Comments to the Author(s)

Evaluation of the manuscript: "Experimental evidence that matching habitat choice drives local adaptation in a wild population".

Overview

This manuscript investigates the occurrence of matching habitat choice in a colour polymorphic grasshopper species living in an urban area. While the experiments and analysis are simple, the theoretical background from which the authors put their work is very clear and interesting. By painting the body coloration of animals, the authors show that grasshopper are able to change their choice towards a new matching background in order to maintain cryptic. This experimental manipulation is very promising since it enables to alter animal's phenotype

without changing their own genotype, which opens a range of new questions to be addressed on this and other biological systems. The authors discuss their findings based on matching habitat choice theory on which animals may detect the substrate where their fitness is enhanced. This is a new and little studied issue from the camouflage theory and studies like this are important to point new directions at this growing research area. The manuscript is well written and all statistical analysis appropriated, although I found some parts in the Methods section hard to follow, especially during the explanation of the statistical models used (I suggest below the inclusion of a table in order to show all variables included in each model). Still in Methods, I found the way the authors measure grasshopper colour very subjective (estimating a ranking of blackness), mainly because we currently have many different and easy methods to record animal coloration accurately. Even not possible to have these accurate measurements in this study, I think the authors can discuss the probable shortcomings of this kind of subjective ranking. In addition, I missed some citations both in the Introduction and in Discussion, mainly because the authors have focused on studies about grasshoppers and did not generalize their findings to other taxa or ecosystems (especially to marine habitats). Finally, I missed some alternative explanation about the possible sensory cues grasshoppers may use to perceive their own phenotype (aside vision), especially some related to thermal cues.

Response: We would like to express our gratitude to reviewer 2 for his/her insightful and detailed comments on our submitted manuscript.

Details are given below:

Introduction

1 – How different is your work compared to that of Edelaar et al. (2019) [REF 45]? I found both studies very similar, including the experimental alteration of grasshopper colour. Would the big difference this work being conducted entirely in the field while the other in laboratorial conditions? This needs to be clear in your Introduction in order to show that you are not repeating the same protocols from the Edelaar et al. (2019) paper.

Response: We apologize for not including a detailed explanation of the added value of the current work in the first draft.

Edelaar et al.'s (2019) previous study [reference 35 in the revised version] differs from the present study in several critical aspects. First, the previous study did experiments in lab and field, but in contrast to our study, field experiments were restricted to lab-bred nymphs with no prior knowledge of the study area, whereas here we used wild-born grasshoppers whose response is likely to be more relevant if we want to know what is happening in the field. Second, the colour of grasshoppers used by Edelaar et al. (2019) was manipulated only in one single direction (darkening), and by the injection of a hormone (corazonin) during the nymph stage, whereas here we used paint to change adult colour in both directions to either pale or dark. Corazonin is suspected to function as a stress hormone too, so painting is probably a better manipulation to get reliable, unbiased data on grasshopper behaviour. It is even possible that previously the hormone treatment, or even the act of injection itself, caused individuals to make greater use of dark substrates, independent of their colour and their degree of crypsis. By manipulating all individuals in this study, and in both directions (darker and paler), these alternative interpretations can be avoided. One final aspect that makes our current approach more powerful and relevant than the one(s) used in the past is that the natural pattern and the experimental pattern are based on the same individuals. Overall, then, the experimental approach used in this study is a substantial improvement over the procedure

used in Edelaar et al. 2019 (which is why we undertook this additional study), and unequivocally demonstrates that body colour directly influences adaptive microhabitat selection of individuals. To make clear how the present manuscript advances on previous work and what the novelty is, we have added the following sections to the introduction and the discussion, respectively:

> (L.81-100): "Matching habitat choice has received considerable research attention in the last decade and, although several tests have been conducted under controlled indoor conditions [32-34], examples of phenotype manipulation experiments are restricted to a couple of studies on grasshoppers. For example, in a series of laboratory experiments using a mosaic of solar radiation, Karpestam et al. [30] and Wennersten et al. [31] showed that pale-painted and dark-painted grasshoppers tended to settle in the thermal zone offering the better fitness prospects given their susceptibility to radiation. No such tendency was however seen in unmanipulated naturally dark and pale morphs, thus obscuring the applicability of these findings to more realistic natural scenarios. More recently, Edelaar et al. [35] demonstrated that lab-reared grasshoppers released in the field after a unidirectional hormone-induced cuticle darkening made greater use of dark substrates than unmanipulated (uninjected) individuals. Clearly, this finding provides support for matching habitat choice, but its relevance to nature is somewhat uncertain due to the use of lab-reared nymphs lacking prior knowledge of the study area. In addition, the hormone used (corazonin) for darkening also functions as a stress hormone, so injected grasshoppers could have preferred dark substrates for other reasons unrelated to their own colour and crypsis. Combining evidence from observational data and a phenotype manipulation experiment in both directions using free-ranging animals in their local natural environment is probably the best inferential approach, but no such study has been explicitly conducted. Consequently, the operation of matching habitat choice in natural populations remains to be conclusively demonstrated".

> (L.351-360): "Our finding that grasshoppers adjust their movement patterns to choose the substrate that confers an apparent improvement in camouflage given their individual-specific colour strengthens the interpretations of previous studies […], suggesting a role of matching habitat choice as a mechanism of local adaptation. More specifically, our study supports the findings of earlier phenotype manipulation experiments using lab-reared individuals [30, 31, 35]. But, importantly, the approach used in this study is more relevant to natural populations because, unlike in previous studies, it combines evidence from observational and experimental data on the same sample of wild-born individuals in their local, natural habitat".

2 – Lines 38-39: What type of trade-offs do you expect here?

Response: Crypsis is generally thought to confer survival benefits and, therefore, dark and pale grasshoppers are expected to experience survival trade-offs between distinctly coloured substrates. This has been clarified in the introduction:

> (L.125-128): "(iv) previous evidence indicates that they are indeed more colour-matched on their local urban pavement than they are on alternative pavements, supporting the potential for a trade-off in survival between distinctly coloured substrates [35, 43]".

3 – I suggest you include a new citation as an example of phenotype-environment match mediate by habitat choice in marine habitats, as a way to show how widespread this mechanisms can be in nature (Green et al 2019: http://www.nature.com/articles/s42003-019-0465-8)

Response: Done, thanks.

4 – Line 61: Survival is for individuals, maybe for population, the best term to use is "population persistence".

Response: Right, thank you. Changed as suggested.

5 – Better to combine the 4th and 5th paragraphs in a single one since both focus in the colour traits of the grasshopper species and how such variation is important to the context of your research questions.

Response: Done!

6 – Lines 89-90: The REF 38 is about the role of animal behaviour influencing camouflage. Here, when talking about background matching, I suggest you cite the Merilaita & Stevens (2011) chapter about crypsis by background matching (REF 55).

Response: Thank you for pointing out this mistake. Merilaita & Stevens (2011) is now cited instead.

Methods

7 – The method you used to measure grasshopper colour is very subjective. How does this blackish scale work? Do you have any correlation between your assignment and the real colour (measured as brightness or colour reflectance) of the animal?

Response: The scale we used to measure grasshopper colour has now been included in the electronic supplementary material – 3. Basically, it is just a sheet with 100 grey tones printed on it, and one then visually matches the grasshopper with one of those grey tones. We decided to not take standardised pictures for automated colour scoring (we have done this in some of our prior studies), in order to not stress the individuals even more as they were going to be painted already.

The fact that we find an effect of this original colour on habitat use for the resighted individuals shows that this visual scoring at least partly reflected the real colour of individuals. To further corroborate this, the same person who collected the data scored a set of grasshopper images which had been measured objectively, and we found a good correlation betwee the two (see electronic supplementary material – 3 for details). So while the visual method is not optimal, it provides a good compromise given the trade-offs between reliability, stress to individuals, and speed (i.e. catching more individuals and increasing statistical power).

8 – Line 139: Does the pen marking persist even after grasshopper moulting? How do you control possible moulting of the marked animals?

Response: Only grasshopper nymphs, but not adults, moult, and this is the reason why we used adults only in this study. This has been clarified in the main text:

> (L.131-132): "...adults do not moult anymore and only change colour very slowly".

9 – Lines 141-143: I did not understand whether you recapture marked individuals or not. You explain that you recaptured grasshoppers without handling them because you observed the marked animals with binoculars, right? Please, make this clearer in the text.

Response: Marked grasshoppers were not really recaptured, but identified from a distance using binoculars, as explained above in our response to the editor's comment on the same issue. To make this point clearer, the term 'recapture' has been replaced by 're-sighing' or 'visual recapture' throughout the text and, also, we have explicitly mentioned that marked were visually identified from afar:

(L.170-174): "Marked grasshoppers were not recaptured, because in virtually all cases the letter code could be identified from a distance using binoculars. For visual recaptures, we also recorded the type of substrate upon encounter, coordinates, date, and time of resighting using the same field methods as for first captures".

10 – Have you compared the colour of painted grasshopper with the correspondent original coloration of the animals? Is the coloration of painted grasshoppers similar to the natural colour of animals? Do you have the brightness value of painted animals?

Response: Blackness of unmanipulated grasshoppers ranged between 20% and 80% in the study population, and so we painted individuals to resemble these extreme phenotypes, regardless of their original colour. The reason for this is twofold. First, under matching habitat choice, the spatial responses of individuals are expected to be stronger in extreme than in intermediate phenotypes, because mismatched decisions are predicted to be most costly for the former (Camacho & Hendry 2020). Second, given that the degree of crypsis on a certain background –and, ultimately, local performance– depends on the current appearance of an individual, performance-based habitat selection decisions should be influenced primarily by the current (acquired) colour of individuals. No measurements of brightness were taken in the field after colour manipulation because wet individuals look slightly different, but paint was always applied by the same person to ensure consistency in the external appearance (blackness) of pale-painted and dark-painted individuals. This has been clarified in the main text:

(L.176-186): "Upon first capture, individuals were alternatingly assigned to receive a dark (approximately 80% blackness) or pale (approximately 20% blackness) colour that resembled the extremes of the natural cline of colour variation, regardless of their original colour, sex or type of substrate on which they were found. The reason for manipulating the overall colour of individuals to either pale or dark is that, under matching habitat choice, the spatial responses of extreme phenotypes are expected to be stronger than that of intermediate phenotypes [48]. Furthermore, performance-based habitat selection decisions should be influenced primarily by the current colour of individuals, independent of the magnitude of the difference between the original and newly acquired colour. To experimentally mimic the natural colour of the palest and darkest individuals in the population, one of us (A.S-F.) applied white or black water-based (aquarelle) paint […]".

11 – Have you painted both adults and nymphs? Have you recorded the size of individuals? You need to be careful about the importance of animal's size controlling mechanisms of habitat selection.

Response: As explained above, we only used adults. We did not measure the size of individuals. Statistically controlling for body size in the analysis might reduce some noise in the data, for instance, if smaller individuals were less mobile than larger ones (so fitting an effect of size in interaction with body colour). Nevertheless, we don't think body size variation can be an alternative explanation for the observed results given the small spatial scale of our study compared to the movement capacity of grasshoppers, and there is no relationship between size and colour. In addition, males are quite a bit smaller than females, yet we found no statistical difference between the sexes. Due to space limitations, we have made no mention of this in the discussion of the improved version, but we will be happy to do so if requested.

12 – The explanation about the recapture model is hard to follow since you have many different variables. I suggest you summarize all factors you included in both models (model 1 – capture; model 2 – recapture) in a table or schematic design.

Response: The description of the set of models used in this study is now provided in Table S2 in electronic supplementary material – 5.

13 – It is not clear for me what is your dependent variable for both models. Is it the substrate chosen by grasshoppers? Make it explicit for the readers.

Response: This has been clarified as follows:

> (L.221-222): "For both models, the dependent variable was the type of substrate chosen by grasshoppers, coded as dark asphalt (1) or dark asphalt (0)".

Results

14 – Lines 231-233: Please provide the standard deviation for your mean estimate of grasshopper colour.

Response: Done.

15 – The number of recaptured painted grasshoppers is very different according to the treatment. You recaptured the double of pale-painted animals compared to the black-painted. Do you think this is a result of differential mortality between treatments? I think this need to be included somewhere in the Discussion.

Response: Dark-painted individuals might have suffered greater mortality due to overheating (we made them very dark). However, any effect this selective mortality may have on observed habitat use would go in the opposite direction. This has been clarified in the discussion:

> (L.341-345): "Dark-painted grasshoppers were resighted less often than pale-painted ones, and this difference might be related to mortality due to overheating. However, mortality of dark individuals would be highest on hot, dark asphalt, which would create the opposite pattern of what we actually observed".

16 – In Figure 2, does the shaded grey area represent the 95% CI? Put this information in the legend.

Response: Yes, sorry for the omission. This information has now been included in the figure legend.

Discussion

17 – Line 264: All papers you cite here showed indirectly that background matching confers higher survival of camouflaged individuals. I suggest you include the work of Duarte et al. (2018) - https://www.nature.com/articles/s41598-018-34470-z - in which the authors show experimentally that better camouflaged marine prawns are less predated by seahorses confirming visual modelling predictions.

Response: This reference is definitely relevant to our study, and so it has been incorporated into the manuscript, thank you for the suggestion.

18 – Line 267: I think here you can add an observation: "the mechanism causing this association is unknown (but see… and include some references)". I suggest you cite here the Green et al. 2019 paper.

Response: Done, thank you.

19 – Do you have any alternative explanation about how grasshoppers "know" their actual coloration? Do they have colour vision? Aside colour assessment by vision, is it possible they

use “thermal” cues to perceive their coloration (black grasshoppers feel warmer than pale ones and this “feeling” guide their habitat choice)?

Response: Grasshoppers can see blue, green and ultraviolet, and should be able to compare the darkness of their own body against that of the substrate using their large eyes. This has now been explained in the discussion:

> (L.380-383): “Grasshoppers have tricolour vision, and at any rate should not have any trouble comparing the darkness of their own body against that of the substrate, given their large eyes which are placed somewhat on top of their mobile heads, enabling them to see many parts of their body and the substrate”.

The use of visual rather than thermal cues seems the most likely mechanism to us. Even though darker individuals indeed warm up more, how would they know that this ‘feeling’ is due to them, and not due to the environment (more sunshine)? This mechanism would cause pale grasshoppers to move to darker environments on warmer days with more sun, and dark grasshoppers to move to pale surfaces on cold days, thus reducing the degree of crypsis. We would also not expect darker individuals to prefer dark backgrounds for thermal reasons – the opposite if anything, to avoid overheating. Blinding the individuals (painting over their eyes) would be a way to test this, but that manipulation could change normal behaviour and survival in the field drastically.

Appendix B

MS RSPB-2019-2671: Experimental evidence that matching habitat choice drives local adaptation in a wild population

Dear Dr. Camacho,

I am pleased to inform you that your manuscript RSPB-2020-0721 entitled "Experimental evidence that matching habitat choice drives local adaptation in a wild population" 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.

REFEREE: 2

Comments to the Author(s)

Overview

This new version of the manuscript was clearly improved by the authors mostly in terms of being clearer on the existent gaps in the topic it addresses and the benefits it provides to the area, and by clarifying important questions in the methodology and results. All the major and minor concerns I had raised in my first review were answered and the necessary changes provided. I have only minor points that needs consideration:

Response: Thank you ever so much for your critical comments of earlier drafts, which have substantially contributed to improve the quality of this paper.

Minor points

1 – Line 144: Do you know for how long the urban-like habitat at your sampling has been there? In the text, you define it as “recent”, but how much is this?

Response: This area was developed between 6-8 years before our sampling. This information has been added to the text.

2 – Line 222: Better to list “pale” first (since it is the 0 code).

Response: Right, done!

3 – Line 249: You say here that you Z-transformed all continuous variables, but do you have more than one variable of this type? I have understood that your single continuous variable was the original grasshopper colour (in %). Is it right?

Response: Thanks for catching this; “all continuous variables” has been replaced by “original colour values”.

4 – Lines 289, 293: “table” instead of “Table”.

Response: Changed.

5 – Line 296: Would not it be “time since manipulation”?

Response: Indeed, changed.

6 – Line 322: Order the references here.

Response: Done.

7 – Lines 341-345: Indeed, I think this is a good explanation about the observed bias in the number of resighted animals. A higher mortality of black-painted grasshoppers maybe have occurred since they tended to be associated to darker substrate (asphalt), which possibly has caused an overheating of the animals.

Response: It's great that you agree!

8 – Line 380: change to "trichromatic colour vision" and add a reference for this information. Is the visual system of grasshoppers conserved among different species?

Response: Changed. To support this information, we have added the reference Briscoe AD, Chittka L. 2001. The evolution of color vision in insects. *Annu. Rev. Entomol.* **46**, 471–510. Based on the information presented in this review, colour vision across grasshoppers appears to be quite conserved, so extrapolation to our species seems justified.

The final version of the manuscript with 'track changes' can be found from the next page onwards. To facilitate assessment, modified sections have been highlighted in yellow. No figures or tables are included in the 'track changes' version, because no change has been made to them since the previous version.