Biased movement drives local cryptic coloration on distinct urban pavements

Pim Edelaar, Adrian Baños-Villalba, David P. Quevedo, Graciela Escudero, Daniel I. Bolnick and Aída Jordán-Andrade

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

Review History

RSPB-2019-1343.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?
Excellent

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.
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
The is clearly a very interesting and well-written manuscript. I am not sure if I fully agree on the use of the term urbanization (are it urbanized when the sit on asphalt in a deserted area and not urbanized when they sit on the open ground next to it?), but that's fine with me. There is just one point left that I think needs to be corrected. I had made this point before, but the response does not convince me.

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it should not exclude sites where the animal has been seen. In fact, the observed data should count as one possible realization of the randomization.

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Minor

L66: I find "effectively counter-act random habitat use" a somewhat odd expression.

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Figure 2: Suggest to remove "confirmed" in "contributing to the grasshopper-pavement colour match"

Figure 3: A) The caption mentions white tick marks, but I can't see any. (B+C) The colours are hardly distinguishable in greyscale (suggest to choose colours with more different grey values). Also include a space before "vs." in the title.

Supplementary L91-99: Did you use log10 or ln? The default in R using log would be ln, in which case the back-transformation would have e in the base.

Decision letter (RSPB-2019-1343.R0)

16-Jul-2019

Dear Dr Edelaar:

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 the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

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.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
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.

Use of animals and field studies:
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.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

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.

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.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

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. 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,

Professor Victoria Braithwaite
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
The authors have produced a substantial revision, that reads and looks better than the previous version. The revision was seen by one of the previous reviewers, who made a few more helpful comments, in particular about one of the statistical analyses. These suggestions are all sound, and should be easy to implement.
The new overview figure 2 is helpful. I also suggest to remove 'confirmed' in "contributing to the confirmed grasshopper-pavement colour match" and to alter the title sentence of the two top panels e.g. "Putative observed pattern" -> "Hypothesis" "Confirmation of putative pattern" -> "research questions". Also, change "Do grasshoppers change to a more matching substrate" to "Do grasshoppers change to a better-matching substrate"
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Reviewer(s)' Comments to Author:

Referee: 1

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Simulation script

```
nrep = 1000
resT = rep(NA,nrep) # test statistics for normal t tests
resLMM = rep(NA,nrep) # test statistics from mixed models
resLMMvar = rep(NA, nrep) # individual identity variance component
n = 50 # The samples size of observed individuals
treat= rep(1:2, each=n)
id = rep(1:n,2)
for(i in 1:nrep) {
presVal = runif(n, 0, 1) # assuming that individual settle randomly
absVal = 1 - presVal # contrasts at absence sites is dissimilar to contrasts as presence sites
resT[i] = t.test(presVal-0.5)$statistic # t value for the t test
LMM = summary(lme4::lmer(c(presVal, absVal) ~ treat + (1|id)))
resLMM[i] = LMM$coefficients[2,3] # t value from mixed models
resLMMvar[i] = summary(m)$varcor$id[1,1]^2 # individual identity variance component
}
mean(abs(resT)>1.96) # expected number of significant cases when using t tests
mean(abs(resLMM)>1.96) # inflation of significance when using mixed models in this case
plot(resT, resLMM, abline(0,1)) # not sure why there is a strong negative correlation, but you see
more extreme values for mixed models
sort(resLMMvar) # individual identity variance components are pretty much all zero
```

Author's Response to Decision Letter for (RSPB-2019-1343.R0)

See Appendix A.

Decision letter (RSPB-2019-1343.R1)

11-Sep-2019

Dear Dr Edelaar

I am pleased to inform you that your manuscript entitled "Biased movement drives local cryptic colouration on distinct urban pavements" 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. Please carefully check the quality of the figures in this proof.

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

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

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,
Victoria Braithwaite

Professor V A Braithwaite
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor, Comments to Author:
None - the revisions all seem in order, and the manuscript much improved in the course of the review process.

Appendix A

Author response to editorial and reviewer comments

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.
　　　*** We thank the editor and the reviewer for their constructive comments. Below we respond to all comments, and specify the changes we made to the ms, which we hope is now ready for publication.

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.
　　　*** We have uploaded this document with 'tracked changes' as requested.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.
　　　*** We revised the material previously uploaded, and the new upload includes all changes to the code and data used.

Please try to submit all supplementary material as a single file.
　　　*** We have now combined the supplementary methods and supplementary figures into a single file.

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.
　　　*** Due to an international stay, holidays and conference attendance, we have applied for and received an extension.

Associate Editor
Board Member: 1
Comments to Author:
The authors have produced a substantial revision, that reads and looks better than the previous version.
　　　*** Thank you.

The revision was seen by one of the previous reviewers, who made a few more helpful comments, in particular about one of the statistical analyses. These suggestions are all sound, and should be easy to implement.
　　　*** See below for our replies.

The new overview figure 2 is helpful. I also suggest to remove 'confirmed' in "contributing to the confirmed grasshopper-pavement colour match"
　　　*** Changed as such.

and to alter the title sentence of the two top panels e.g. "Putative observed pattern" -> "Hypothesis" "Confirmation of putative pattern" -> "research questions".
　　　*** Changed into: "General hypothesis", and "Specific tests".

Also, change "Do grasshoppers change to a more matching substrate" to "Do grasshoppers change to a better-matching substrate"
　　　*** Changed as such.

Because of the length of the manuscript, some figures were moved to supplement - this has not affected the main message. Fig. 1 is quite repetitive, and either panel B or C should be placed in supplement also.
        *** We moved panel C to the supplement, and adapted reference to figures in the main text where necessary.

p. 8, l. 279: change "Simulation of necessary mortality rates to obtain observed population divergence" to "Simulation of mortality rates necessary to obtain observed population divergence"
        *** Changed as such.

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Referee: 1
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This clearly a very interesting and well-written manuscript.
        *** Thank you.

I am not sure if I fully agree on the use of the term urbanization (are it urbanized when the sit on asphalt in a deserted area and not urbanized when they sit on the open ground next to it?), but that's fine with me.
        *** This is partly a discussion we had in previous rounds of review, and no changed made to the ms.

There is just one point left that I think needs to be corrected. I had made this point before, but the response does not convince me.

The point is the use of presence and absence data in the analysis of colour matching. The authors claim that the random effect "individual identity" takes care of the non-independence. I don't think this is the case for this particular kind of non-independence. The point is that random effects in mixed models take care of similarities within groups. But this is not the case here. The response is visual contrast between the animal and the background. Then there is presence data for the sites where animals have been found (the number of data points for this is N which the sample size of independent data). The authors then generate pseudo-data for sites for the animal could be sat. They are likely to be quite different to where the animal is. The visual distance will NOT be similar within individuals. On the contrary, if an individual sits on a matching background, its visual distance will be small, whereas for absence data the distance will be large. There is no correlation within random effect groups and the mixed model can't take care of this. I guess this could be seen form a small individual identity variance component. In effect, the model is fitted with 2N data points and this is likely to give erroneous (anti-conservative) significance tests. I am pasting a script with a very crude simulation at the end that suggests that significance could be inflated about threefold.
        *** We thank the reviewer for the extra effort to explain the issue, and the added code.

I am also not sure if it is a good idea to compare contrast on presence sites to contrasts on absence sites averaged according to availability. This is likely to produce heterogeneity in the residuals.

I think what is needed is a randomization procedure in which the contrast for presence sites is calculated as a test statistic and individuals are then assigned to sites according to availability (and

```
the test statistic calculated). The randomization should not average
across different sites and it should not exclude sites where the
animal has been seen. In fact, the observed data should count as one
possible realization of the randomization.
        *** OK, we now understand the issue raised, and we think the
reviewer is correct that modelling a consistent individual effect
doesn´t make too much sense here and thereby that a random ID variable
does not correct for multiple data per individual. We appreciate this
detailed explanation and the script to simulate its effect. We
therefore changed the analyses and tested the relevant hypotheses by
randomisation, as suggested. Below we paste the text as written in the
expanded (online) methods. (One thing to take into account is that two
randomisations were necessary to test the two hypotheses: assigning
random pavements to focal grasshoppers, or random grasshoppers to
focal pavement. This tests for the two different aspects of local
adaptation, as explained in the original text and associated
reference).
```

"Following [35], and because local adaptation depends on the interaction between organisms and their environments, we tested in two complementary ways for local adaptation. The home-away contrast (Figure 3B) tests for local adaptation from the view point of the individuals, whether the current habitat is the one providing a better match for them or whether they would be better off in other habitats. This contrast is particularly suited to test if individuals have selected their personal best environment, thus representing a test of optimization. We tested this by randomisation. We first calculated the observed average RGB difference across all individuals. By simulation, we then randomly distributed them across the four available pavements (= drawing a random alternative pavement for each individual with replacement), in proportion to the surface area of each pavement in the study area. For this random realisation we again calculated the average RGB difference across all individuals. This process was repeated 10,000 times, and we calculated the proportion of times that the random distribution produced a RGB difference that was as small or smaller as the observed one (i.e. a one-sided test). This proportion was used as the p-value to test the null hypothesis that grasshoppers are not more cryptic on their home pavement than on random pavements. The same procedure was used for the four subsets of grasshoppers from each pavement separately. (Note that this is a conservative test, since we don´t test home versus away, but home versus random, i.e. including the home pavement within the randomisation).

The resident-immigrant contrast (Figure 3C) tests for local adaptation from the view point of the local environment, whether the individuals present in the focal environment are the ones that match best with it or whether individuals present in other environments would match better. We also tested this by randomisation. We first calculated the observed average RGB difference across all individuals, as before. By simulation, we then randomly drew individuals from the total pool of individuals, until we obtained the same number per habitat as the number of individuals originally observed there. For this random realisation we again calculated the average RGB difference across all individuals. This process was repeated 10,000 times, and we calculated the proportion of times that the random distribution produced a RGB difference that was as small or smaller as the observed one (i.e. a one-sided test). This proportion was used as the p-value to test the null hypothesis that resident grasshoppers are not more cryptic than random grasshoppers. The same procedure was used for each pavement separately. (Note that this is a conservative test, since we don´t test resident versus potential

immigrant, but resident versus random, i.e. including the resident grasshoppers within the randomisation).”

```
        ***In addition, we followed the reviewer suggestion to assign
individuals to habitats randomly, but this is not quite in line with
the literature on local adaptation – a more pertinent comparison would
be between the realised situation (e.g. grasshopper 1 is on asphalt)
versus the non-realised alternative (grasshopper 1 is on one of the
other three pavements). However, using random habitats/grasshoppers
simplifies the simulations and perhaps also interpretation, and in
fact provides a statistically more conservative test for local
adaptation since the presumably adaptive subset is now included in the
simulated set. We have noted this in the description of the methods.

        ***Finally, we mention doing one-sided tests, but this is
really due to the nature of the hypothesis (we predict and are only
interested in local adaptation, not greater maladaptation than
random), and not in order to try to make non-significant results
significant (as one-sided testing sometimes is (ab)used). In fact, the
significance and interpretations of the results remain virtually
unaltered, since generally there either is a very significant effect,
or it is not close to significance.

Another general remark is that it is rather difficult to find out
sample size. I expect to find them in the methods section, but they
are mostly presented in the results section. I suggest to had samples
size in brackets, for example (but not limited to) in L268 (N = xx)
for the habitat selection after manipulation.
        *** In some cases we feel it is more useful to have sample
sizes in the results section whenever statistical tests are performed,
since this helps to assess the significance of the reported test
statistic. In other cases we added information on sample size in the
methods, as requested. We also found one sample size misreported (it
was larger at 27 instead of 17, a typo for which we apologise).

Minor

L66: I find "effectively counter-act random habitat use" a somewhat
odd expression.
        *** We removed the word "effectively".

L133: "Below we elaborate"
        *** Changed accordingly.

L268: "on a 115-metre long street"
        *** Changed accordingly.

L331: Regular intervals are equally spaced, I would think. You could
write "frequently" instead (although every few weeks isn't really
frequent).
        *** We removed "regular"

L348: Effect size estimate would be nice (as in L253).
        *** We provided some extra effect sizes (in mean RGB colour
differences).

L350: revise the statement in brackets, I think p < 0.0001 is for all
types except pale tiles, something like (all p < 0.0001, except for
pale tiles p = x.xx) would do.
        *** All two-sided p are actually < 0.0001, but for pale tiles
the direction of the effect is opposite. Since we performed one-sided
```

tests (as discussed above), we now report that the result is not
significant, as p = 1.00, and mention the effect in opposite direction
as before.

L673: I don't see dashes, but I see stars and circles.
        *** Correct, we now describe the difference in the legend.

Figure 2: Suggest to remove "confirmed" in "contributing to the
grasshopper-pavement colour match"
        *** Removed.

Figure 3: A) The caption mentions white tick marks, but I can't see
any. (B+C)
        *** That is strange – we do see them? We will check this in the
proofs (if the paper is accepted). By the way, as described in the
legend, the tick marks are and should only be present in panel A.

The colours are hardly distinguishable in greyscale (suggest to choose
colours with more different grey values).
        *** Good suggestion – we chose a pale blue colour for the blue
boxes.

Also include a space before "vs." in the title.
        *** We don´t understand this comment: both in the figure and
the legend there is always a space before vs. Perhaps something went
wrong in conversion to the pdf for review? We keep an eye open in the
proofs, if the paper is accepted.

Supplementary L91-99: Did you use log10 or ln? The default in R using
log would be ln, in which case the back-transformation would have e in
the base.
        *** Thanks for the warning. The back-transformation was
actually done in excel, with the correct base.

Simulation script
        *** Again, we very much appreciate the effort.

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nrep = 1000
resT = rep(NA,nrep) # test statistics for normal t tests
resLMM = rep(NA,nrep) # test statistics from mixed models
resLMMvar = rep(NA, nrep) # individual identity variance component
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t tests
mean(abs(resLMM)>1.96) # inflation of significance when using mixed
models in this case
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negative correlation, but you see more extreme values for mixed models
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```
sort(resLMMvar) # individual identity variance components are pretty
much all zero
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****