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Genetic coupling of signal and preference facilitates sexual isolation during rapid speciation

Mingzi Xu and Kerry L. Shaw

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Review timeline

Original submission:	8 July 2019
1st revised submission:	13 September 2019
2nd revised submission:	30 September 2019
Final acceptance:	1 October 2019

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

Review History

RSPB-2019-1607.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?
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?
Yes

Is it clear?
No

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Xu and Shaw fine map variation in male song traits and corresponding female preferences in Laupala crickets, and test whether loci underlying these two traits are physically linked. Their results suggest that this is indeed the case, and so provide one of the most robust examples where corresponding signal and preferences are controlled by loci that are physically very tightly linked (within ~1.6cM), or even controlled by pleiotropic alleles.

I can't identify any major methodological problems, and only have a few general comments. Overall, I think this is a fantastic paper, which should certainly be published in Proc B.

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1) Use of the term 'coupling'. I understand that the term is used here to imply a physical relationship between alleles (i.e. tight linkage or pleiotropy) underlying cue and preference. I think this usage stems from the Butlin and Ritchie (1989) paper, cited in the manuscript, or perhaps older. Unfortunately, I think with more recent usage – particularly within the speciation literature – it has taken on a broader (and in my mind more useful) meaning: Butlin & Smadja (2017, AM Nat) define it as 'any process that generate coincidence of barrier effects'. Both LD resulting between unlinked loci as a result of assortative mating *and* LD resulting from tight physical linkage would fit this definition (as would coupling of cue and preferences as a result of one-allele mechanisms). Neither definition is *wrong*, but to avoid this ambiguity, my *suggestion* would be to avoid 'coupling' and refer to 'tight linkage' and 'pleiotropy'. For example, in the abstract:
"we tested two contrasting hypotheses for the genetic architecture underlying signal-preference coevolution: linkage disequilibrium between unlinked loci and genetic coupling (pleiotropy of a shared locus or tight physical linkage)"
might be changed to:
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Minor comments.
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I applaud the authors for including these scripts – though some annotation would be helpful.

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Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
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.
No

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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
I believe this to be a significant paper in the continuing study of the genetic basis of male trait and female preference in a group undergoing rapid speciation. Theory predicts circumstances under which premating isolation can arise and persist in the face of gene flow, and "genetic coupling" is one important possibility. However, there are few examples from natural systems. This paper provides important support for close linkage between a trait locus and a preference locus, supporting coupling theory. In addition, we know almost nothing about the genetics of divergence in preferences (mating biases) and this system represents one case in which it might be possible soon to identify genes and snps that underlie divergent preferences. I feel that the paper's results are useful now and also point to further riches ahead.

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Decision letter (RSPB-2019-1607.R0)

09-Aug-2019

Dear Dr Xu,

Thank you for submitting this manuscript to Proc B - it looks like a really interesting set of results!

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:

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

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 Loeske Kruuk
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This study presents fine-scale quantitative trait locus (QTL) mapping of a female acoustic signal preference in a species pair of crickets. It is positioned very close to a QTL for male pulse rate, representing a second case of a shared QTL underlying both signal and preference in these crickets. The study is framed in terms of testing alternative hypotheses about the genetic architecture underlying signal-preference co-evolution - which is a topic of broad interest and

significance. It also represents one of very few studies that have identified QTLs for both signal and preference. Both reviewers agree that it makes an important contribution to our understanding of the genetic architecture of signal-preference coevolution. The reviewers have a number of suggestions to fine-tune the manuscript, which will make a very nice contribution to PRSLB.

Reviewer(s)' Comments to Author:

Referee: 1

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Author's Response to Decision Letter for (RSPB-2019-1607.R0)

See Appendix A.

Decision letter (RSPB-2019-1607.R1)

23-Sep-2019

Dear Dr Xu

I am pleased to inform you that your manuscript RSPB-2019-1607.R1 entitled "Genetic coupling of signal and preference facilitates sexual isolation during rapid speciation" has been accepted for publication in Proceedings B.

The Associate Editor has recommended publication, but has also suggested some minor revisions to your manuscript. Therefore, I invite you to respond to the AE'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.

Yours sincerely,
Professor Loeske Kruuk
Editor, Proceedings B
mailto:proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
This is an excellent study. The authors have, in most cases, addressed the reviewer comments well. However, there are some minor comments that still need to be addressed:

1. Original reviewer comment: Line 52 "Hidden within the divergence of sexual signaling systems is a co-evolutionary process: while signals and preferences diverge among lineages, they are functionally constrained to maintain effective communication, and thus, to co-evolve within a lineage [6, 7]...What genetic architecture facilitates signal-preference coevolution'. This is obviously important for setting up the paper, but I think it could be rephrased in a simpler way. It's not immediately clear to me what the authors are trying to say.

Author response: We think the sentence is clear and prefer to leave it as written. Our intent was to evoke a sense of biological mystery.

BM comment. I agree that this sentence can be clearer, and is also not necessarily true. The functional 'constraint' can be very loose. For example, divergence of color signals without associated divergence in preferences, is common. More accurate to say that 'Divergence of sexual signalling systems often entails co-evolution of signals and preferences within a lineage because they are functionally constrained to maintain effective communication'

2. Scripts - the reviewer noted insufficient annotation, but this doesn't seem to have been addressed. I too am a big fan of including scripts to ensure repeatability and transparency. However, if these scripts are not sufficiently annotated, they are of little use. Please carefully annotate the scripts.

3. Original reviewer comment: L275 and Table 1: 'Similar magnitudes of effect"? In each case the effect size for pulse rate seems ~twice that for preference? Are these really similar?

Author response: Here, we were referring to the order of magnitude of the effect, i.e., whether it is on the 1/100 magnitude or 1/10 magnitude. In addition, both effect sizes are minor to moderate in a broad sense.

BM comment: I agree that 'relatively similar magnitude of effect' is too vague and should be clarified. If you mean 'order of magnitude, please say so, though I'm not sure why you'd expect effect sizes to differ by orders of magnitude. Otherwise, you can say that effect sizes were both minor to moderate though in each case the effect size for pulse rate is approximately twice that for preference.

4. Original reviewer comment: line 121: Say how many offspring resulted.

Author response: We do not have the total number of offspring that resulted. We only have the number of offspring we have phenotyped, which is in the Result section.

BM comment: The reader needs to have a clear understanding of sample size in the methods, rather than having to read results before knowing sample size. At line 121, please add…..from which we phenotyped XX individuals (Results).

5. Original Reviewer comment: line 334: You are probably right about the traits, but I'd be surprised if genetic difference in the underlying gene, a pleiotropic one at that, would not also be under natural selection.

Author response: These behaviors are used in the highly specialized context of mating, which is why it has been argued that Laupala is a case of non-adaptive radiation and specifically, speciation by sexual selection (Mendelson and Shaw 2005, Nature 433: 375-376).

BM comment: I appreciate the point you are making but even traits that 'function exclusively in a reproductive context' are very likely to also be under natural selection (e.g. because calls attract predators), which will affect the evolution of sexual signalling systems. It's worth acknowledging this possibility, rather than conveying a likely artificial dichotomy between 'magic traits' and exclusively sexual traits.

Author's Response to Decision Letter for (RSPB-2019-1607.R1)

See Appendix B.

Decision letter (RSPB-2019-1607.R2)

01-Oct-2019

Dear Dr Xu

I am pleased to inform you that your manuscript entitled "Genetic coupling of signal and preference facilitates sexual isolation during rapid speciation" 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.

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

Sincerely,

Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

09-Aug-2019

Dear Dr Xu,

Thank you for submitting this manuscript to Proc B - it looks like a really interesting set of results!

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.

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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. 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 Loeske Kruuk
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This study presents fine-scale quantitative trait locus (QTL) mapping of a female acoustic signal preference in a species pair of crickets. It is positioned very close to a QTL for male pulse rate, representing a second case of a shared QTL underlying both signal and preference in these crickets. The study is framed in terms of testing alternative hypotheses about the genetic architecture underlying signal-preference co-evolution - which is a topic of broad interest and significance. It also represents one of very few studies that have identified QTLs for both signal and preference. Both reviewers agree that it makes an important contribution to our understanding of the genetic architecture of signal-preference coevolution. The reviewers have a number of suggestions to fine-tune the manuscript, which will make a very nice contribution to PRSLB.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Xu and Shaw fine map variation in male song traits and corresponding female preferences in Laupala crickets, and test whether loci underlying these two traits are physically linked. Their results suggest that this is indeed the case, and so provide one of the most robust examples where corresponding signal and preferences are controlled by loci that are physically very tightly linked (within ~1.6cM), or even controlled by pleiotropic alleles.

I can't identify any major methodological problems, and only have a few general comments. Overall, I think this is a fantastic paper, which should certainly be published in Proc B.

General comments.

1) Use of the term 'coupling'. I understand that the term is used here to imply a physical relationship between alleles (i.e. tight linkage or pleiotropy) underlying cue and preference. I think this usage stems from the Butlin and Ritchie (1989) paper, cited in the manuscript, or perhaps older. Unfortunately, I think with more recent usage – particularly within the speciation literature – it has taken on a broader (and in my mind more useful) meaning: Butlin & Smadja (2017, AM Nat) define it as 'any process that generate coincidence

of barrier effects'. Both LD resulting between unlinked loci as a result of assortative mating *and* LD resulting from tight physical linkage would fit this definition (as would coupling of cue and preferences as a result of one-allele mechanisms). Neither definition is *wrong*, but to avoid this ambiguity, my *suggestion* would be to avoid 'coupling' and refer to 'tight linkage' and 'pleiotropy'. For example, in the abstract:
"we tested two contrasting hypotheses for the genetic architecture underlying signal-preference coevolution: linkage disequilibrium between unlinked loci and genetic coupling (pleiotropy of a shared locus or tight physical linkage)"
might be changed to:
"we tested two contrasting hypotheses for the genetic architecture underlying signal-preference coevolution: i) linkage disequilibrium, arising from assortative mating, between unlinked loci and ii) linkage disequilibrium resulting of tight physical linkage or pleiotropy"
To reiterate this is a suggestion and I am more than happy I the authors disagree. That said, as it stands, I do think it is a bit confusing and it might be better to be more explicit.

We think a shorter, more memorable term would be better for disseminating the concept. We clearly define "genetic coupling" when it first appears in line 33-34 and refer to "genetic coupling" as the short hand thereafter. We also added "linkage disequilibrium resulting from" in the definition of genetic coupling in line 33. As the reviewer notes, our usage does have precedence in the literature, and we prefer not to change it. We thank the reviewer for pointing this out but being gracious by making it only a suggestion to change it.

2) Table 2 is missing.

It is a mislabeling of the table in the main text (it should be Table 1). We have corrected it.

3) What is the evidence that these differences in song actually result in assortative mating? Are there any data on this – i.e. mate choice trails rather than trials measuring preference? Perhaps these experiments have not be done (and it does not affect my view of this paper), but if these data are available perhaps this could be discussed (perhaps in association with the information in L88).

We have a manuscript in preparation that documents that song preference reflects a motivation to mate, but it will not be through the review process in time for the publication of this paper.

4) I'm a little confused by the estimate of genetic distance between cue and preference loci given (for example, in the abstract L37 'estimates of the pulse rate and preference loci are 0.06-0.23 cM apart', and elsewhere throughout the manuscript). These seem to refer to the position of the QTL peaks – but if so why the range? Presumably markers at different position have the same LOD score, which must result from recombination between the markers in the male but not female mapping populations? I think it would be better to report the cM distance between the two peaks and then the range based on the 1.5 lod interval, which I think would be: "0.06 (0-1.65) cM".
Related to this, how does this compare to previous estimates for QTL on other linkage groups (from the Shaw and Lesnick paper I think) using the same methods to estimate the degree of linkage?

The range comes from the fact that the maximum female preference peak is not a single point but a plateau between 26.17-26.34 cM. Since the male pulse rate peak location is estimated at 26.40 cM, the distance between the male and female peaks is thus a range, between 0.06 (26.40-26.34) and 0.23 (26.40-26.17) cM. It is difficult to explain why there is a range in the short abstract so we changed the wording to "as close as 0.06 cM apart" to avoid confusion. In the main text, we pointed out that the female peak is a plateau in the LOD profile in line 211 and added an explanation of where this range comes from in lines 271-272.

Minor comments.
Introduction.

P52 'Hidden in the divergence of sexual signalling … What genetic architecture facilitates signal-preference coevolution'. This is obviously important for setting up the paper, but I think it could be rephrased in a simpler way. It's not immediately clear to me what the authors are trying to say.

We think the sentence is clear and prefer to leave it as written. Our intent was to evoke a sense of biological mystery.

L77 'are' -> 'is'

Corrected.

L82 Pleiotropic ‘alleles’ rather than ‘genes’

We have made the suggested change.

L98 What previous study? Citation needed.

We have added a citation.

L110 ‘finding illuminates the quantitative dynamics of co-evolution in sex-limited traits under sexual selection’ – It is not clear to me what this sentence adds.

We have changed the wording to clarify.

Methods

L117 Although this is in the authors’ previous (Genetics) paper, I think a figure describing the crossing design might help this paper stand alone. In general, I think this manuscript relies too much on methods described elsewhere – I understand the benefits of brevity, but found myself constantly having to look up important details.

Thank you for this suggestion. We have added a figure (the new Figure 1) for the crossing design. This figure is reproduced with modifications from our Genetics 2019 paper with permission from the Genetics Society of America. We have cited the original paper in the figure legend and included the full citation in the reference list.

Figure 1a is not very well described in the legend. Is the tube below an enlargement of the tube above? Why are there three tubes on top of each other?

We have added more detailed description of this figure in the legend.

L178 Very small point – changing from past to present tense (‘kept’ then ‘is’) is a little distracting to read.

Corrected.

Scripts and data: I was unable to obtain the scripts with:
https://github.com/MingziXu/QTL4-colocalization-scripts
though did find them eventually, I think the link should be:
https://github.com/MingziXu/QTL4-colocalization-scripts-and-data
I applaud the authors for including these scripts – though some annotation would be helpful.

We corrected the link.

Results

Figure 2. Are these cumulative plots? i.e. does the near normal distribution for the pink f2 individuals represent both ‘pure species’ and hybrid individuals?

We have added explanation in the figure legend.

Discussion

L265 Mis-citation? Based on the paragraph starting 327, and the critique of ref 27 in ref 28, I think this should be “[26, 28]’

Corrected.

L275 and Table 1: ‘Similar magnitudes of effect”? In each case the effect size for pulse rate seems ~twice that for preference? Are these really similar?

Here, we were referring to the order of magnitude of the effect, i.e., whether it is on the 1/100 magnitude or 1/10 magnitude. In addition, both effect sizes are minor to moderate in a broad sense.

L281 I feel like this statement could do with a reference?

We have added citations.

L300 'super gene' – Again just a suggestion, but I feel 'super gene' is typically confined to situations where an apparently complex phenotype, controlled by a seemingly simple genetic basis, is polymorphic *within a population*. I think it's a little distracting here - I would *suggest* simply referring to tight physical linkage.

From the gene evolution point of view, the selective forces favoring the evolution of tightly linked signal and preference genes are consistent with that expected for the evolution of super genes (Schwander et al. 2014, Current Biology 24:R288-R294). We have modified the sentence to say "gene cluster" first, and then, liken it to a supergene. We think referring to this term will benefit the discussion by linking the genetic architecture of signal-preference coevolution to a well-developed topic.

L327 perhaps insert 'loci for'?

Done.

L330 The Heliconius study was looking at variation in 'visual preferences' rather than 'courtship time'.

We have made the suggested change.

L332 'sexual signals' -> 'mating cues' – again a small point but I think 'sexual signal' implies that the trait has 'evolved to' transfer information relating to mate choice, which is not necessarily the case with the stickleback and Heliconius cues (which have diverged due to ecological selection).

We have changed it to "mating signal or cues" to be accurate.

L333 'Magic trait' implies a little more than just functioning in a 'both a ecological and mate choice context'. Importantly magic trait models depend on trait under *divergent ecological selection* that contribute to assortative mating (perhaps as a mating cue).

We are not providing a definition of "magic traits" but rather, a contrast to the type of traits we studied, as evident by the "In contrast" in the next sentence.

L347 'recently' Actually these (broad) ideas are quite old – Maynard-Smith certainly referred to 'pleiotropism' in his 1966 'Sympatric Speciation' paper (though I suppose he was referring to pleiotropy between traits under divergent selection and those for mate choice, so perhaps it is a little different).

The pleiotropism in the Maynard-Smith 1966 paper is not exactly the same as what we are discussing in this paper. In this sentence, we emphasize a need to further understand the process and consequences of genetic coupling through rigorous theoretical treatment and formal models (as opposed to verbal models). Therefore, we think the use of "recently" is appropriate in our intended context.

Referee: 2

Comments to the Author(s)
I believe this to be a significant paper in the continuing study of the genetic basis of male trait and female preference in a group undergoing rapid speciation. Theory predicts circumstances under which premating isolation can arise and persist in the face of gene flow, and "genetic coupling" is one important possibility. However, there are few examples from natural systems. This paper provides important support for close linkage between a trait locus and a preference locus, supporting coupling theory. In addition, we know almost nothing about the genetics of divergence in preferences (mating biases) and this system represents one case in which it might be possible soon to identify genes and snps that underlie divergent preferences. I feel that the paper's results are useful now and also point to further riches ahead.

The paper is clear, brief, and sound. I had only a few minor comments.

line 120: remind us here this is LG5

Done.

line 121: Say how many offspring resulted.

We do not have the total number of offspring that resulted. We only have the number of offspring we have phenotyped, which is in the Result section.

line 194: Say whether this is a genome wide significance level. For testing predictions on LG5, you'd be justified in using a table-wide alpha level instead, where the table consisted only of markers within the introgressed sequence.

We agree. This is not the genome wide significant level because our QTL mapping analyses were focused on LG5 only. We have added the description to clarify that this significance level is "Linkage-group-wide".

line 334: You are probably right about the traits, but I'd be surprised if genetic difference in the underlying gene, a pleiotropic one at that, would not also be under natural selection.

These behaviors are used in the highly specialized context of mating, which is why it has been argued that *Laupala* is a case of non-adaptive radiation and specifically, speciation by sexual selection (Mendelson and Shaw 2005, Nature 433: 375-376).

Appendix B

23-Sep-2019

Dear Dr Xu

I am pleased to inform you that your manuscript RSPB-2019-1607.R1 entitled "Genetic coupling of signal and preference facilitates sexual isolation during rapid speciation" has been accepted for publication in Proceedings B.

The Associate Editor has recommended publication, but has also suggested some minor revisions to your manuscript. Therefore, I invite you to respond to the AE'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.

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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:
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- Phylogenetic data: TreeBASE accession number S9123
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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.

Yours sincerely,

Professor Loeske Kruuk
Editor, Proceedings B
mailto:proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:

This is an excellent study. The authors have, in most cases, addressed the reviewer comments well. However, there are some minor comments that still need to be addressed:

1. Original reviewer comment: Line 52 "Hidden within the divergence of sexual signaling systems is a co-evolutionary process: while signals and preferences diverge among lineages, they are functionally constrained to maintain effective communication, and thus, to co-evolve within a lineage [6, 7]...What genetic architecture facilitates signal-preference coevolution'. This is obviously important for setting up the paper, but I think it could be rephrased in a simpler way. It's not immediately clear to me what the authors are trying to say.

Author response: We think the sentence is clear and prefer to leave it as written. Our intent was to evoke a sense of biological mystery.

BM comment. I agree that this sentence can be clearer, and is also not necessarily true. The functional 'constraint' can be very loose. For example, divergence of color signals without associated divergence in preferences, is common. More accurate to say that 'Divergence of sexual signalling systems often entails co-evolution of signals and preferences within a lineage because they are functionally constrained to maintain effective communication'

We have made the suggested change.

2. Scripts - the reviewer noted insufficient annotation, but this doesn't seem to have been addressed. I too am a big fan of including scripts to ensure repeatability and transparency. However, if these scripts are not sufficiently annotated, they are of little use. Please carefully annotate the scripts.

We have added qtl mapping script files with detailed annotations. The QTL forced call script already contains annotation and we also included more detailed explanation of what each command does in this script in the supplementary information.

3. Original reviewer comment: L275 and Table 1: 'Similar magnitudes of effect"? In each case the effect size for pulse rate seems ~twice that for preference? Are these really similar?

Author response: Here, we were referring to the order of magnitude of the effect, i.e., whether it is on the 1/100 magnitude or 1/10 magnitude. In addition, both effect sizes are minor to moderate in a broad sense.

BM comment: I agree that 'relatively similar magnitude of effect' is too vague and should be clarified. If you mean 'order of magnitude, please say so, though I'm not sure why you'd expect effect sizes to differ by orders of magnitude. Otherwise, you can say that effect sizes were both minor to moderate though in each case the effect size for pulse rate is approximately twice that for preference.

We have changed the wording here to " both preference and song QTL contribute relatively minor to moderate effect in a largely additive way to the differences in acoustic behaviors between the two species " to avoid confusion.

4. Original reviewer comment: line 121: Say how many offspring resulted.

Author response: We do not have the total number of offspring that resulted. We only have the number of offspring we have phenotyped, which is in the Result section.

BM comment: The reader needs to have a clear understanding of sample size in the methods, rather than having to read results before knowing sample size. At line 121, please add…..from which we phenotyped XX individuals (Results).

We have made the suggested addition in line 128-129.

5. Original Reviewer comment: line 334: You are probably right about the traits, but I'd be surprised if genetic difference in the underlying gene, a pleiotropic one at that, would not also be under natural selection.

Author response: These behaviors are used in the highly specialized context of mating, which is why it has been argued that Laupala is a case of non-adaptive radiation and specifically, speciation by sexual selection (Mendelson and Shaw 2005, Nature 433: 375-376).

BM comment: I appreciate the point you are making but even traits that ‘function exclusively in a reproductive context’ are very likely to also be under natural selection (e.g. because calls attract predators), which will affect the evolution of sexual signalling systems. It’s worth acknowledging this possibility, rather than conveying a likely artificial dichotomy between ‘magic traits’ and exclusively sexual traits.

We have changed "exclusively" to "primarily" and added "(ecological function of pulse rate or preference for pulse rate has yet to be discovered)" at the end of this phrase.