

Polygenic basis for adaptive morphological variation in a threatened Aotearoa|New Zealand bird, the hii (*Notiomystis cincta*)

Laura Duntsch, Barbara M. Tomotani, Pierre de Villemereuil, Patricia Brekke, Kate D. Lee, John G. Ewen and Anna W. Santure

Article citation details

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Review timeline

Original submission: 26 April 2020
Revised submission: 18 June 2020
Final acceptance: 3 August 2020

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

Review History

RSPB-2020-0948.R0 (Original submission)

Review form: Reviewer 1

Recommendation

Major revision is needed (please make suggestions in comments)

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

Acceptable

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

The manuscript RSPB-2020-0948 studies the genetic basis of three morphological traits in hihi by first looking at heritability of the trait and then doing chromosome partitioning and genome-wide association analyses. Authors did not find any significant associations which is most probably due to low heritability and high polygenic background of the traits. The analyses are mostly sound but the manuscript failed to convince why to study these three traits in hihi. Thus, the manuscript needs some re-writing and additional analyses in order to be suitable for publication.

I suggest a correction of the work considering the following points.

Introduction

Lines 115-120: Could you write more about how nestling beak and tarsus length and weight are important for fitness and adaptation? Because that is your main story in the manuscript but now it isn't clear why those traits are important to study and why in hihi.

Methods

Lines 173-174: what QC metric were used in the Axiom Analysis Software? The default settings or hihi specific?

Lines 177-178: Did you check the structure with PCA plotting, both including all the populations and just having Tiritiri? This is to be sure that there isn't any hidden genetic structure or outliers that could affect the results.

GWAS: Do you know the linkage disequilibrium in hihi? If LD is really small that could also explain why you won't find anything as you will need many more SNPs to detect associations.

Line 256: Please give the annotation database version.

Results

Please start with giving the trait measurement variation like the mean, SD, sex and how many individuals. Especially it is interesting to see how is the variation around the mean of the traits. It might be also good to calculate the evolvability of the traits in addition to heritability especially if

there is little phenotypic variation.

Figure 1: Please explain “a, b, c” micro-chromosomes in the caption.

Discussion

I think one additional discussion point is how difficult trait especially nestling weight is as non-genetic factors affect it quite a lot (weather, environment, parents) which in turn makes the heritability lower and also it's difficult to catch associations in the GWAS.

Line 426: Lard = Lars

Review form: Reviewer 2

Recommendation

Accept with minor revision (please list in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

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

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

This ms explores the genetic basis of three morphological traits under selection in a small threatened population of hihi from New Zealand. 523 birds were genotyped on a 50K SNP array which formed the basis for probing past the quantitative genetic basis of these traits using chromosome partitioning as well as GWAS. The key findings of this work is that 1) heritability is low for all three traits inferred from a animal model with reconstructed pedigree (from microsatellite markers) as well as when using the GRM from the SNP array as relatedness matrix (using both MCMCglmm and RepeatABEL) as well as BayesR to infer effect size distribution. 2) Chromosome partitioning analyses demonstrate polygenic basis to all traits and 3) GWAS results also support an infinitesimal basis to these traits with no genome wide significant marker. None of these results should come as a surprise given what we know about the genetic architecture of morphological traits in other avian and non avian systems however.

The ms is well written and clearly presented. The analyses have been expertly carried out and I have no issues with the methods section of the ms. It might not be the most surprising or exciting results, but I agree with the authors that demonstrating a polygenic basis to traits in small threatened populations can be informative for conservation purposes and for that reason I think this will make a nice addition to the literature. I just have a few minor comments on the ms that I would like to see the authors address:

Table 1 and line 361-362: the difference between the pedigree estimated h^2 and GRM based h^2 is particularly large for tarsus and head-bill length. Since its not a consistent difference across traits (though it is hard to draw much inferences from only three traits of course) I was wondering if there is anything specific about these traits (or their measurement) that could explain this? The differences between GRM and pedigree is of course the same for all traits given that the authors restricted the analysis to the same set of individuals (if I interpret the methods correctly) so it should not be due to differences in pedigree structure between traits.

line 280-281: A correlation of 0.75 between the pedigree and GRM was less than what I had expected given that the pedigree is actually reconstructed based on 13 SSRs. I was wondering if you have explored this further? Might be interesting to do given that even some close relationships seems to have been erroneously inferred from the SSRs.

line 174: filtering on maf 0.01 is often used in studies but it does mean that one can have very few individuals that are homozygotes of the rare genotype depending on the sample size, which of course in this study is low ($0.01^2 * 523 = 0.05$ ind expected under HWE) which can cause problems in association analysis. It would be interesting to report the number of individuals for each genotype class for the reported "outlier" loci.

Line 355: collared flycatchers also analysed by Silva et al

Arild Husby

Decision letter (RSPB-2020-0948.R0)

04-Jun-2020

Dear Ms Duntsch:

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.

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

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

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

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

Best wishes,
Professor Gary Carvalho
mailto:proceedingsb@royalsociety.org

Associate Editor
Comments to Author:

Both reviewers found the analyses presented here to be sound, and one noted that the study provides a nice addition to the literature. The reviewers had a few suggestions for clarifying the methods and further interpreting the results. One reviewer raised concerns about the framing of the manuscript, which should not be too difficult to address. You mention that the three morphological traits of interest are known to be under selection in the abstract but not in the introduction or the discussion.

In addition to the comments from the reviewers, I would more explicitly discuss the limitations of GWAS with small sample sizes and provide more information on the model used for your GWAS. Also, I would tweak the BayesR results to present the proportion of variance explained by SNPs of each effect size and include the proportion of SNPs that have no effect on each trait.

I look forward to seeing the revised version.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

The manuscript RSPB-2020-0948 studies the genetic basis of three morphological traits in hibi by first looking at heritability of the trait and then doing chromosome partitioning and genome-wide association analyses. Authors did not find any significant associations which is most probably due to low heritability and high polygenic background of the traits. The analyses are mostly

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Referee: 2

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Arild Husby

Author's Response to Decision Letter for (RSPB-2020-0948.R0)

See Appendix A.

RSPB-2020-0948.R1 (Revision)

Review form: Reviewer 1

Recommendation

Accept as is

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

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

Thank you for the corrections. I have no further comments. Well done!

Review form: Reviewer 2

Recommendation

Accept as is

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

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No

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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

I congratulate the authors on a nice paper and a good job with the revisions!

Decision letter (RSPB-2020-0948.R1)

03-Aug-2020

Dear Ms Duntsch

I am pleased to inform you that your manuscript entitled "Polygenic basis for adaptive morphological variation in a threatened Aotearoa | New Zealand bird, the hihi (*Notiomystis cincta*)" 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

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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,
Professor Gary Carvalho
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:

Comments to Author:

I and both reviewers think the authors have done a good job with the revisions. Thank you and nicely done!

Appendix A

Dear Editor Professor Gary Carvalho and Associate Editor Dr Nancy Chen,

Many thanks for the encouraging news that our manuscript may be suitable for publication in Proceedings of the Royal Society B following some revisions.

We found the comments of the Associate Editor and the two reviewers very helpful and apologise that we neglected some important details in our original submission. We have modified our manuscript to address the Associate Editor and reviewers' insightful comments and suggestions, and respond fully to these comments below. In particular, we have added details of the selection acting on the three examined morphological traits and discussed more fully the sample size constraints of the genome wide association analyses (including adding an analysis of linkage disequilibrium and power). Moreover, we have added an analysis of population structure within our dataset, and have further investigated the correlation between pedigree and genomic relatedness values, in addition to adding more details and edits as requested. The main manuscript including track changes can be found at the end of this document, following the responses.

Please note that the line numbers in our point-by-point responses below refer to the separate clean version of the main document without tracked changes. In addition, our two figures are now uploaded as separate files but the captions can be found on *manuscriptcentral* and at the end of the clean main document.

Thank you for providing a link to Dryad, we have now included our datasets on this repository and added the details to our manuscript.

In addition, we would be delighted for you to consider the uploaded hihi photo as cover image for this Special Issue. Photographer Oscar Thomas confirmed that we have full permission to reproduce the image online and in print in perpetuity.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you might have.

All authors have read and approved of the revised manuscript.

With best wishes,
Laura Duntsch, on behalf of all of the authors.

Associate Editor
Comments to Author:

Both reviewers found the analyses presented here to be sound, and one noted that the study provides a nice addition to the literature. The reviewers had a few suggestions for clarifying the methods and further interpreting the results. One reviewer raised concerns about the framing of the manuscript, which should not be too difficult to address. You mention that the three morphological traits of interest are known to be under selection in the abstract but not in the introduction or the discussion.

Our apologies, we indeed completely neglected to provide information about the selection pressure on these traits. We have added the following to the introduction, lines 112-118: *"While previous hihi studies have examined the effect of body size on reproduction (e.g. Brekke et al., 2015) and survival (e.g. Low and Part, 2009), the large dataset (>2,000 birds) analysed by de Villemeureuil et al., (2019b) revealed that all three morphological traits had*

positive linear selection and negative non-linear selection gradients. These gradients suggest that there are optimal values for nestling tarsus length, head-bill length and mass that maximise fitness, but that all trait means are smaller than these optima, and hence are under directional selection to increase body size.”

And discussion, lines 376-378:

“The three morphological traits studied here are all under selection as previous analyses suggest that the population mean of all three traits is smaller than the fitness optimum (de Villemereuil et al. 2019b).”

In addition to the comments from the reviewers, I would more explicitly discuss the limitations of GWAS with small sample sizes

Thank you, this is an excellent suggestion. We have added to the discussion lines 356-373, which now includes a power analysis based on our calculation of linkage disequilibrium between our markers (please see response to referee 1 below):

“The sample size, number of markers and low heritabilities is also likely to have limited power to detect association in the GWAS analyses. Linkage disequilibrium between neighbouring markers is moderate (mean of 0.518, calculated with all 523 individuals using plink --r2 and reporting all pairwise values with --ld-window-r2 0.0) and suggests that power to detect a locus explaining all of the trait heritability ranges from 0.306 to 0.516 (Wang and Xu 2019; electronic supplementary material, table S6). As expected, nestling mass, with the lowest heritability due to substantial maternal and month effects compared to the other traits (de Villemereuil et al. 2019b), has the lowest power to detect association.”

and provide more information on the model used for your GWAS

Our apologies, we overlooked this important information. We have clarified lines 249-251 that the model used is the same as that for the preceding heritability analyses:

“SNPs are then tested for association by accounting for these random effects in the “rGLS” function in RepeatABEL, using the same fixed and random effects as in the heritability analysis.”

Also, I would tweak the BayesR results to present the proportion of variance explained by SNPs of each effect size and include the proportion of SNPs that have no effect on each trait.

Rather than provide the proportion of SNPs that have no effect, we have adjusted the last column of table S5 (for space reasons table 2 has been moved to the supplements and merged with table S5) to show the proportion of SNPs that **do** have an effect on the trait; we hope that this is acceptable. With regard to the proportion of variance explained by SNPs in each effect size, we have also added these details to the supplementary information (Supplementary Table S5) with a brief reference to this table on line 268/269. The reason we would prefer not to add this information to the main text is we feel it is a little unreliable as calculations of variance explained by genotyped SNPs (i.e. $\sum 2p(1-p)\beta^2$) rely on accurate estimation of both effect sizes (β) and of allele frequency (p), as well as assuming Hardy Weinberg equilibrium, and any inaccuracies in estimates of β and p are exponentially increased (i.e. p^2 and β^2).

I look forward to seeing the revised version.

Reviewer(s)' Comments to Author:
Referee: 1

Comments to the Author(s)

The manuscript RSPB-2020-0948 studies the genetic basis of three morphological traits in hihi by first looking at heritability of the trait and then doing chromosome partitioning and genome-wide association analyses. Authors did not find any significant associations, which is most probably due to low heritability and high polygenic background of the traits. The analyses are mostly sound but the manuscript failed to convince why to study these three traits in hihi. Thus, the manuscript needs some re-writing and additional analyses in order to be suitable for publication.

I suggest a correction of the work considering the following points.

Introduction

Lines 115-120: Could you write more about how nestling beak and tarsus length and weight are important for fitness and adaptation? Because that is your main story in the manuscript but now it isn't clear why those traits are important to study and why in hihi.

Our apologies that we completely neglected to describe the selection pressures on these traits of this threatened species. We've added details to lines 112-118 in the introduction: "*While previous hihi studies have examined the effect of body size on reproduction (e.g. Brekke et al., 2015) and survival (e.g. Low and Part, 2009), the large dataset (>2,000 birds) analysed by de Villemereuil et al., (2019b) revealed that all three morphological traits had positive linear selection and negative non-linear selection gradients. These gradients suggest that there are optimal values for nestling tarsus length, head-bill length and mass that maximise fitness, but that all trait means are smaller than these optima, and hence are under directional selection to increase body size.*"

With respect to the query of 'why hihi', with all biases included, we do feel that hihi are an important species globally because of the quality and quantity of the long-term data available for this threatened species. We therefore describe hihi as a '*model species for studying the genetics of endangered birds, and species with small population sizes more generally*' (line 102-104).

Methods

Lines 173-174: what QC metric were used in the Axiom Analysis Software? The default settings or hihi specific?

Thanks, we've clarified that we used the default settings to select only PolyHighResolution SNPs, line 173.

Lines 177-178: Did you check the structure with PCA plotting, both including all the populations and just having Tiritiri? This is to be sure that there isn't any hidden genetic structure or outliers that could affect the results.

As we don't have trait data for many individuals from the other populations, and to reduce the problems associated with combining data across populations (population structure, different impacts of fixed and random effects on the traits) we felt it was very important to restrict the dataset to only individuals from Tiritiri Matangi. However, it is an excellent idea to assess the population structure within Tiritiri Matangi; we have done so and found that there is no structure on the island (which matches what we know about dispersal, which is effectively random across the island). We have added a supplementary figure (S1) with these results and referenced this figure on lines 178-179.

GWAS: Do you know the linkage disequilibrium in hihi? If LD is really small that could also explain why you won't find anything as you will need many more SNPs to detect associations.

Thanks – we actually had this calculation in an earlier version of the manuscript but removed it when editing to try and reduce the word count! We have added this into the discussion along with a discussion of the limitations of GWAS in this system, lines 365-371:

“The sample size, number of markers and low heritabilities is also likely to have limited power to detect association in the GWAS analyses. Linkage disequilibrium between neighbouring markers is moderate (mean of 0.518, calculated with all 523 individuals using plink --r2 and reporting all pairwise values with --ld-window-r2 0.0) and suggests that power to detect a locus explaining all of the trait heritability ranges from 0.306 to 0.516 (Wang and Xu 2019; electronic supplementary material, table S6).”

Line 256: Please give the annotation database version.

Thanks, we have added, lines 258-259.

Results

Please start with giving the trait measurement variation like the mean, SD, sex and how many individuals. Especially it is interesting to see how is the variation around the mean of the traits. It might be also good to calculate the evolvability of the traits in addition to heritability especially if there is little phenotypic variation.

Thanks for this suggestion, this made us realise that we completely forgot to reference our supplementary tables S2 and S3 with much of this information (i.e. trait mean), we have now added this reference to line 267. We have added the number of individuals and number of males + females to the legend of Table 1 and added the trait means to this table. We haven't added the SD, as this is captured by the variance V_P given in Table 1. We have added the evolvability of the traits to Supplementary Tables S2 and S3 with the outputs of the *MCMCglimm* pedigree and GRM analyses. We are happy to also add this for the other two estimates of heritability (*RepeatABEL* and *BayesR*) on the advice of the editor, but feel it may be getting a bit confusing to have so many different estimates. We have not added discussion of the evolvability nor overall variation to the main text as selection analyses are more fully explored with a larger (pedigree-only) dataset in de Villemereuil et al. (2019b): “Little adaptive potential in a threatened passerine bird”.

Figure 1: Please explain “a, b, c” micro-chromosomes in the caption.

Great catch, thanks we have done so in the caption of figure 1, found in the main document in line 633-634 (figures were removed from the main body of the text as required for re-submission).

Discussion

I think one additional discussion point is how difficult trait especially nestling weight is as non-genetic factors affect it quite a lot (weather, environment, parents) which in turn makes the heritability lower and also it's difficult to catch associations in the GWAS.

Thanks for this suggestion, we have added to the discussion in lines 371-373:

“As expected, nestling mass, with the lowest heritability due to substantial maternal and month effects compared to the other traits (de Villemereuil et al. 2019b), has the lowest power to detect association.”

Line 426: Lard = Lars

Thanks!

Referee: 2

Comments to the Author(s)

This ms explores the genetic basis of three morphological traits under selection in a small threatened population of hihi from New Zealand. 523 birds were genotyped on a 50K SNP array which formed the basis for probing past the quantitative genetic basis of these traits using chromosome partitioning as well as GWAS. The key findings of this work is that 1) heritability is low for all three traits inferred from an animal model with reconstructed pedigree (from microsatellite markers) as well as when using the GRM from the SNP array as relatedness matrix (using both MCMCglmm and RepeatABEL) as well as BayesR to infer effect size distribution. 2) Chromosome partitioning analyses demonstrate polygenic basis to all traits and 3) GWAS results also support an infinitesimal basis to these traits with no genome wide significant marker. None of these results should come as a surprise given what we know about the genetic architecture of morphological traits in other avian and non-avian systems however.

The ms is well written and clearly presented. The analyses have been expertly carried out and I have no issues with the methods section of the ms. It might not be the most surprising or exciting results, but I agree with the authors that demonstrating a polygenic basis to traits in small threatened populations can be informative for conservation purposes and for that reason I think this will make a nice addition to the literature. I just have a few minor comments on the ms that I would like to see the authors address:

Table 1 and line 361-362: the difference between the pedigree estimated h^2 and GRM based h^2 is particularly large for tarsus and head-bill length. Since it's not a consistent difference across traits (though it is hard to draw much inferences from only three traits of course) I was wondering if there is anything specific about these traits (or their measurement) that could explain this? The differences between GRM and pedigree is of course the same for all traits given that the authors restricted the analysis to the same set of individuals (if I interpret the methods correctly) so it should not be due to differences in pedigree structure between traits.

Thanks for this suggestion. We were indeed a little puzzled when we first noticed the discrepancy and have already included some discussion of the reasons we felt the GRM and pedigree based heritabilities may be different, lines 350-360. We actually had a theory that perhaps the GRM allowed maternal and paternal effects to be more accurately estimated, and that some of the pedigree heritability was 'shifted' to maternal/paternal effects in the GRM model. However, this turned out not to be the case – maternal and paternal effects are relatively concordant between the pedigree and GRM models. For space considerations (and since our hunch wasn't correct!) we didn't mention this in the submitted manuscript. It is correct that exactly the same animals and GRM are used for each trait analysis. We can't think of a reason that these two traits would be more or less accurately measured compared to nestling mass, or any other feature of these traits that would lead to systematic over-estimation using pedigree data rather than the GRM. As you point out, with only three traits it is difficult to explore this comprehensively. We have added a further sentence (line 358) to highlight that the credible intervals are overlapping so another option is that it is simply chance that all three traits have lower GRM heritabilities.

line 280-281: A correlation of 0.75 between the pedigree and GRM was less than what I had expected given that the pedigree is actually reconstructed based on 13 SSRs. I was wondering if you have explored this further? Might be interesting to do given that even some close relationships seems to have been erroneously inferred from the SSRs.

Yes good point – although we note that the correlation is higher at 0.847 when self-self is included (which is what seems to be typically reported). We have examined in more detail the 9 pairs of individuals that seem to have a particularly low genomic relatedness where the pedigree predicts relatedness of around 0.5 (i.e. predicted siblings, parent-offspring). This

revealed that six of the pairs were predicted from our microsatellite-verified pedigree to be parent-offspring and three pairs to be full siblings, but the genomic data does indeed suggest that the microsatellite pedigree (inferred using 19 microsatellites, rather than the mentioned 13) is incorrect. In hindsight it might have been better to have removed these individuals from our pedigree analysis, but we note that this would most likely only slightly underestimate our pedigree heritabilities (these 9 pairs represent a proportion $\sim 7 \times 10^{-5}$ of all pairs), and will have not impacted our GRM heritabilities nor the chromosome partitioning or GWAS as none of these include pedigree information in the models. For the remainder of the pairs where the genomic relatedness is not exactly equal to the pedigree relatedness: based on analyses we have experience with in other bird datasets (e.g. zebra finch¹, great tit², kakī³ and kākāriki karaka³), we are confident that this is just a reflection of the difference between expected and realised genome sharing due to recombination and independent assortment during meiosis, as well as due to differences in pedigree depth and completeness per individual and the uncertainty around the relatedness of the population founders.

1. Santure, A.W., et al. (2010) *Molecular Ecology* 19: 1439-1451. 2. Robinson, M.R., et al. (2013) *Molecular Ecology* 22: 3963-3980. 3. Galla, S., et al. (2020) *Evolutionary Applications* 13: 991-1008.

line 174: filtering on maf 0.01 is often used in studies but it does mean that one can have very few individuals that are homozygotes of the rare genotype depending on the sample size, which of course in this study is low ($0.01^2 \times 523 = 0.05$ ind expected under HWE) which can cause problems in association analysis. It would be interesting to report the number of individuals for each genotype class for the reported "outlier" loci.

Excellent suggestion, thanks! We've added the minor allele frequency and counts of genotype classes for each of the top 10 hits for each trait to electronic supplementary tables S4 a, b and c. As can be seen, some SNPs indeed have a relatively small proportion of rare homozygotes, but the SNP we discuss in the main text for tarsus length, and the three SNPs mentioned in relation to head-bill length, have 13, 30, 20 and 29 individuals with the rare homozygote respectively.

Line 355: collared flycatchers also analysed by Silva et al.

Thanks, added!
(Now line 344)

Arild Husby