

THE UNIVERSITY OF BRITISH COLUMBIA



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Dear Dr. Edwards,

Thank you for your very helpful comments and request for a major revision of our manuscript “Migratory orientation in a narrow avian hybrid zone.” We appreciate the efforts of you and the two reviewers in providing suggestions of how to improve the manuscript.

We have now thoroughly revised the manuscript in accordance with your suggestions. In particular, we have carefully modified our conclusions to follow closely from the data presented, and have added explicit consideration of the possibility that birds in the orientation cages are not displaying their actual migratory orientation. We provide detailed point-by-point responses to the reviewer comments below.

Thank you for considering this manuscript for publication in *PeerJ*.

Sincerely,

A handwritten signature in black ink that reads "Darren E. Irwin".

Darren Irwin, PhD

Professor, Department of Zoology, and Biodiversity Research Centre

(On behalf of all authors)

Response to reviewers (comments of reviewers italicized):

Editor's Comments

The two reviews I have received are both very thorough. Whereas reviewer 1 sees some positives in the paper, reviewer 2 is very concerned that the observed data represents artifacts. In your revision, you need to address the possibility that the data collected represents artifacts and not real data, and rebut this as well as you can. Otherwise the paper should surely be rejected.

Please also address reviewer 2's comment about selection against hybrids. Reviewer 1 also had comments on this point. Your revision will definitely be sent out for re-review.

Thank you for this summary. We have now explicitly raised and considered the possibility that the movements in the orientation chambers might not indicate migratory orientations. This is a possibility in all orientation chamber studies. We have also refocused the manuscript on the initial research question that the study was well designed to address: does genetic ancestry predict orientation as measured by movements in the chambers? The data are real in the sense that they allow a valid statistical test of that prediction and were carefully collected from birds in the chambers, but we acknowledge the possibility that the lack of a significant relationship might be due in part to the movements not being good indicators of actual migratory orientation in free-flying birds. We feel that the paper should not be declined on that basis, since it is important to include results of valid tests of hypotheses in the published literature, whether or not results show significant relationships.

We have now removed the comment about the results being consistent with selection against hybrids, and agree that our results provide no support for (nor evidence against) that idea.

Reviewer 1's comments

Basic reporting

The study should provide more information on the frequency of hybrids known for this population. If hybridization is very common and hybrids and backcrosses are more common than pure genotypes, then the prediction that hybrids may show maladaptive behavior might need to be revised. I elaborated on this idea in my comments for the authors.

Thanks for this comment. We have now provided more description of the details of the hybrid zone and previous research on it; for example: "Within this hybrid zone there are a full range of hybrid genotypes, indicating that multiple generations of hybrids and backcrosses are present and that reproductive isolation is far from complete. However, the narrowness of this hybrid zone and sizeable amounts of linkage disequilibrium in

the center of the zone suggest that some form of moderately strong selection maintains it (Brelsford and Irwin 2009). Assortative mating and other pre-mating reproductive barriers are unlikely to be strong between these taxa (Brelsford and Irwin 2009; Brelsford et al. 2011; Toews et al. 2014a), implying a potentially sizeable role for post-mating selection against hybrids, possibly based on inferior migratory behaviour.”

Experimental design

The study is conducted in a single site, which is a strength but also a limitation in this kind of research. To some this would mean a fatal lack of replication. I believe the authors can discuss this problem but it needs to be clearly pointed out in the paper.

The reviewer is correct that this would be a fatal lack of replication for a study examining the role of particular geographic features (such as mountain valleys). For this reason we have refocused our emphasis to be entirely on the question that we set out to address: whether there is an association between genetic background and orientation. That question is best addressed at a single site, such that birds of different genetic backgrounds are tested under the same conditions.

Validity of the findings

Related to my comment above on experimental design, the paper reports a negative result, but it was done at a single site. This may represent lack of replication and requires appropriate discussion in the paper. I believe it is not a fatal problem if the authors clearly recognize the limitations of the study. I elaborated on this issue in my letter to the authors.

The reviewer’s concern about the single site was likely raised primarily by our previous emphasis on the conclusion that birds tended to be orienting down a mountain valley, and that this was consistent with migratory orientation in a particular direction and somehow consistent with selection against hybrids. We have removed much of that conclusion from the manuscript and now focus almost entirely on the test that is appropriate at a single site: whether genetic background and orientation are associated. (We do still mention that the average orientation direction is down the valley, but mention this in the Discussion just as an interesting observation that would require testing at multiple locations to investigate further).

Comments for the Author

The most evident strength of the paper is the description of a refined method for scoring orientation in the field, which solves many problems of the many former approaches based on Emlen funnels. Otherwise the study reports negative results, which are interesting but need to be properly framed and described to avoid confusion with conclusions being drawn on true lack of effects. My most important criticisms go precisely to this central issue. In order to tackle with it properly, the paper needs at least the following major modifications:

1. The title should be changed to clearly state that the result (lack of the expected effect of genotype on orientation) may be just a local occurrence, rather than a general phenomenon across the hybrid zone. Most evidently, the birds could orient differently according to their genetic background in another place on the contact zone that is less influenced by the mountains. From this perspective, the study lacks replication and the negative result turns out to be weak evidence of true lack of effects. In relation to this, the introduction (and also the discussion) should be revised to clearly state what can and what cannot be tested with this species and setup. The paper needs to make an effort to clearly distinguish true lack of patterns (such as “hybrids between these two genotypes do not show maladaptive migration, as they orient the same as pure genotypes”) from negative results (“possible maladaptive migration of hybrids could not be detected”). Note that in this study negative results could be due to various things, most importantly the possibly “wrong” choice of a single location with strong influence of mountains.

We agree that it is important to clarify that the negative results don't mean that we can “accept the null” of no actual relationship between genetic ancestry and migratory orientation, and we have greatly modified the discussion to clarify this and provide possible explanations for the negative results (1: no real migratory divide, or one too broad to be detected; 2: cage orientation shows short-term orientation down the valley, away from mountains as the reviewer suggests; 3: cage orientation as measured in our study does is not a good indicator of orientation of free-flying birds; we provide more full discussion of each possibility in the new manuscript).

Regarding the suggestion of changing the title, we tried to incorporate the points of the reviewer into the title, but found that it become rather long and awkward, e.g. “No significant association between genetic background and orientation chamber directionality at a single locality in a narrow avian hybrid zone.” We prefer the current title because it concisely describes the scope of the study but makes no claims about the results or interpretation: “Migratory orientation in a narrow avian hybrid zone.” We would be willing to defer to suggestions of the editor regarding the title.

2. The paper needs to test for age effects. Birds were aged in the field but no use is apparently made of age data. However, first year birds probably show innate orientation while older birds enrich their behavior with previous migration experience (which is admitted by the authors; L. 343-344). This issue requires statistical treatment or at least an explanation as to why age was not included in the analysis despite its recognized importance in studies of the genetic control of migration.

We agree that age would be good to include, if possible. We considered including age as a factor in the analysis, but originally based our aging based on feather quality and wear. Having spent more time in this hybrid zone and using skull ossification as an

aging metric, we are not as confident in our original age classifications. While our data are consistent with having sampled many hatch-year birds, we feel it would be irresponsible to conduct a formal analysis using our age data for this sample. We doubt that including accurate age classes would affect our finding of no relationship between genetic background and orientation.

3. The fact that hybrids are more abundant in the sample than pure A and H genotypes somewhat tells that hybrids are not in real disadvantage. This should be discussed appropriately. If this circumstance was already known before the study, it should be put forward already in the introduction. It does not matter if this information would change the predictions of the study (maladaptive migration of hybrids would be less expected in a contact zone where mixed genotypes make 88% of captured birds). This information is important to appropriately frame the study of migration in relation to genotype under the state-of-the-art paradigm of hybrid disadvantage.

We agree that the first submission should have described the makeup and known dynamics of the hybrid zone more clearly, and have now added that (see above).

Specific comments:

L62-63: “our understanding of how these migratory behaviors are expressed under natural settings is unclear.” This is true, and this paper is not going to contribute much on this regard given that cages are not natural settings. It is true the birds are expressing their behavior in cages at the place where expressing this behavior makes sense, but for some questions more controlled settings may be more advisable. I wonder if Helbig’s blackcaps would have showed the same behavior in a setting of the kind used in this paper.

We have now removed that comment.

L131. Song playback was used to attract birds into mist-nets. Please clarify which song was used, if it belonged to Audubon’s or myrtle warblers. Hybrids that respond to one but not the other parental song (if that is possible) could also have the parental migration pattern... or not, in any case the information is relevant to correctly evaluate the method. The issue deserves a comment, given the fact that tape luring usually creates various kinds of bias in this type of sampling (such as in relation to sex in this case, but there might be other more cryptic ones).

We have added clarification that playback was done “using a tape of a variety of yellow-rumped warblers as well as begging calls from nestlings; previous experience showed that similar recordings could be used to attract all phenotypes in the hybrid zone”

L. 134. Please specify which age classes were distinguished. I assume these were first year and older birds.

We have removed the comment about aging birds, given that we are less confident of the ages and do not use them in the analysis.

L. 151. Remove one “traditional” (typo).

Good catch. Done.

L. 265-269. I guess these groupings were meant to capture the different degrees of genetic introgression between species that birds may score at the contact zone (hybrids and backcrosses to simplify). Please clarify. If I am right, then the backcrosses are expected to have parental behaviors as they are likely selected through the survival of their parents with “non-lethal” migratory behaviors.

We have now clarified in the introduction that intermediate migration is not likely to be strongly lethal in this case, but rather just fractionally reduce the probability of survival and/or reduce fecundity (due to being in poor condition following migration).

Regarding the statistical tests using the groupings, we have added this clarification: “While the linear model would assume that each hybrid class as an intermediate migratory orientation compared to the two classes on either side of it, the ANOVA does not make that assumption, allowing situations in which hybrid classes might have more extreme orientations than either parental group.”

L. 275-277. This is informative but does not say much about the proportion of myrtle alleles of each bird if no information is given on the variance of this average estimate. I would rather produce a graph in which each bird captured is represented by a dot in Fig. 4A, so that the genetic background of each bird (and the variation around this 0.4 estimate) can be clearly visualized.

The number of birds within each genetic class is provided in two places in the paper: at the end of the results section, and visually in Fig. 6. We feel that showing each bird as a dot in Fig. 4 would complicate that figure and not contribute to conveying the main message, which is simply that there is not a general increase or decrease in h-index during the season. We will defer to the editor regarding this figure.

L. 358-360. Compared to Ilieva’s, this study has the advantage that different genotypes are sampled at the same place, but it has the other problems I explained above: age effects not tested, single site may be good but also leads to lack of replication, etc. Apart from this, in this system hybrids (as identified by their mixed genotype) are very abundant, which implies that hybridization has low impact on fitness via expression of maladaptive migration, either because migration is not genetically controlled in this

species or because hybrid behavior is not so disadvantageous compared to the behavior of the parents as it has been reported in studies of other species (especially those this paper is being compared with).

We have modified the discussion to more clearly state the possibility that this hybrid zone does not closely correspond to a narrow migratory divide. Our study cannot directly address whether intermediate migratory routes are disadvantageous, and we are careful not to imply that our study addresses that question.

L. 363. The statement “Our data were consistent with selection against hybrids in the non-breeding season” is contradictory with L. 353 “Mean orientation direction did not differ between the different genotype classes”. This contradiction is also seen in the summary (L. 40-41).

We agree, and have now removed the statement that the results are consistent with selection against hybrids.

Reviewer 2's comments

Basic reporting

The manuscript is structured and written in a very proper way. In this respect in my opinion it does fully meet your requirements.

Experimental design

please see below. No other comments here.

Validity of the findings

I had the opportunity to review an earlier version of this manuscript. At that time I had rather fundamental concerns about the validity of the results. This new version has been improved in many respects and some aspects are explained and discussed in a more appropriate way, however, the main problems I see could not be healed:

(1) Northward orientation during autumn migration: besides all the explanations through reverse migration and behavior at ecological barriers the most probable thing is that the measurements simply are artefacts which seems not to be uncommon in tests of wild birds in Emlen-like setups, no matter whether we look at films, scratches or bill marks for the analysis. The time pattern of the activities with the sharp end in all individuals but one is another indication that any unnatural trigger influenced the birds activities and thus presumably also orientation in the cage. Migration behavior patterns of at least the European warblers look completely different e.g. in radar studies. There is a lot more individual variation in onset and end of the migration activities. I am still convinced: whatever has been measured in this study is rather not a proxy for the natural behavior.

We thank the reviewer for these comments and agree that there is a possibility that the orientation in the chambers is not a good proxy for natural orientation. We have now acknowledged that possibility very clearly in the manuscript. Nonetheless, we feel that the experiment was well designed as a test of whether genetic background was predictive of cage orientation, and we feel the behavior observed in the cages was consistent enough with *zugunruhe* to be considered potentially valid orientation data. We feel that it is important to publish the study, because there is much concern over the “file-drawer” problem in science: if only the statistically significant results get published, then we end up with a biased impression of the magnitude and generality of various relationships. We considered abandoning this manuscript, but feel we have a duty to the community, funding and permitting agencies, and the birds themselves to do our best to publish the results, with a clear acknowledgement that the orientation behavior seen in the cages might be related to long-distance orientation, short-distance orientation, or neither. We also feel that the methodological developments provided by our study may be of significant assistance to other researchers.

(2) Similar directional preferences: Even if the preferred mean direction should be resulting from a natural trigger (like valley orientation) and not just be an artefact the directional preference of both “pure” species groups are identical and not different from hybrids. Therefore the substrate on which selection could act is the same for all groups and cannot act differently on hybrids and “pure” species individuals. Thus I do not understand how this is “consistent with selection against hybrids on migration” (as said e.g. in the abstract).

Agreed. We have remove the two instances of that comment.