Answer to the reviewers

Round 1

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Contents

Decision

by Sébastien Lavergne, 2019-05-25 18:25

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Novel study but needs some substantial revisions

> First, I would like to apologize this overly long delay for taking an editorial decision. As I found this study quite intriguing, I wanted to take the time necessary to take a careful decision about it. Both reviewers found your study interesting and quite novel, and raised no substantial issue on how the study is executed. I fully agree with them. Nevertheless, they expressed a number of concerns about how the rationale of the study is being explained, how the theoretical and empirical background of the study are elaborated (mainly in the introduction), and how some interpretations are drawn from the results. At this stage, it is important to take all reviewers' comments into account in order to make this paper have the impact it deserves. I think that your work explores novel questions in evolutionary community ecology and will contribute to opening up a new field of research. Therefore it deserves further consideration and very likely publication.

Thanks for your comments! We have carefully revised our manuscript and answered all comments from the reviewers.

Additional requirements of the managing board

> As indicated in the 'How does it work?' section and in the code of conduct, please make sure that:
>
> - Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad (to pay) or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data.
>
> - Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.
>
> - Details on experimental procedures are available to readers in the text or as appendices.

Our scripts and data are shared via GitHub and Zenodo (http://doi.org/10.5281/zenodo.3355444), as now stated on L107-108.

> Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

We have added a mention that Marianne Elias is a recommender and is part of the managing board of PCIEvolBiol (L339-340).

Reviews

Reviewed by anonymous reviewer, 2019-05-12 02:12

> I have carefully read the pre-print "Distribution of iridescent colours in hummingbird communities results from the interplay between selection for camouflage and communication". I was intrigued by this work which addresses an interesting question in a charismatic group of birds. The manuscript is generally well written, however I do have issues with how the hypotheses are presented, how some of the analyses are done and the conclusions that are drawn from the results.

Thanks for your comments! Please see our answers inline.

Major comments:

> In the introduction the rationale and theory behind the predictions is not at all clear, which makes it very hard to understand what supports them, and also the results and importantly conclusions that are drawn from the results. An example is ln 57-59: where it is stated that co-occurring species are expected to converge in coloration because of predation risk. There are many implicit "all else being equal" here that should be made explicit.

We have updated this section of the manuscript (L50-52). In short, we expect co-occurring species to be under the same selective pressures, thereby producing a similarity in the colour patterns harbored by co-occurring species (which appears as a convergence pattern), all else being equal.

> Also, it is not stated why different patches might be under different selection pressures and this seems highly important. What evidence is there for some colors in hummingbirds being related more to crypsis, while others might be more associated with species recognition? I would think this is key to

> the hypothesis that different patches would present different patterns of dispersion within a community. This refers to the predictions presented in lns 86-89. There is no theoretical support presented for these predictions and the lack of such theory buttressing the predictions makes the author's rationale hard to follow.

Good point, we added a couple of sentences and references to better explain why dorsal patches, which are exposed to aerial predators, are expected to be involved in camouflage, whilhe facial and ventral patches, which are often exposed to intended receivers, are expected to be involved in communication. Our framework and predictions in this area match perfectly those of the recent pre-print from Delhey (2019) on the distribution of colour patches in Australian birds. We have also added a sentence and references to explain why we expect this pattern to hold in hummingbirds more specifically (L75-78).

> In ln 65-66 the authors state that "misidentification can also lead to misdirected aggression and costly fighting when individuals compete for resources or territories". I am not an expert in hummingbird behaviour, but I think that inter-specific aggression is somewhat common in this group, there are descriptions of dominance structures between different species based on size.

Yes, you are correct. However, in spite of this, we expect that similar appareances would lead to even higher levels of interspecific aggression, as shown in Grether et al. (2009), Anderson and Grether (2010).

> Ln 79: the authors state that previous studies relied on human vision, which is likely to have biased the results. It is not stated how or why. In addition recent work suggests that for some purposes human vision can detect much of the variation in coloration in the visible range (see e.g. Bergeron and Fuller 2018, Dale et al 2015).

We have revised the wording in the manuscript and instead of unadvertantly implying that human vision is unfit for all cases, we now explain why spectrometric measurements are warranted in our case: "which did not allow them to analyse colours as perceived by the intended receiver, in this case birds." (L68-70)

We are precisely in one of the cases where Bergeron and Fuller (2017) highlighted the necessity of spectrometry and visual models: "Below is a list of important questions that require spectrophotometry and visual detection models: How do females perceive male color patterns? What makes a color pattern conspicuous to a viewer? How do predators perceive their prey?"

Indeed, many studies showed that human vision fails to detect differences that are perceived by birds, especially when these differences occur in the UV range (Bennett, Cuthill, and Norris 1994; Cuthill et al. 1999; Eaton 2005; Montgomerie 2006; Armenta, Dunn, and Whittingham 2008), which are very important in hummingbirds. Our data show that UV can make up to 38% of the total reflectance for some species/patches (S1U index in Montgomerie 2006).

> In Ln 85: The authors introduce the model system but it comes a bit out of the blue, without any justification to why hummingbirds are a good system. This only comes up in the Methods.

The presentation of the study system was originally in the introduction but it disrupted quite badly the presentation of the theoretical background. We have tried to address your comment nonetheless by adding a couple of sentences that provide some context about hummingbirds as early as the introduction (L75-78). However, for the clarity of the introduction, the bulk of the presentation of the study system remains in the methods section.

> Ln 136: a single male was measured for each species. This is worrisome as no information is provided on how said male was chosen, nor on the degree of within species variation as opposed to among species variation. Readers might worry to what degree a single male can be representative of the whole species.

While this is true that it is often better to include multiple individuals from the same species when possible, in order to sample a large number of species and answer community-level questions, we had to sacrifice the number of samples within species (a single individual took between 1 and 2 hours). We however did our best to limit this issue by selecting adult individuals in good plumage condition, and which had a colouration that seemed representative of the whole species to the human eye (as now stated L127-130). Additionally, as always in this kind of study, the amount of intraspecific variation is much lower than the interspecific variation.

We verified this on an independant dataset of hummingbird colours from French Guiana (unpublished at the moment), where all measurements were performed with a bifurcated fibre at 45°. The measurements included 1111 patches, spread across 35 species, with each patch measured between 1 and 3 times. We find that the median coefficient of variation (standard deviation divided by mean) was 0.84% for hue ($H1$) and 24.9% for brightness ($B2$). These values did not significantly change if we only included male individuals (1.14% for $H1$ et 25.1% for $B2$).

> **"** Also related to this, were several measures taken of the same patch? What was the repeatability among measurements for the same individual and patch? Do patches vary in the repeatability? **"**

As mentioned, we ensured that all variables used in this study were repeatable, meaning that interspecific variability (even between similar looking patches!) is greater than intraindividual variability. To test this with a high level of confidence, we specifically picked patches that looked the same with human vision, making it harder for us to produce repeatable measurements. We managed to reliably repeat our measurements even for these patches. Colour variables do indeed vary in repeatibility depending on the patch but this is not necessarily due to biological differences between patches. More specifically, brightness of directional iridescent patches was often less repeatable than the brightness of diffuse iridescent patches, mainly because precise measurements were more difficult to achieve. We have expanded the table in supplementary information to display repeatabilities for diffuse patches alone, directional patches alone and both types pooled (table S1). We now also explain with more details how repeatibility was computed in the main text (L165-167).

> **"** Ln 194-195: random assemblages from a species pool containing all species from all communities were used as null models. It would be good to justify the choice of null model and make explicit what such null model entails, from an evolutionary or ecological point of view. The choice of null model is likely to have an important influence on the results. **"**

We agree with the reviewer and have expanded this part, which now reads: "In other words, actual assemblages are compared to fictional assemblages with exactly the same number of species but no abiotic or biotic constraints on the species composition." (L178-180)

This null model was used for both the accumulation curves, which show how hue and brightness diversity changes with the increase of the species number in the community. It was also used to compute the p-values for Π_{ST} and τ_{ST}. For Π_{ST} and τ_{ST}, we considered at some point using another null model that would only include species from similar habitats and/or elevations. We finally opted against this approach as this meant that the null model would already include the very pattern we were trying to test.

> **"** Ln 200-201: analyses are undertaken for all patches together, i.e. creating a color volume per species (if I understood correctly), and then repeated for each patch independently. The latter increases the number of analyses, and there is no justification for repeating the analyses for each patch other than the fact that the whole-species color volume might not capture subtleties of particular patches. Why not run a preliminary analysis to see whether some patches tend to present similar colours repeatedly across all species? This would enable the authors to justify grouping some patches and analysing others separately. **"**

Two reasons call for the use of patch specific analyses:

- the fact that different patches may be under different kind of selective pressures (predation on the back, communication on the belly – see our previous answer to our comments) and thus display different patterns (as in Delhey 2019)

- the fact that patch location on the body (and not just the combination of colours) may serve in species recognition, as shown in the figure below.
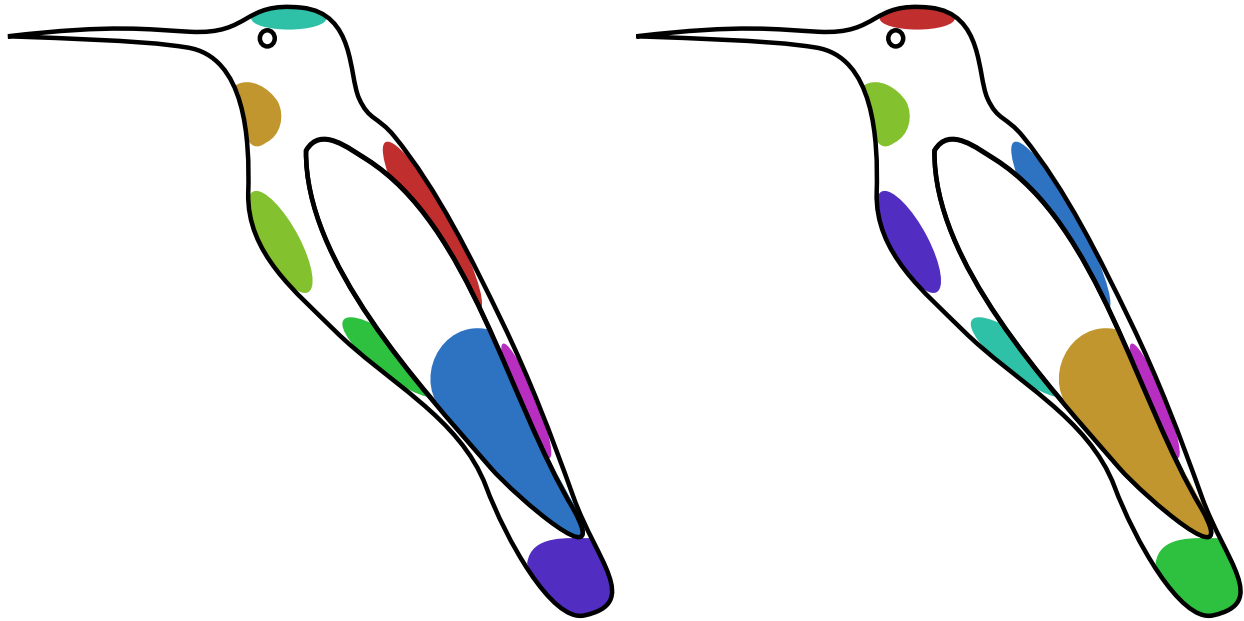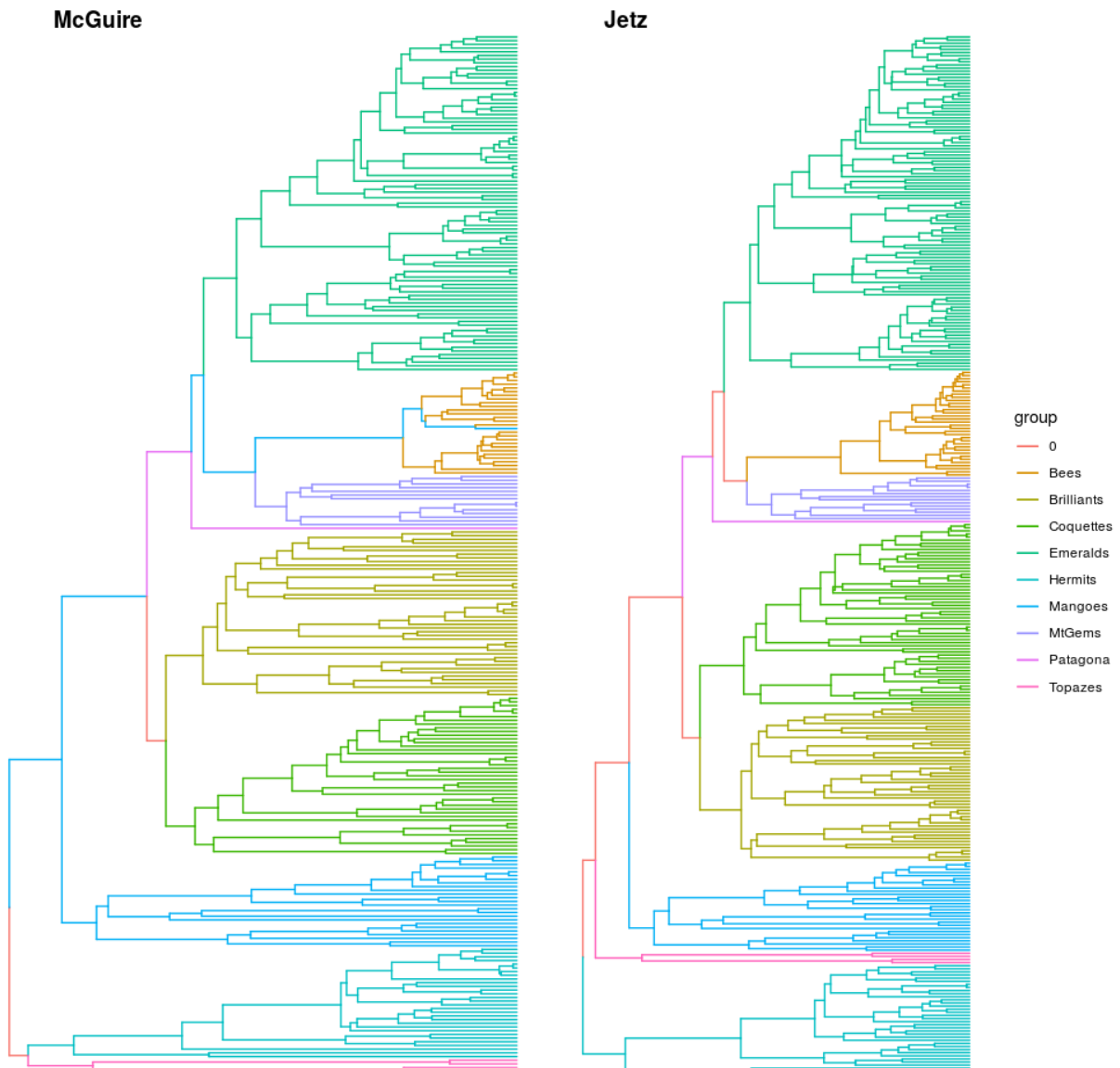
In this figure, the two birds have exactly the same colours but on different patches. They cannot be mistaken for one another, meaning that analyses that do not take into account patch location are not sufficient to discriminate between species. We also reworded slightly this part so it should hopefully be clearer now.

Regarding why we didn't group patches for analyses: cursory observations during the measurements revealed that no patches were repeatedly similar across all species (we have 112 species spanning the complete hummingbird phylogeny). Additionally, our results show different patterns for each one of the 8 major patches so it shows that grouping any of these patches wouldn't have made sense.

> ❝ Ln 204: the authors use a subsample of phylogenies for the group of interest from birdtree.org Why did they prefer to use the trees from birdtree.org rather than the McGuire et al 2014 phylogeny? ❞

Ideally, we would love to use the McGuire et al. (2014) phylogeny. However, this phylogeny is not publicly available, whereas the Jetz et al. (2012) phylogeny is, and allows us to get a tree distribution which takes into account the uncertainty instead of a consensus tree (Pagel and Lutzoni 2002; family=Villemereuil et al. 2012). Nevertheless, this should not have a strong impact on results, as the hummingbird phylogenies from birdtree.org and McGuire et al. (2014) are very similar and mainly differ in the position of one clade (Topazes, see figure below). This is not surprising given that Jetz et al. (2012) phylogeny is built from the same genetic sequences as McGuire et al. (2014).

McGuire **Jetz**

group
— 0
— Bees
— Brilliants
— Coquettes
— Emeralds
— Hermits
— Mangoes
— MtGems
— Patagona
— Topazes

> ❝ Ln 239-242: I don't understand the rationale behind "removing" the effect of shared ancestry and then analysing clustering or overdispersion in coloration. The authors already found that species are composed more commonly by closely related species, so I wonder whether it actually makes sense to even attempt to "remove" the effects of shared ancestry. Also, it is unclear what exactly the authors are doing here. What exactly is being "removed"? The fact that the results change notably between the analyses does lead one to wonder whether it is a statistical artefact or whether these results reflect a biological pattern of interest. ❞

The word "remove" is indeed confusing, and we changed all occurrences of the word "remove" for the more precise and more accurate "decouple".

Comparing the phenotypic structure of the communities taking into account (decoupled indices; $dc\tau_{ST}$) or not (original indices τ_{ST}) the shared ancestry between species allows us to disentangle processes and mechanisms that lead to the observed patterns, and specifically whether these processes involve filtering or character evolution. This is detailed in table 1 that we have moved from the supplementary material to the main text, following reviewer 2's

suggestion.

> Ln 253-255: the authors state that results suggest there is a trade-off between selection for camouflage and species recognition. However, I do not see which results suggest there is any trade-off, nor which results suggest some coloration is used for camouflage and which is used for species recognition. This is partly due to the fact that the theory behind the hypothesis and predictions has not been presented to the readers, so we cannot draw the same conclusions as the authors do.

After reading your and reviewer 2's comments, we realise that "trade-off" was probably not the best choice of words, as it implies a direct, mechanistic link between two traits. Here, we instead wanted to say that we see two opposite patterns, depending on the patch location on the bird body, and that these two patterns are likely the result of two selective pressures acting in opposite directions. We have therefore removed all occurrences of the word "trade-off" in the manuscript.

> Ln 284-285: the authors state that a previous work, on the same dataset, but using different methods, found similar results. But the authors do not contrast their results with those of the previous work, nor enlighten the readers as to whether the different methodology might impact the results, how or why.

We have reworded this part to better express the fact that this concerns only the phylogenetic clustering, which is not the main objective/result of this study (L261-264). We have also added some metrics of comparison between the two studies (% of local communities with significant phylogenetic clustering) and we briefly comment on the differing methodologies (Π_{ST} versus NRI).

Minor suggestions:

> ln. 18 and ln 54-55: the authors state that co-ocurring species that share the same environment would be expected to have similar appearances due to selection for crypsis. However, merely overlapping in the distribution is likely insufficient for selection on predator avoidance to lead to similar coloration. Surely there are other factors that are hugely important beyond the environment. For example, size of the prey will have an important impact on predation risk, habits (diurnal vs nocturnal) will also have an important influence, as will behaviours, and other factors.

We agree with the reviewer but there is not enough data in the literature to do a rigorous analysis of hummingbird predators. In fact, there are few accounts of adult predation in hummingbirds. We mention in the introduction that we assume they share similar predators.

> ln 31. It is unclear why it is concluded that over dispersion observed in some patches and clustering on others suggests one may counter-balance the effect of character displacement. If different patches respond to different selection pressures, i.e. colors in some patches are signals for species recognition, while other patches function for crypsis, no counter-balance is expected, or?

You are right and we have removed this part.

> ln 60 (and elsewhere): "species assortment locally", I guess you mean local species sorting.

> ln 72: worth noticing, change to worth noting.

> ln 100: replage large with long.

> ln 260: As predcited in our prediction 5, redraft.

Thank you for your valuable comments which have been taken into account in the updated version of the manuscript.

Reviewed by anonymous reviewer, 2019-05-06 16:50

> This manuscript investigates the community-level and phylogenetic distribution of iridescent colors in hummingbird communities in Ecuador. The authors use spectrometry data and a set of field observations to examine the distribution of different color variables within and between communities and to elucidate which variables of color change at variable phylogenetic and community scales. The topic is fairly original in that it attempts to examine color macroevolution in an ecological framework, aided by the fact that the study system contains many co-occurring taxa with varying degrees of relatedness which have diversified using an understudied and complex plumage coloration mechanism. This is an informative and novel study and the reviewer therefore recommends that the authors revise the manuscript to reflect the enclosed suggestions. The conclusion that character displacement acts on certain patches and certain plumage color axes makes intuitive sense and is supported by the evidence presented.

> However, the conclusion which assumes that co-occurrence of similar phenotypes represents convergence or environmental filtering due to camouflage may be aided by some description or quantification of the colors which are exhibited in the differing light environments of the canopy and understory.

This is a fair point and we have addressed it as follows:

- As detailed below, we have added a phylogeny of hummingbirds with the colour on each one of the 8 "maint patches" for each species. One can easily notice that (as opposed to the throat) the back is green/brownish on most species, suggesting a possible role in crypsis against a vegetation background.

- We have nonetheless revised the wording of the manuscript in several places (such as L328-329) to make clear that convergence/environmental filtering may be caused by selection for camouflage but also other environmental factors (e.g. thermoregulation, protection against damaging UV radiation, etc.)

- Finally, we have added references to Delhey (2019), in addition to others (e.g. Gomez and Théry 2007), whose findings also suggest a role of selection for camouflage on dorsal patches, in birds in general.

> For the most part this paper is clear and readable. The most confusing aspects of the paper involve the use of jargon specific to methodology. There is a clear distinction made between phenotypic and phylogenetic dispersion, but when these terms are used in close proximity it is sometimes hard to mentally track these terms and the matrix of hypotheses. It potentially might be clearer to refer to biological hypotheses in plain text as opposed to numerically or to include table S2 as a main-body table.

As mentioned below, we moved table S2 to the main text. We've also made sure that all numerical references to our predictions are matched by plain text descriptions.

> Additionally, it would be nice to have some description of the actual colors which are on these birds. If colors on the back are being conserved are these mostly brown or gray patches?

We have added in ESM a phylogenetic tree with all species from our assemblages, showing the colour of each of the "main" patches for each species.

> When they vary within a community what are the color axes of the variation?

The aforementioned figure should give you an overall idea of the axes of variation among and across communities for each one the the "main" patches.

To answer this question in a more quantitative manner, we also looked at the variance of quantum catches (UV, short, medium and long wavelengths photoreceptors) in our dataset. We found that most variation happened in the medium and long wavelengths (var = 0.11 and 0.12 respectively) rather than the ultraviolet and short wavelengths (var = 0.005 and 0.006 respectively). This pattern stays qualitatively the same when we split this analysis by patch. In other words, no matter whether the patch displayed a clustering or overdispersion pattern, the largest amount of variation was also due to the medium and long wavelengths. This variation was simply smaller in the case of patches that displayed phenotypic clustering at the community level.
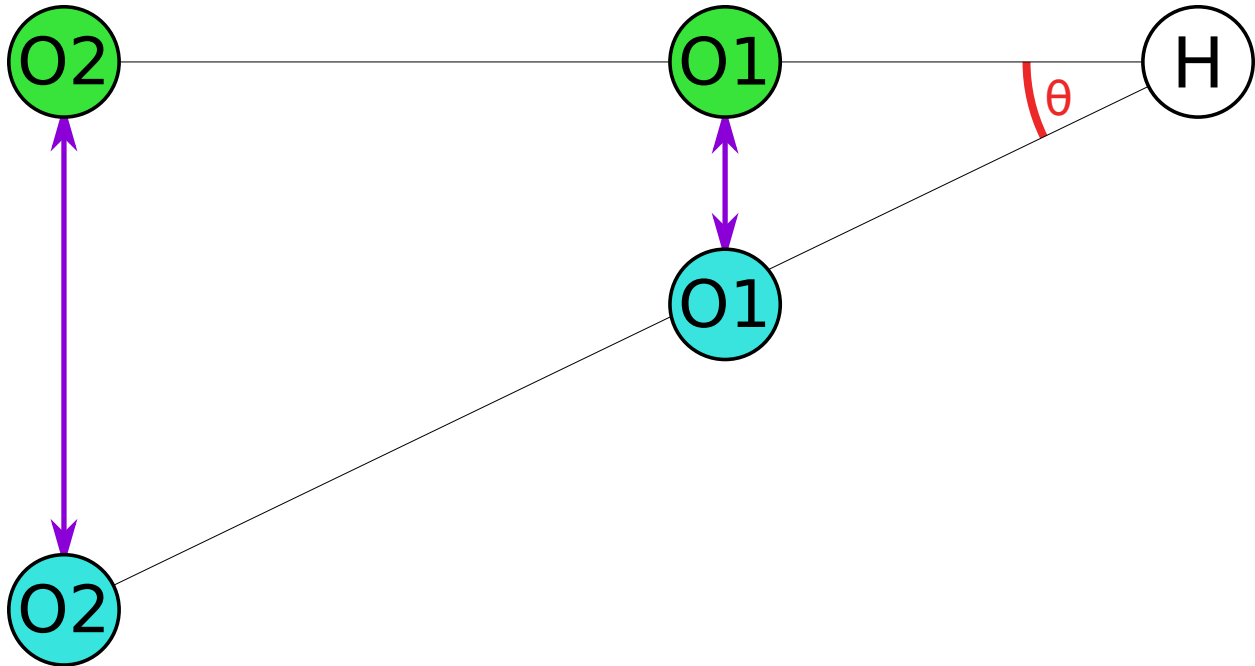
Figure 1: In this figure, an observer O2, placed further from the hummingbird H than another observer O1, has to move a larger distance to observe the same hue shift as O1.

Line-By-Line Comments

> 130: Interesting approach to look at environmental organization data for hummingbird assemblages, I'd wonder if these accounts are biased and perhaps may not provide a clear picture of co-occurrence, especially for rarer taxa.

Good observation. As in any sample, rare species might indeed not be accurately represented. Even though these are inventories, surely there will be species left out in some of them. Nonetheless, previous studies using this dataset (Graham et al. 2009, 2012; Parra et al. 2011, 2011) ensured that the lists were concordant with the expectations from the sites and the spatial scale being sampled.

Additionally, rare species probably have little influence on community structure and possibly on trait evolution, assuming they have always been rare.

> 196: Good justification for the patch-specific analysis.

Thank you!

> 100: This is an interesting prediction. I do wonder if as a human the hue shift effect between close and long-distance is visible and if there would be a way to model the distance-based effect. Or is it more a function of the angle?

The hue shift occurs when the angle between the incoming light rays and the observer changes. So simply because a larger movement is required to achieve the same angle at long distance, the hue shift is less visible, as illustrated in the attached figure.

> 126: For the species which coexist, is hybridization common? How closely related are the "closely related" co-occurring species? Are they con-generics? Overlapping subspecies?

Yes, hybridization among co-occurring hummingbird species is quite common (non-exhaustive citation list below), even between different genera (ref #4). We have a comment about this in the section of the methods where we present

the study system (L116-118).

1. Lynch, J. F., & Ames, P. L. (1970). A new hybrid hummingbird, *Archilochus alexandri* x *Selasphorus sasin.* The Condor 72:209-212.

2. Wells, S., Bradley R. A., & Baptista L. F. (1978). Hybridization in *Calypte* hummingbirds. The Auk 95:537- 549.

3. Wells, S., & Baptista L. F. (1979). Displays and morphology of an Anna x Allen hummingbird hybrid. Wilson Bulletin. 91:524-532

4. Graves, G. R., & Zusi, R. L. (1990). An intergeneric hybrid hummingbird (*Heliodoxa leadbeateri* × *Heliangelus amethysticollis*) from northern Colombia. The Condor, 92(3), 754-760.

5. Clark, C. J., Feo, T. J., & Bryan, K. B. (2012). Courtship displays and sonations of a hybrid male broad-tailed × black-chinned hummingbird. The Condor, 114(2), 329-340.

6. Stiles, F. G., & Cortés-Herrera, J. O. (2015). Diagnosis and observations of a hybrid hummingbird (*Metallura tyrianthina* x *Aglaiocercus kingi*) in the Eastern Andes of Colombia. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales, 39(153), 481-490.

7. Perez-Eman, J. L., Ferreira, J. P., Gutierrez-Pinto, N., Cuervo, A. M., Cespedes, L. N., Witt, C. C., & Cadena, C. D. (2018). An extinct hummingbird species that never was: a cautionary tale about sampling issues in molecular phylogenetics. Zootaxa, 4442(3), 491-497.

To give you a rough idea of the relatedness of the species in our dataset, McGuire et al. (2014) estimated the age of the most recent common ancestor to all extant hummingbirds to about 22 million years ago. We have also added a time scale to the new tree with the patch colours in ESM.

> **141: How did you measure homology of these "extra" patches. If they were present in multiple taxa why didn't you include them as key patches while circling patches?**

The conditions of our measurements did not allow us to easily identify more "extra" patches that would be present in many taxa. Indeed, the measurements were performed during two campaigns in two different museums and we had no way to have a look at the complete sample before starting the measurrements. The main "patches" were not intended as an exhaustive list but simply a subsample of patches that might be under different selective pressures, and that is commonly used in such studies [e.g. @Gomez2007_simultaneouscrypsisconspicuousness]. It is also worth noting that in the colour volume analyses (by pooling all patches), this does not matter in any way since the "main" and "extra" patches has exactly the same status.

> **169: Remove "called" here.**

Done.

> **175: Was a shearwater the closest related visual-model taxon you could find? This ecologically seems like an odd choice.**

As now written in the manuscript, the shearwater was not chosen because of its ecologically or phylogenetic proximity to hummingbirds. We picked it because:

- the absorbance peak of the V photoreceptor is at 405nm, very close to the absorbance peak for hummingbirds reported in Ödeen and Håstad (2010), as now explained L160-161 in the manuscript.

- it is the standard model for VS vision, and the species for which optical parameters (such as optical media transmittance; Hart 2004) are known best.

Another common practice is to use a theoretical average V visual system, but we are reluctant of using this approach as averaging may produce a biologically impossible visual system.

> **176:: Are you referring here to the actual illuminant you used or a habitat-simulation model you used to transform your data? If so, wouldn't this prevent you from performing meaningful standardized environmental comparisons?**

This is the actual illuminant we used. We believe this actually allows for meaningful comparisons between different environments, whereas using the same illuminant for every species (e.g. D65) would not. Here, all colours are seen as they would be by a conspecific or a predator in a natural setting.

> It might be good to find a way to compare the canopy and understory birds which co-occur as that could be an important source of variance.

As some point during this study, we ran the analysis by splitting the communities into subcommunities depending on the strata (understory or canopy). We had to give up on this approach as it created very small communities, which lead to a very low statistical power. We could thus not determine if the lack of observed phenotypic structure revealed a true absence of structure or simply an insufficient statistical power.

> Table 1: I like the color scheme here and find the data presentation intuitive but the pluses and minuses may be a little bit hard to see. It may be helpful also to include a statement about the point you're trying to show with this table. For example, are you trying to show the difference between Phenotypic structure and decoupled phenotypic structure? Between the different variables? What patterns can be illuminated to guide the reader?

We have updated the figures to only display one big minus or plus for each patch, which should be as intuitive but more legible.

> Also, which aspects of hue best describe the clustering (eg, light to dark, brown to gray)? Do the three variables which describe hue have similar variance or is variance more distributed along certain hue axes?

We have looked at variance for quantum catches, which are equivalent to x, y and z variables we used to describe hue but more easily interpreted from a biological perspective in this case. To reiterate our findings, detailed above in the present document, we found that the largest amount of variation in hue was due to medium and long wavelengths.

> 253: The suggestion that colour structure results in a camouflage-dependent tradeoff depends on the assumption that the environmental clustering represents selection on camouflage, but these patterns could be driven by mimicry as in woodpeckers or environmental adaptive gradients such as gloger's rule that may not be driven by crypsis. One powerful way you could fix this is by comparing the canopy and understory birds to see if color differences in patches supposedly used for crypsis are best explained by habitat.

That's an interesting and fair point. We've toned down a bit this part. Regarding Gloger's rule specifically, it is unlikely to act at this spatial scale, since we're focusing on Ecuador, which spans ~720km in latitude. But it is true other environmental adaptive gradients might come into play.

> 290: Low dispersal ability as compared to what?

We have modified this sentence, stating that this hypothesis is not as probable as the ones presented previously (L268-270). Although low dispersal ability is a potential hypothesis, the few evidence available suggests that hummingbirds may encompass a wide variety of dispersal capabilities.

> Also in reference to line 285 could this result be biased by the selection of sample sites in contact zones? Just because these birds co-occur now in some parts of their range doesn't mean that their plumage didn't evolve in allopatry. Testing evolutionary models for each patch may give you a finer grained approach to understand the evolutionary history of the integrated phenotypes.

This is true that our theoretical framework and predictions assumes that plumage colour evolution evolved given the current species distribution. This is indeed a common assumption in phylogeographic studies. We are not entirely sure how we could verify this assumption.

> 292: I'm still not sure I understand why this pattern would indicate filtering on another trait and not ?

We have clarified this point in the manuscript (L271-275): because we observe an overdispersion when we take into account the phylogeny, this suggests that there is a character displacement process (first row in Table 1). So the fact

we observe clustering when we decouple the effect of the phylogeny (last column in Table 1) must be due to another trait (e.g. flight ability at high elevation, where lift is weaker due to decreased atmospheric pressure).

> 302-314: It would be helpful to mention some of the elaborate behavioral adaptations that hummingbirds use to take advantage of iridescent gorget's for instance.

This is a good point. We have added relevant references to this section (L281-282).

> 309: Once again, you have the canopy/understory data, why don't you compare the effect of these two habitats? That would be a really compelling test or an interesting note if you tested this and it didn't matter. Would you see the same pattern if you only tested understory or canopy birds?

While this could be interesting in theory, it was not possible to test it with our dataset, as detailed in our answer above.

> 332: It is stated that hue shift may be less variable to predators but wouldn't any iridescence on the wings and back have a flashing reflective effect, attracting attention?

On the contrary and unexpectedly, recent studies show that iridescence actually impairs object detection in bumblebees (Kjernsmo et al. 2018) as well as birds and humans (Kjernsmo et al., in prep.)

> 335: The allusion to variable layer structure seems speculative, you should note that nano-scale imaging of the layer structure would be necessary to know if the layer variation you allude to is real.

This is now associated with a reference to a recent preprint where we used transmission electronic microscopy to indeed show that the chemical nature of the multilayer structures producing iridescent is highly variable among species (Gruson et al. 2019) (L309).

> 336: Once again, the numbering of predictions is difficult to follow and the frequency with which you refer to them suggests that figure S2 is more of a key guide for the reader than its placement in the supplement would suggest.

You are completely right and we now moved it to the main text.

> 359: How conserved are these patch colors across the phylogeny? Do they represent perhaps a historically conserved patch? Once again, evolutionary model fits may elucidate these historical questions.

As mentioned above, we have added a tree with all species from our assemblages, showing the colour of the "main" patches (crown, back, rump, tail, throat, breast, belly and wing) for each of them. This should help the reader get an idea of the variance and the phylogenetic signal of these traits.

References

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