Peer Community In
Zoology

Experimental evidence for asymmetrical species recognition in East African Ophthalmotilapia cichlids

Ellen Decaestecker based on reviews by George Turner and 2 anonymous reviewers

A recommendation of:

The initial response of females towards congeneric males matches the propensity to hybridise in Ophthalmotilapia

Maarten Van Steenberge, Noemie Jublier, Loic Kever, Sophie Gresham, Sofie Derycke, Jos Snoeks, Eric Parmentier, Pascal Poncin, Erik Verheyen *(2022), bioRxiv, 2021.08.07.455508, ver. 3 peer-reviewed and recommended by Peer Community in Zoology* ***https://doi.org/10.1101/2021.08.07.455508***

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Recommendation

I recommend the Van Steenberge et al. study. With over 2000 endemic species, the East African cichlids are a well-established model system in speciation research (Salzburger 2018) and several models have been proposed and tested to explain how these radiations formed (Kocher 2004). Hybridization was shown to be a main driver of the rapid speciation and adaptive radiations of the East African Cichlid fishes (Seehausen 2004). However, it is obvious that unrestrained hybridization also has the potential to reduce taxonomic diversity by erasing species barriers. In the classical model of cichlid evolution, special emphasis was placed on mate preference (Kocher 2004). However, no attention was placed on species recognition, which was implicitly assumed. There is, however, more research needed on what species recognition means, especially in radiating lineages such as cichlids. In a previous study, Nevado et al. 2011 found traces of asymmetrical hybridization between members of the Lake Tanganyika radiation: the genus Ophthalmotilapia. This recommended study by Van Steenberge et al. is based on Nevado et al. (2011), which detected that in one genus of Ophthalmotilapia mitochondrial DNA ‘typical’ for one of the four species (*O. nasuta*) was also found in three other species (*O. ventralis*, *O. heterodonta*, and *O. boops*). The authors suggested that this could be explained by the fact that females of the three other species accepted *O. nasuta* males, but that *O. nasuta* females were more selective and accepted only conspecifc males. This could hence be due to asymmetric mate preferences, or by asymmetric abilities for species recognition.

This is exactly what the current study by Van Steenberge et al. did. They tested the latter hypothesis by presenting females of two different Ophthalmotilapia species with con- and heterospecific males. This was tested through experiments, making use of wild specimens of two species: *O. nasuta* and *O. ventralis*. The authors assumed that if they performed classical “choice-experiments”, they would not notice the recognition effects, given that females would just select preferred, most likely conspecific, males. Instead, specimens were only briefly presented to other fishes since the authors wanted to compare differences in the ability for ‘species recognition’. In this, the authors followed Mendelson and Shaw (2012) who used “a measurable difference in behavioural response towards conspecifics as compared to heterospecifics’’ as a definition for recognition. Instead of the focus on selection/preference, they investigated if females of different species behaved differently, and hence detected the difference between conspecific and heterospecific males. This was tested by a short (15 minutes) exposure to another fish in an isolated part of the aquarium. Recognition was defined as the ‘difference in a particular behaviour between the two conditions’. What was monitored was the swimming behaviour and trajectory (1 image per second) together with known social behaviours of this genus. The selection of these behaviours was further facilitated based on experimental set-ups of reproductive behaviour or the same species previously described by the same research team (Kéver et al. 2018).

The result was that *O. nasuta* females, for which it was expected that they would not hybridize, showed a different behaviour towards a con- or a heterospecific male. They interacted less with males of the other species. What was unexpected is that there was no difference in behaviour of the females whether they recognized a male or (control) female of their own species. This suggests that they did not detect differences in reproductive behaviour, but rather in the interactions between conspecifics. For females of *O. ventralis*, for which there are indications for hybridization in the wild, they did not find a difference in behaviour. Females of this species behaved identically with respect to the right and wrong males as well as towards the control females. Interestingly is thus that a complex pattern between species in the wild could be (partially) explained by the behaviour/interaction at first impression of the individuals of these species.

References

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Steenberge MV, Jublier N, Kéver L, Gresham S, Derycke S, Snoeks J, Parmentier E, Poncin P, Verheyen E (2022) The initial response of females towards congeneric males matches the propensity to hybridise in Ophthalmotilapia. bioRxiv, 2021.08.07.455508, ver. 3 peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2021.08.07.455508

Reviews

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Evaluation round #1

DOI or URL of the preprint: ***https://doi.org/10.1101/2021.08.07.455508***

Version of the preprint: 1

Author's Reply, None

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Decision by Ellen Decaestecker, 14 Nov 2021

Dear Dr. Van Steenberge and colleagues, your manuscript has been reviewed by 3 reviewers. Most of them think your manuscript merits publication but still have some issues. Therefore I suggest a revision of your manuscript integrating the issues the reviewers have brought up. Kind regards, Ellen Decaestecker

Reviewed by anonymous reviewer, 29 Oct 2021

In the paper "The initial response of females towards congeneric males matches the propensity to hybridize in Ophthalmotilapia", Van Steenberge and colleagues test differences in initial behavioral responses of female Ophthalmotilapia ventralis and Ophthalmotilapia nasuta towards conspecific males (relative to heterospecific males, conspecific females, or no fish). As predicted from previously reported introgression asymmetries found in natural populations, female O. nasuta are "pickier" than female O. ventralis. The manuscript reads very well, is well organized and the discussion is balanced (although a bit lengthy). The methods and statistical analyses of the data are detailed, well-founded and produce clear results. Nonetheless, I have one important suggestion regarding the analyses and one correction of the figure that illustrates the experimental setup. I have a few minor additional comments and suggestions that I detail next:

- Asymmetries in pre-zygotic isolation are not only observed in several species (as the authors indicate and for which several references are provided) but they are also expected. This is an important nuance but I think it is worth making and referencing. Coyne and Orr (2004) discuss this but more references should also be available.
- I feel that Figures 2 and 3, which explore focal female behavior before and after visual contact is established with stimuli, would be more informative if the analyses (PCA and CVA) would be ran on the combined (before and after) dataset (ON experiments and OV experiments analyzed separately). The authors would still plot 'before' and 'after' separately, but both would be on the same coordinate system and, importantly, any change in female behavior in response to stimuli would be easier to perceive.
- I detected one mistake in Figures 2 and 3 (and supplementary files) where the different experimental comparisons are depicted. In particular, only conspecific females seem to have been presented to focal females. Thus, in the figures for the OV experiments, the non-focal part of the figure should depict O. ventralis females as well.
- Line 101. I suggest "feeding schools"
- Line 177. I suggest "monospecific aquaria"
- Line 273. I suggest "terracotta flowerpots"
- Line 403. I suggest "We carried out CVAs on the same datasets"
- Line 450-451. Do non-focal females and conspecific males differ in weight from each other or from the focal females? Please specify.
- Line 489-490. I suggest "'flee' behaviour when presented to an O. ventralis male"
- Line 518. I suggest "did not"
- Line 527. I suggest "more" or "more strongly"
- Line 649. I suggest "stereotypical"

- Line 665. I suggest “does not”
- Line 674. I suggest “ecological range”

Reviewed by anonymous reviewer, 04 Nov 2021

This experiment aims to examine whether asymmetric hybridization discovered in wild populations has a behavioural basis that can be uncovered in the lab. The aim is to test a case of prezygotic, behavioural isolation. There is a nice experimental design, and some clear predictions that one could make about female behaviours in response to conspecific and heterospecific males (and females). One notable limitation of the study is the lack of comparison between conspecific and heterospecific males and females in both species, acknowledged in the Discussion. Another important limitation acknowledged by the authors is that their experiment seemed to “observe(d) the routine behaviour of a (isolated) female that encounters a conspecific individual, rather than sexually motivated behaviour”. I have some concerns that this rather undermines the main premise of the work, since sexually motivated behaviour would be crucial to understand in order to successfully address the main question the authors identify.

The paper says “we may expect that the early response to conspecific and heterospecific mates will predict the outcome of the mating process to a substantial degree. We test this hypothesis using a cichlid model.” but this is contradicted by the acknowledgement that they are not assessing sexually motivated behaviour.

From the point of view of the statistical analysis, I found the approach lacking a clear direction and hypothesis test. There is a very clear prediction here - that specific behaviours should reduce. Why is the exploratory analysis needed? Why “without defining a priori in what variable specimens would differ.” ? I would consider replacing it with a GLMM which tests specific defined behaviours as a dependent variable, and includes gonad weight, before/after1/after2, treatment and species in the model (as well as non-focal male behaviour, which could be confounding the experiment). If you think that multivariate analysis is important, PCs could be used instead of single behaviours of interest.

In general, although the PCs might be helpful for exploring the behaviours, I don’t think they are very helpful in presenting the results. There were some specific aspects I found confusing and which might benefit from more clarity:

“However, heterospecific males were (somewhat) separated from all other specimens by their higher values for PC1 (ON experiment) or PC2 (OV experiment). This difference was due to a more active swimming behaviour (Sp, SpX, SpY) higher up in the water column (height) for *O. ventralis* males (ON experiment) and a higher number of point events (ram, sand, bite) performed at the floor of the aquarium (height) for the *O. nasuta* males (OV experiment), prior to their presentation to a heterospecific female”

Are these separate plots but from the same PC analysis? Or different PC analyses for each species? Perhaps these data would be more clearly visualised as boxplots showing the (lack of) difference between species at specific behaviours?

There are a couple of other points or small issues that might be helpful to address:

- “which raises the question how can they coexist.” needs a question mark.
- hybridise/hybridize needs a consistency throughout
- “ the question remains what mechanisms keep incipient species separated.” needs a question mark as well I think?
- Are these really incipient species? Evidence needed.

- “In scenarios of sympatric, closely related species, the ability to correctly distinguish between conspecific and heterospecific mates is probably crucial (Sullivan 2009).” I would add something like “if the speciation process is to be complete” to clarify what it is crucial for.
- “By snapping at the egg dummies, which are situated close to the genital opening of the male, the intake of sperm is facilitated, increasing the fertilisation rate of the eggs within the female’s mouth (Salzburger et al. 2007).” - this reference doesn’t evidence this claim. It might be better to add “the intake of sperm is thought to be facilitated” since to the best of my knowledge there isn’t a study that has directly tested this (if there is, please cite it here instead!).
- “we predict to see an interspecific difference in female response to conspecific and heterospecific males.” - but not females? Surely this is an important control, if this really is about mate choosiness?
- “that a sex change did indeed took place in several specimens” = take place
- “The later was conducted to maximize” = latter
- Before, after1 and after2 - clarify how after1 and after2 were defined and what the biological meaning was.
- “For this, in view on the size of the dataset” = of
- “*O. nasuta* specimens, on average, spent more time closer to the bottom whereas O. ventralis specimens were more often found higher up in the water column” presumably because nasuta is a bower builder?
- Figure 1B is a good opportunity to visually outline the experimental design, but I think it could perhaps be improved so that actual fish pictures are used and the reader doesn’t have to rely so heavily on the text?

Overall, if there is good evidence that these are incipient species, and that sexual behaviour can be adequately quantified, I would think that this is a nice study (if not optimal, given some limitations of the design).

Reviewed by George Turner, 11 Oct 2021

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